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Understanding the role of plant-microbe symbiosis in the cycling of carbon in temperate forest ecosystems

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Understanding the role of plant-microbe symbiosis in the cycling of carbon in temperate forest ecosystems

A thesis submitted to Bangor University by

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In candidature for the degree

Philosophiae Doctor

January 2020

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“I may not have gone where I intended to go, but I think I have ended up where I needed to be.”

Douglas Adams

“It is a miracle that curiosity survives formal education.”

Albert Einstein

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Abbreviations

SOM	Soil Organic Matter
SOC	Soil Organic Carbon
TON	Total Organic Nitrogen
DOC	Dissolved Organic Carbon
MB	Microbial Biomass
CMN	Common Mycorrhizal Network
EMF	Ectomycorrhizal Fungi
AMF	Arbuscular Mycorrhizal Fungi
NSC	Non-structural carbohydrates

Abstract

Soil microorganisms and their symbiotic relationships with plants are fundamental to nutrient cycling in temperate forest ecosystems. This highly diverse microbiome contains up to a quarter of Earth's biodiversity, but our understanding of how this affects the function of forests is not well understood. This thesis investigated the role of plant symbionts on the allocation of C to belowground microbial symbionts and to ground vegetation via microbial symbionts. Radio-isotope pulse labelling was used to determine the belowground C dynamics of these highly complex systems by allowing us to quantify pools and fluxes within the plant-microbe-soil continuum. In Chapter 3, the role of arbuscular and ecto-mycorrhizal fungi in belowground allocation of C in three temperate tree species was investigated by destructive harvesting of trees 336 days after a pulse label had been applied. The results suggested that *Alnus glutinosa* and *Betula pendula* allocated C belowground to microbes, whereas *Castanea sativa* transferred the C to the soil where it was sequestered. In Chapter 4, inter- and intra-specific C transfer was studied using trees connected via a common mycorrhizal network (CMN), the results suggested that more C was transferred between inter- than intra-specific species combinations. In Chapter 5, C transferred via three "donor" tree species to the root nodules of *A. glutinosa* "receiver" tree connected with a CMN was investigated using the methodology pioneered in Chapter 3. The plant: fungal amalgam preferentially allocated C from the donor trees to the root nodules of the receiver *A. glutinosa* tree. We postulated that this was due to the considerable energetic demands of nitrogen-fixation by *Frankia alni* in the root nodule creating a strong C sink. In Chapter 6, the transfer of C from 13-year-old coppiced *A. glutinosa* and *C. sativa* trees to ground vegetation via CMN was investigated. ^{14}C activity in the ground vegetation under the *A. glutinosa* trees was expected to be greatest, as *A. glutinosa* share arbuscular mycorrhizal partnerships with the ground vegetation. No difference in ^{14}C activity was found in the hyphae, soil solution or ground vegetation under *A. glutinosa*. We postulated that this could be due to root grafting, mycorrhizal types exchanging nutrients, or reabsorption of tree rhizodeposits. Overall this study suggests that the plant: microbe symbiosis that is ubiquitous across the temperate biome is both important for nutrient cycling and C storage, but also that the sharing of resources via CMNs could be altering plant competition dynamics that have previously been based on the assumption that plants are not physically connected and actively sharing resources. Further work to determine how plants or mycorrhizae control belowground resource sharing could lead to a paradigm shift in our understanding of competition and facilitation in plant community dynamics.

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Chapter 1: Introduction

1.1. General Introduction and Rationale

Since Darwin first described the evolution of species, the focus of ecological research has been on competition or survival of the fittest as the dominant driver of biological diversity (Ryan, 2002). This term is widely misinterpreted and often symbiosis is seen as incommensurable with the competitive model (Ryan, 2002). However, others disagree and suggest that the co-operation enables the organism to do this better and thus increases its fitness (Axelrod and Hamilton, 1981). The model of evolution described by Darwin is typified by gradual changes though natural competitive advantage gained by multiple diminutive mutations (Roossinck, 2005). Darwin (1859) stated “Nature ... can never take a leap but must advance by the slowest and shortest steps” and yet fossil records and phylogenetic analyses show that leaps forward have historically occurred (Ryan, 2002). It is now generally accepted amongst evolutionary theorists that competition alone cannot account for the speed of evolution and vast diversity of life on our planet (Tilman, 1994). One possible explanation, known as the symbiosis model, suggests that two or more previously symbiotic individuals merge and become new taxonomic groups (Ryan, 2002). For example, the discovery that mitochondria and chloroplasts contain both DNA and ribosomes reminiscent of those located in bacteria has led to a distillation of these ideas, that the importance of facilitation and symbiosis may have been underestimated (Sagan 1967; Gaia et al., 2018). In 1975, analysis of rDNA confirmed that a chloroplast’s 16S RNA oligonucleotide was more closely related to that of cyanobacteria than to the 18S RNA of the chloroplast containing plant (Zablen et al., 1975). Plant mitochondrial rDNA was finally linked to the organelles of alphaproteobacteria (Yang et al., 1985). A recent hypothesis known as the eocyte hypothesis suggests that the nucleus and cytoplasm of eukaryotic cells originate from an ancient archaeon, which enveloped a bacterial mitochondrial ancestor at some point in evolutionary history (Archibald, 2008).

The natural world is full of co-operative partnerships that do not obviously sit comfortably within the competitive framework proposed by Darwinian Theory. For example, the lichens that cover the rocks and exposed surfaces of our ecosystems are complex symbiotic relationships between cyanobacteria and often multiple species of fungi (Margulis and Barreno, 2003), in some cases totalling four partners in stable equilibrium (Spribille et al., 2016; Tuovinen, 2019). Traditionally, it was thought that coral reefs were comprised of microbial algae called *Symbiodinium* and a basic animal from the *Anthozoa* group. However, DNA

analysis now suggests that coral reefs that inhabit the World's seas include a third player, a group of animals normally associated with the *Apicomplexa* (Richards and McCutcheon, 2019). Normally these organisms are thought to be associated with parasitic diseases, such as malaria (Kwong et al., 2019). Clements as far back as 1916 suggested that facilitation or mutualistic symbiosis was more fundamental to ecological theory and, to plant succession particularly. Unfortunately, since then this has been largely overlooked by ecologists due to the popularity of the alternatives; the competition (Tilman, 1982; Grime 1974) and individualistic theories (Gleason 1926; Whittaker 1956). In the mid-nineties interest in facilitation was renewed after a series of experiments in extreme conditions suggested facilitation was more fundamental to ecosystem stability (Michalet and Pugnaire, 2016). In 1994, Bertness and Callaway hypothesised a new ecological model whereby the importance of competition gives way to facilitation, under periods of ecological stress. Since this theory was proposed, the number of experiments only concerned with competitive drivers of ecosystems has dropped from 90 % to 76 % (Michalet and Pugnaire, 2016). Facilitative symbiosis is also prevalent in the belowground component of our ecosystems and recent research is suggesting that in some cases mitigates aboveground competitive pressures between plants (Fonseca, 2017). It would now seem that facilitative relationships are more widespread and particularly during periods of environmental stress (McIntire and Fajardo, 2014). Ecological theory probably needs to be modified to include relationships which undermine competitive pressures amongst biological communities and more research needs to be conducted to see if their importance increases under stress (Bruno et al., 2003).

In temperate forest ecosystems, ectomycorrhizal (ECM) fungi dominate the belowground mutualistic symbiotic landscape with over 7000 known isolated species forming relationships with a range of trees species (Tedersoo et al., 2012). Although the presence of these complex interactions has been known since the 1850s, facilitative symbioses were seen as exceptional and as a result were largely overlooked as a selective pressure by Darwinian Theory (Ryan, 2002). The Earth's biosphere is a fundamental component of nutrient and water cycling and as a result global carbon (C) budgeting (Fahey et al., 2010). Although most studies have investigated the aboveground C stored in forest ecosystems as either live biomass, woody debris and detritus or as soil organic matter. However, the belowground component (i.e. soil) represents the largest reservoir of organic C within terrestrial ecosystems and the world's forests are of particular importance as C storage pools. The belowground processes of forests mediate many key biogeochemical processes, which have an important role in regulating

climate, and are major hotspots of biodiversity. Understanding the mechanisms controlling soil C sequestration and stability are essential in maximizing soil C sequestration potential. Knowledge gaps still exist in quantifying the contribution of plant: microbe symbiosis, specifically; mycorrhizal hyphal turnover, hyphal biomass, root exudation, rhizodeposition, and the contribution of microbial derived C to soil C stocks (Clemmensen et al., 2013; Gougoulas et al., 2014; Averill et al., 2014). Within the heterogeneous soil matrix microbial hotspots, have been identified that preferentially sequester C (Vogel et al., 2014), and show disproportionately high reactions rates relative to the surrounding soil matrix (Johnson et al., 2014), but the mechanisms surrounding their formation, function, and ecological relevance remains poorly understood. To maximize the provision of ecosystem services from our forests and to inform policy makers there is a need to improve our understanding of belowground processes influencing the C cycle (Hulvey et al., 2013; Cotrufo et al., 2013). Increasing forest diversity and exploiting species' functional traits offers the potential to enhance ecosystem resilience and resistance to our changing climate (Lavorel, 2013; Forrester, 2014), but will also likely modify belowground microbial transformations and ecological interactions altering C cycling and long-term C storage.

This thesis investigates the role and contribution of belowground plant-microbe symbiosis to C storage and cycling, by utilising novel radio-isotopic pulse chase experimental set-ups. A greater understanding of the belowground microbial components of temperate forest ecosystems will enable us to choose appropriate species and silvicultural management techniques to further increase terrestrial C storage.

1.2. Carbon Dynamics Terminology

Studies that have investigated C dynamics in forest ecosystems have often used inconsistent terminology (Litton et al., 2007), and, as a result, the terminology used in this thesis may benefit from clarification with reference to the terminology used in the scientific literature. Studies frequently refer to different nouns e.g., apportionment, allocation, distribution, partition, transportation, translocation, that are frequently used interchangeably causing widespread confusion (Gower et al., 1995). The term allocation is used with reference to everything from the growth of biomass, to the flux of C to a specific plant part to the distribution of fractions of the net primary production (Ryan et al., 1996; Giardina et al., 2003). During this thesis, I propose to accept the standardised definitions proposed by Litton et al., (2007), which are as follows:

Allocation: a wide-reaching term that can be referring to a diverse range of the physiology of both plants, fungi and ecosystems, including a flux of C to a tree partition, an allometry of live biomass or the delivery of a portion of the products of photosynthesis to a particular plant organ (Litton et al., 2007; Epron et al., 2012). C allocation is the result of many complex mechanisms (Cannell and Dewar, 1994).

Biomass: the mass of any or all organic components of an ecosystem (Odum et al., 2005) and therefore biomass allocation refers to the distribution to different plant structure, for example root: shoot (Litton et al., 2007).

Compartment: there is no unambiguous definition for the word compartment within the biological literature. Nevertheless, for the purpose of this thesis a compartment is a subdivision of a larger biological unit based on unique or characteristic function or physiological structure (Hauri and Schweizer, 1992).

Flux: the rate at which C is moved or moves from one compartment to another and the processes or mechanism underpinning this transfer.

Partition: is the division of photosynthetically-fixed C into subdivisions (to particular forest ecosystem compartments) and this is a fraction of total (gross) primary production (GPP) (Odum et al., 2005) and usually expressed a percentage or a proportion (0–1, no units). C partitioning- is an overarching definition that can cover all three of the above (Litto et al., 2007).

Pool: a compartment where in this case, C is stored.

1.3. Plan of Thesis

This thesis was divided into six chapters commencing with the literature review, which critically evaluates the role that plant microbial symbiosis has in the cycling and storage of C belowground in temperate forest ecosystems. It was used to identify the knowledge gaps addressed in this thesis.

The experimental work was divided into four chapters. As each is intended to be a stand-alone research article, there is inevitably some repetition of introductory material, methodological techniques and the use of references. The first experimental chapter quantifies the relative importance of the soil microbial community to the three tree species studied, by tracing recently fixed C to quantify the allocation of C to different belowground biomass partitions.

The second and third experimental chapters build on the first, by investigating the role of common mycorrhizal networks to C exchange in both intra- and inter-specific species combinations. The third chapter more specifically looks at the importance of the presence of nitrogen-fixing bacteria, which are located in actinorhizal nodules in the tree roots of the species *A. glutinosa* used in this study. The fourth and final experimental chapter investigated the spatiotemporal transfer of C in field conditions using *A. glutinosa* and *C. sativa* but not *B. pendula*.

Finally, the last chapter includes a synthesis of the experimental chapters and a general discussion of the aims and objectives of the thesis and of the results from each experimental chapter. Overall conclusions are drawn, and areas of future research priorities identified. Appendices include a selection of photographs of the experimental work.

1.4. Aims and Objectives

The aims and objectives as framed by the context of the above were as follows:

- 1) To investigate the differences in belowground C allocation between early and late-successional temperate tree species (Chapter 3 and Chapter 6)
- 2) To investigate the intra and interspecific transfer of C via common mycorrhizal networks (CMNs) in temperate forest ecosystems (Chapter 4)
- 3) To investigate the effect of trees with tripartite mutualistic symbiotic relationships on the magnitude and allocation of C transferred via CMN (Chapter 5)
- 4) To investigate the differences in belowground allocation between glasshouse-based and field-based experiments (Chapter 6)

1.5. Hypotheses

H₁: Belowground allocation of C will be greater in species with the largest diversity of microbial symbiotic associations (Chapter 3).

H₂: The transfer of recently assimilated C to the soil microbial community will be greater in early (*B. pendula* and *A. glutinosa*), than late successional trees (*C. sativa*) (Chapter 3).

H₃: Transfer of C by CMNs is greater inter-specifically than intra-specifically, as a result of differences in C demand between species. (Chapter 4).

H₄: Greater mycorrhizal diversity per unit of rhizosphere soil will result in more C transfer between trees via CMN (Chapter 4).

H₅: The abundance of mycorrhizal hyphae is positively correlated with C transfer between trees (Chapter 4).

H₆: Belowground allocation of C via inter- and intra-specific CMNs will be greatest in “receiver” trees with actinorhizal associations, due to an increased C sink strength generated by the metabolic processes of the root nodule (Chapter 5).

H₇: Transfer of C to ground vegetation will be greatest via the CMN of *A. glutinosa* that shares an arbuscular mycorrhizal symbiosis than via the obligate ectomycorrhizal species *C. sativa* (Chapter 6).

H₈: The percentage transfer of recently fixed C to mycorrhizal fungal hyphae in the field will be similar to those calculated in laboratory experiments (Chapter 6).

1.6. Methods Overview

C flows through the air-plant-soil continuum by first being photoassimilated by the plant, allocated to plant organs whereby a proportion is autotrophically respired and returns to the atmosphere. The remainder is either released into the rhizosphere or mycorrhizosphere where it is rapidly consumed by soil micro-organisms and either respired heterotrophically or retained as quasi-inert compounds which are stored in the soil. These processes are highly complex and understudied but represent a fundamental component of forest C budgeting (Epron et al., 2012). Further, C allocation to the plant organs and microbial component of the air-plant-soil continuum is of particular interest as this can adjust the trees growth pattern and allometry by mediating competition for C between the above- belowground sinks (Litton et al., 2007). The change in C partition size is vital, as in the case of the leaf biomass/area, as this obviously alters the light intercepted by the tree and the resulting primary production of the tree (Gower et al., 2001; Sinsabaugh et al., 2017). C partitioning is also essential for facilitating, the acquisition of nutrients and trace elements required for healthy tree growth through modifying the belowground biomass such as root volume and which effects C sequestration as both soil organic matter and plant material (above and belowground standing biomass) (Litton et al., 2007; McCormack et al., 2015).

C allocation was historically estimated using mass balance calculations, which traditionally are the addition of measured aboveground biomass and respiration measurements. The belowground component including the importance of fine-root turnover, mycorrhizal hyphal growth and root exudation is then estimated by subtracting the C inputs from aboveground (litter and woody debris inputs) from the total cumulative soil CO₂ efflux (Giardina and Ryan, 2002). In some cases, measurements of annual fluctuations in aboveground respiration from tree organs and allometric relationships are used to calculate aboveground C flux (Ryan et al., 1996). Eddy covariance can also be used to measure net CO₂ flux and belowground C fluxes, which are then subtracted from gross primary production to estimate total aboveground C flux (Navarro et al., 2008). Although these techniques have been useful in the quantification of C budgets for an entire ecosystem or area it is near impossible to disentangle the autotrophic and heterotrophic respiration components (Carbone et al., 2016). Mass balance approaches whilst useful, do not elucidate the short-term C dynamics essential to understanding the annual allocation dynamics of temperate deciduous tree species or explain the relative roles of phenology climatic drivers of seasonal changes in allocation or how these factors could change in importance in changing climatic conditions (Epron et al., 2012). Mycorrhizal plants present

their own additional methodological challenges especially when attempting to quantify C fluxes and the concealed molecular mechanisms that underpin these processes (Slavíková et al., 2017). Mycorrhizal fungi obtain the majority of their C from plant photosynthesis, alongside a small proportion derived from decomposing plant matter (Olsson and Johnson, 2005; Hobbie et al., 2014; Lindahl and Tunlid, 2015). The colonisation of roots by mycorrhizal fungi can also alter the relative allocation to plant organs, particularly the roots (Wright et al., 1998; Slavíková et al., 2017), as well as altering the root architecture in some cases (Chen et al., 2000; Eissenstat et al., 2015; Chen et al., 2016). The presence of mycorrhizal fungi has also been found to increase photosynthetic activity through providing the tree with nutrients directly or by increasingly the belowground sink strength (Douds et al., 2000; Kaschuk et al., 2009; Valentine et al., 2013). The flux of C from the plant can only be measured directly by employing the use of C isotopes and a percentage of total C budget diverted to the symbiosis calculated (Řezáčová et al., 2017). Studies have shown that the cost of mycorrhizal associations to photosynthetically fixed C ranges from 4 – 20 % (Smith and Read, 2010). The highest value of 20 % has only been observed in one mesocosm, containing young cucumber (*Cucumis sativus*) plants by Jakobsen and Rosendahl (1990) and has unfortunately been extrapolated up to a total global figure (Brzostek et al., 2014). Contemporary research suggests that the actual figure is far lower than even the lowest 4 % suggested previously; both Tomè et al., (2015) and Slavíková et al., (2017) found only a few percent of the plants total C budget was expended on mycorrhizae. The investigation of the true cost of forming a mycorrhizal symbiosis to the plant have only been made possible by the increase of availability of isotopic methodologies and a reduction in the cost of equipment used to analyse them (Řezáčová et al., 2017).

Isotopic C can be used to trace the source and fate of C recently fixed by photosynthesis through the plant partitions, into metabolites and respiration and onto the mycorrhizosphere (Dawson et al., 2002), but the sometimes-low signal strength (especially with ^{13}C) can make resolving lag times and rates of transfer problematic (Epron et al., 2012). However, that said, these techniques have been employed successfully to study C allocation in trees and forest ecosystems (Knohl et al., 2005; Brandes et al., 2006; Keitel et al., 2006; Kodama et al., 2008; Marron et al., 2009; Wingate et al., 2010). Isotopes of C that have been successfully deployed in this type of allocation experiments include stable isotopes (^{13}C) and radio-isotopes (^{14}C and ^{11}C), these have been used for short-pulses or over extended periods, by artificially altering the isotopic C before photo assimilation (Epron et al., 2012). During pulse-chase experiments, the isotope is used as a proxy for recently fixed C and the relative allocation can be estimated by

the amount of isotope retained in the plant organs or lost to the system by exudation, respiration and emissions of volatile organic compounds and calculated as a percentage of the total fixed C. Isotopes can therefore also determine if the C has been allocated to structural or non-structural components of any plant organ (Kagawa, 2006).

Radioisotopes have been used since the 1950s to study the fate of recently fixed C, although their use remains controlled by both Health and Safety and radioisotope safety regulations (Schoor et al., 2016). Initially, radioisotopes were favoured due to the availability of analytical instruments to trace the radioactivity through the air-plant-soil continuum, namely, biological oxidation, autoradiography, liquid scintillation (LS) spectrometry and accelerator mass spectrometry (AMS). These have been used for pot-based experiments, individual branches of field-grown trees and *in-situ* whole tree labelling experiments (Epron et al., 2012). One famous example of this methodology being used includes the experiments conducted by Calvin and colleagues, which led to the discovery of the photosynthetic reduction cycle (Calvin, 1949). Because recent photosynthates are very rapidly transferred to the soil microbial community (Kaiser et al., 2015), either directly or via mycorrhizae, labelled C can quickly be recaptured in root exudates, soil pore water, heterotrophic respiration and microbial biomass (Epron et al., 2012). Soluble C can also be extracted from the soil using a suitable solvent (usually K₂SO₄). The isotopic C transferred to the mycorrhizal hyphae can also be detected through the use of soil cores, hyphal in growth bags or fractions of a mesocosm by excluding roots with a suitably sized nylon mesh (Johnson et al., 2002; Epron et al., 2011). Novel molecular techniques are also now allowing us to trace the isotope into nucleic acids (Ostle et al., 2003), fatty acids and amino acids (Högberg et al., 2010).

Due to availability of analytical facilities at the School of Natural Sciences, Bangor University, in agreement with my supervisors I decided to use a ¹⁴C pulse-chase experiment throughout this thesis. In all experimental data chapters, a radioisotope of C (¹⁴CO₂ with a half-life of 5730 years) was utilised to produce a C pulse-chase label, which was traced through the plant soil continuum to identify the pathway of recently fixed C from the photobiont to the soil microbial community. The advantage of using ¹⁴C over other isotopes is that in a field experiment tiny amounts of isotopic enrichment can be detected partly due to the low detection rates of the analytic devices and also because the atmospheric background readings of ¹⁴C are relatively low (Epron et al., 2012). Rhizon suction samplers were used to capture rhizospheric soil pore water that was analysed by liquid scintillation spectrometry after biological oxidation, which bubbled the resulting gas through liquid scintillation fluid to capture the ¹⁴C, and allow

quantification of C inputs from the roots and hyphae by liquid scintillation spectrometry (Plate 2). All chapters with the exception of the final experimental chapter were mesocosm experiments that used the liquid scintillation technique as the β -radiation remained detectable whilst remaining under safe working limits set by legislation. The heterotrophic and autotrophic soil respiration was captured using sodium hydroxide respiration traps suspended above the soil. After destructive harvesting, the 2-year-old trees (see section 1.7 for species description and provenance information) of the three species studied were separated into plant tissue types (leaves, stem and branches, and roots). These were then analysed for ^{14}C activity by combusting the organic material in the sample with a Harvey Instruments Biological Oxidiser OX400 (R.J. Harvey Instrument Corp., Hillsdale, N.J., U.S.A) (Plate 1). The $^{14}\text{CO}_2$ bubbled through and dissolved into Oxysolve C-400 (Zinsser Analytic GmbH, Frankfurt, Germany) scintillation fluid prior to determination of ^{14}C content using a Wallac 1409 liquid scintillation counter (Wallac EGandG, Milton Keynes, UK) (Plate 2).



Plate 1: The Harvey Instruments Biological Oxidiser OX400 located in the fume hood located, in the radiation laboratory, which was used to combust the vegetation, soil and hyphae in sand, for all experimental chapters.



Plate 2: Wallace 1409 liquid scintillation counter located in the counting room and utilised for all ^{14}C analysis of vegetative, soil sand and liquid samples (Wallac EGandG, Milton Keynes, UK).

1.7. Facilities and Field Site Description

All experiments were conducted in either in the glasshouse, located on the roof of Memorial Building in Bangor, or at Bangor University's Henfaes Research Farm. These facilities will be described in this section. A total of 44 x *A. glutinosa* and 36 x *B. pendula* and 36 x *C. sativa* trees were used in the experimental chapters.

Glasshouse experiments

Chapters 3, 4 and 5 were all conducted *ex-situ* in a glasshouse located on the roof of the Memorial Building, Deiniol Road, Bangor, LL57 2UR (53°13'32.0"N 4°07'55.8"W) (Plate 3). All soil used in the glasshouse experiments was collected from beneath established (15 years old) trees of the same species in the BangorDIVERSE experiment located at Henfaes Experimental Farm, Abergwyngregan (Plate 4). The glacial till derived nature of the soil in this area means that it was extremely stony, so stones were removed on site with a homemade wheelbarrow mounted riddle (Plate 4). After the majority of large stones (cobbles) had been removed, the soil was barrowed to a glasshouse where it was air-dried (Plate 5), before sieving to pass through a 2 mm sieve and utilised in the mesocosm experiments (Chapter 3, 4 and 5).



Plate 3: Memorial Building, Deiniol Road, Bangor with the experimental glasshouses on the roof. The lower level glasshouse on the left of the plate was the glasshouse that experimental chapter 3, 4 and 5 were conducted in.



Plate 4: All soil used in the pot experiments was dug from BangorDIVERSE, due to the glacial till nature of the soil we constructed a homemade, wheelbarrow mounted riddle to remove the majority of large stones before air drying the soil in a glasshouse.



Plate 5: Soil removed from BangorDIVERSE was air dried before being passed through a 2 mm. sieve and utilised in the mesocosm experiments (Chapter3, 4 and 5).

The mesocosms used in the three mesocosm experiments (Chapters 3, 4 and 5) were located in the glasshouses (Plate 4) and laid out on the benches in a randomised block design and rotated around the glasshouse to homogenise conditions and compensate for uneven growth conditions (hotspots, shaded areas, etc.).



Plate 6: Inside the glasshouse during the experimental establishment period of chapters 3 and 4 during the summer of 2016.

Field based experiments

Field based research was conducted at Bangor University's tree diversity experiment (BangorDIVERSE), established in 2004 on two fields totalling approximately 2.36 ha at Bangor University's Henfaes Research Farm, Abergwyngregan, Llanfairfechan, North Wales, LL33 0LB (53°14 N, 4°01 W), located 12 km east of Bangor city centre (Ahmed et al., 2016). The BangorDIVERSE forest experiment is part of a global tree diversity network (<http://www.treedivnet.ugent.be/>) which has already collected data that is of significance to global forest policy and management (Verheyen et al., 2016). BangorDIVERSE was planted on land that had previously been pasture, since the 1980s; one of the fields had been used for small-scale forestry experimentation; the other was ploughed for oil seed rape (*Brassica napus* subsp. *napus*) in 2003. The 60 cm trees were planted in four replicated blocks of one, two and three species mixtures at spacing of 1 m (10,000 stems/ha). The species planted (*Alnus glutinosa* L., *Betula pendula* Roth., *Fagus sylvatica* L., *Fraxinus excelsior*, *Acer pseudoplatanus* L., *Castanea sativa* Mill. and *Quercus robur* L.) were chosen to exhibit contrasting functional traits and successional stages.

The plots are gently sloping (between 1 – 2 °) and approximately 13 – 18 m above sea level with a north-westerly aspect. The underlying geology is alluvial gravel and glacial till with a Eutric Cambisol soil type (Rheidol series) (Smith et al., 2013). Soil has a fine loamy texture (48.2 ± 1.3 % sand, 33.6 ± 0.9 % silt and 18.2 ± 2.1 % clay) as determined by laser diffraction granulometry (Coulter LS particle size analyser) (Gunina et al., 2017). The soil has an Ah horizon (0-40 cm which grades into a Bh horizon with the C horizon apparent at ca. 100 cm). The climate is hyperoceanic, the mean annual precipitation of 1034 mm and mean annual temperature of 11.5 °C (Campbell Scientific Ltd, Shepshed, UK).

1.8. Autecology of the Tree Species Studied

Tree species were selected from the existing species within the BangorDIVERSE plots. The study will determine the C balance of three species possessing contrasting functional traits. The species studied were as follows:

Common alder (*Alnus glutinosa* [L.] Gaertn.; A) (also commonly known as black alder) is a native broadleaf, deciduous, fast growing but short-lived actinorhizal pioneer species. This tree was of particular interest as not only does it have a symbiotic relationship with nitrogen fixing bacteria (*Frankia alni*), but also possesses both arbuscular and ecto-mycorrhizal associations (tripartite symbiosis). *A. glutinosa* tolerates waterlogged soil and in fact needs high water availability to thrive, ideally with annual rainfall above 1500 mm or accessibility to groundwater (Claessens et al., 2010). As such, it is most commonly found in riparian zones and swamps, the seed is cone-like and floats in water allowing for dispersal along watercourses (McVean, 1956). *A. glutinosa* has adaptations which allow the trees survival in the wettest of conditions, the roots are supplied air from above the water level through the xylem, and this supplies the roots with oxygen (Dittert, 2006). Where watercourses are not present, the rate of spread is generally slower than that of other pioneer species such as *Betula pendula* (silver birch) and *Fraxinus excelsior* (common ash). *A. glutinosa* has one of the most northerly ranges of a deciduous tree in the UK, only outdone by *Betula pubescens* and *Alnus incana* (Iverson, 1944) and is found at altitudes of 500 m in Scottish hills (Savill, 2019). To regenerate from seed *A. glutinosa* demand both moisture and high light levels and therefore flourish on disturbed sites. Initially, growth rates are high in years 7-10 after which they slow dramatically; *A. glutinosa* prefer sites with a pH above 6 and are tolerant of salt spray (Savill, 2019).

Timber from *A. glutinosa* is not that dense (530 kg m⁻³ at 15 % moisture) and is used as a source of energy, for paper and to make chipboard, as well as for joinery veneers and traditionally for clogs and charcoal (Savill, 2019; Claessens et al., 2010). A useful characteristic is that the wood is decay resistant when submerged in water and as a result is used for sluice gates, jetties, underwater supports and small boats (Savill, 2019; Hines et al., 2016). Rotation length is normally seventy years if heart rot is to be avoided and mean average growth rate is between 4 to 14 m³ ha⁻¹ year⁻¹ (Claessens et al., 2010). Average lifespan is reported to be only 20- 25 years with a maximum height of 22 m and diameter at breast height (1.3 m) of 0.65 m (Mitchell et al., 1974; Savill, 2019). The commonest pathogen of *A. glutinosa*, *Phytophthora alni* has been

identified in many European countries and is expected to increase in frequency in the near future (Hines et al., 2016).

Silver birch (*Betula pendula* Roth; B) is an early successional, pioneer, broadleaved deciduous native tree species with ectomycorrhizal associations and labile leaf litter, which occurs across the whole of Europe (Hines et al., 2016). *B. pendula* is quite short-lived with lifespans typically between 35 and 100 years. It has an average maximum height of 30 m and its diameter at breast height (1.3 m) tends not to exceed 0.95 m (Mitchell et al., 1974). *B. pendula* trees are extremely hardy and are distributed and flourish at altitudes where only *Sorbus aucuparia* (rowan) will survive (Savill, 2019). *B. pendula* require full sun to thrive but can cope with soils that possess poor nutritional status (Hines et al., 2016), due to their ability to form mycorrhizal associations with a large range of fungal species (Atkinson, 1992). Due to their production of highly labile litter they are generally considered to be soil improvers (Miles, 1986). The flowers are monoecious with both sexes appearing at different times as unisex catkins. Males develop in summer and disperse their seed a few days following the female flowers in spring (Hines et al., 2016). *B. pendula* produce seeds prolifically (between 1,650,000 – 2 million seeds kg⁻¹), which are very small (2 – 3 mm across) and have small wings to facilitate their dispersal by wind (Hines et al., 2016).

Growth rates are 7 m³ ha⁻¹ year⁻¹ on the best sites and *B. pendula* has a characteristic smooth white bark and is used for high value veneers and furniture as well as firewood. It is not however a durable wood (Lorrain-Smith and Worrell, 1992), with an average density of 670 kg m⁻³ (at 15 % moisture content) (Savill, 2019). *B. pendula* has few diseases, although it can be parasitised by *Amillaria* spp. (honey fungus) and can commonly be observed with ‘Witches brooms’ a distorted growth pattern caused by a fungus *Taphrina betulina* (Savill, 2019). Birch is considered to have high wildlife value, supporting a large number of both generalist and specialist phytophagous insects, birds and mammals (Patterson, 1993).

Sweet chestnut (*Castanea sativa* Mill.; C) is a late successional non-native but naturalised tree species with solely ectomycorrhizal associations and recalcitrant leaf litter. *C. sativa* occurs naturally across the Mediterranean region but because of widespread cultivation for its nuts and durable wood now has a wide-ranging distribution (Hemery and Simblet, 2014). It is believed that *C. sativa* was first brought to the UK by the Romans and is relatively long-lived, living up to several thousand years old and has a maximum height of 35 m and a diameter at breast height (1.3 m) of 3.2 m (Mitchell et al., 1974). The species is considered reasonably

shade-tolerant, grows fast and coppices well on a rotation of 12 – 16 years (Savill, 2019). *C. sativa* prefers deep, fertile, light soils with moderate moisture (minimum rainfall required is between 600 and 800 mm) and flowers in late June (Savill, 2019). Large nuts (average weight 239 nuts kg⁻¹) develop during summer and ripen in autumn if the summer has been warm and dry although heavy crops are rare in the UK and trees need to be at least 30 years old for good production (Savill, 2019).

C. sativa is one of the most productive deciduous broadleaved species, with mean annual yield reported up to 8 m³ ha⁻¹ year⁻¹ (Locke, 1978). The timber is naturally durable, but not dense (560 kg m⁻³ at 15% moisture) and is frequently used for green woodwork and furniture, due to its ease of splitting and was traditionally used for pit props and coppices extremely well (Savill, 2019). It is affected by chestnut blight, *Endothia parasitica* (Savill, 2019), ink disease (*Phytophthora* spp.), and the newly introduced chestnut blight (*Cryphonectria parasitica*). Insect pests of *C. sativa* include Chinese gall wasp (*Dryocosmus kuriphilus*), chestnut weevil (*Curculio elephas*) and tortricids (*Cydia splendana*; *Cydia fagglana*; *Pammene fasciana*) (Hines et al., 2016).

The three trees were chosen as they exhibit both types of mycorrhizal symbiosis and *A. glutinosa* is the UK's only native tree which forms an association with nitrogen-fixing bacteria. In addition, the trees differ in their typical successional stages with *B. pendula* considered to be an early successional stage tree whereas *C. sativa* is most typically a late-successional stage tree. The trees were all purchased as 1+1 saplings (20- 40 cm height) from Maelor Forest Nurseries Ltd., Bronington, UK, in November 2015. At the time of planting, the height of all tree saplings was between 0.2 – 0.4 m. The provenance details of the trees purchased and those already established in the BangorDIVERSE plots (used in Chapter 6) are shown in Table 1. Of the three species studied experimentally, two are considered UK native species (*A. glutinosa* and *B. pendula*) and the other studied species is known as a naturalised species. Tree species are considered UK natives if they colonised the UK between the end of the last ice-age (10,000 years ago) and the formation of the English Channel, separating us from Europe several thousand years later. Species that have reached the UK since the formation of the Channel are considered naturalised (e.g., *C. sativa*). All forestry tree species are categorised by origin and provenance, for native species these two terms are synonymous, however for naturalised species the origin would be the origin that is considered the natural range and the provenance would be the location of the tree from which the seed was collected (Hubert and Cundall, 2006). Plate 7 is the UK Forestry Commissions provenance and seed zone map showing the lines of

demarcation, Table 1 is a list of the provenance of the trees used in the mesocosm experiments (Chapter 3, 4 and 5) and the provenance of the trees planted in BangorDiverse experiment in 2004.

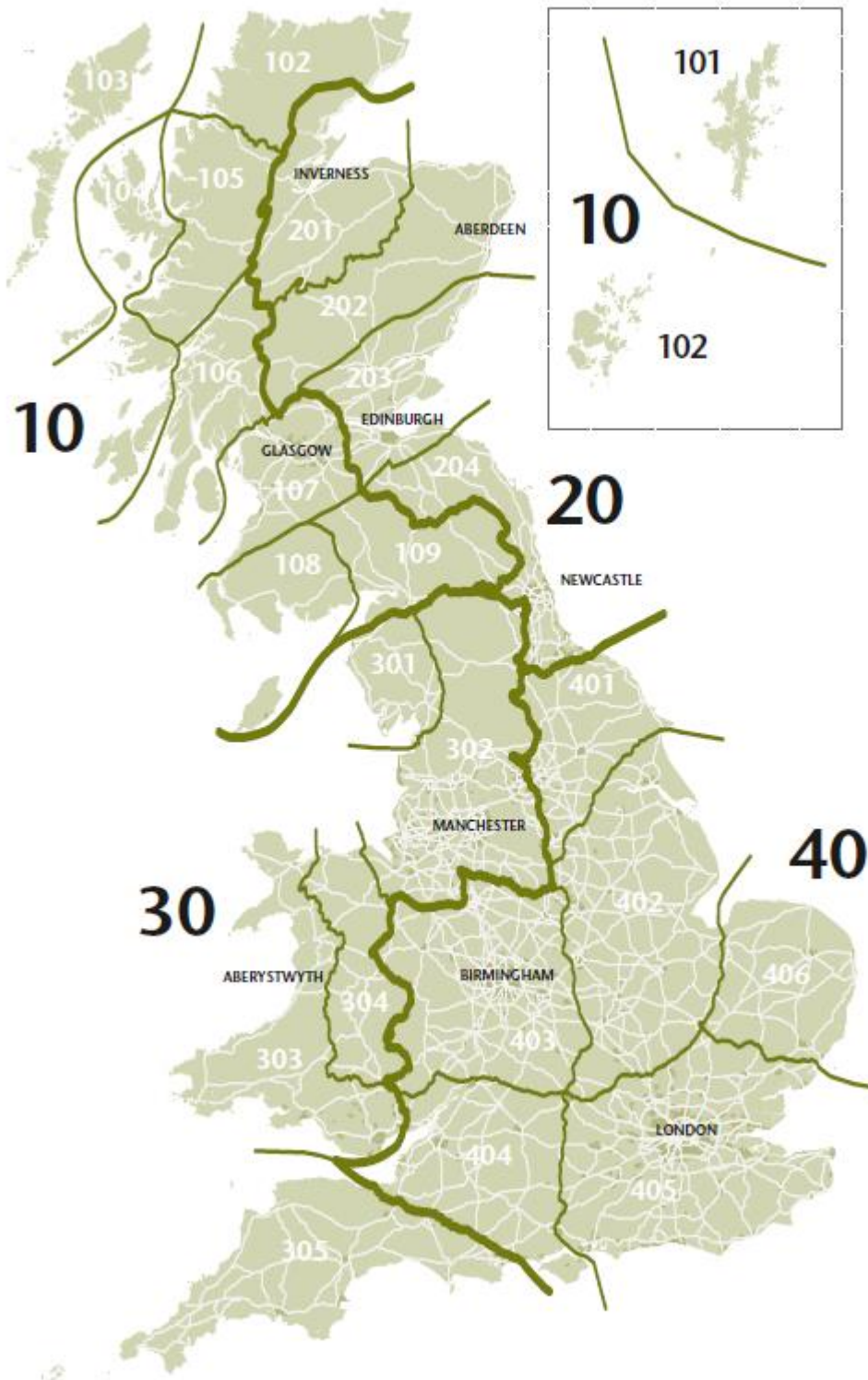


Plate 7: Regions of provenance and seed zones of the UK. Source: Hubert and Cundall (2006)

Table 1: The provenances of the three-tree species studied in both the glasshouse experiments (Chapters 3, 4 and 5) and the field experiment (Chapter 6).

Species Common name (<i>Latin name</i>)	Juvenile 1+1 (20-40 cm) trees ordered from Maelor nurseries, Whitchurch (403)	Mature trees within Bangor DIVERSE plots planted 2004
Silver birch (<i>Betula pendula</i>)	204 (Northumberland)	204 (Northumberland)
Common alder (<i>Alnus glutinosa</i>)	204 (Northumberland)	403 (Shropshire)
Sweet chestnut (<i>Castanea sativa</i>)	404 (South-west England)	303/304 (Wales)

1.9. References

- Ahmed, I.U., Smith, A.R., Jones, D.L. and Godbold, D.L., 2016. Tree species identity influences the vertical distribution of labile and recalcitrant carbon in a temperate deciduous forest soil. *Forest Ecology and Management*, 359, pp.352-360.
- Archibald, J.M., 2008. The eocyte hypothesis and the origin of eukaryotic cells. *Proceedings of the National Academy of Sciences of the United States of America*, 105(51), pp.20049-20050.
- Atkinson, M.D., 1992. *Betula pendula* Roth (*B. verrucosa* Ehrh.) and *B. pubescens* Ehrh. *Journal of Ecology*, 80(4), pp.837-870.
- Averill, C., Turner, B.L. and Finzi, A.C., 2014. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature*, 505(7484), pp.543-545.
- Axelrod, R. and Hamilton, W.D., 1981. The evolution of cooperation. *science*, 211(4489), pp.1390-1396.
- Bertness, M.D. and Callaway, R., 1994. Positive interactions in communities. *Trends in Ecology and Evolution*, 9(5), pp.191-193.
- Brandes, E., Kodama, N., Whittaker, K., Weston, C., Rennenberg, H., Keitel, C., Adams, M.A. and Gessler, A., 2006. Short-term variation in the isotopic composition of organic matter allocated from the leaves to the stem of *Pinus sylvestris*: effects of photosynthetic and postphotosynthetic carbon isotope fractionation. *Global Change Biology*, 12(10), pp.1922-1939.
- Bruno, J.F., Stachowicz, J.J. and Bertness, M.D., 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution*, 18(3), pp.119-125.
- Brzostek, E.R., Fisher, J.B. and Phillips, R.P., 2014. Modeling the carbon cost of plant nitrogen acquisition: Mycorrhizal trade-offs and multipath resistance uptake improve predictions of retranslocation. *Journal of Geophysical Research: Biogeosciences*, 119(8), pp.1684-1697.
- Calvin, M. and Benson, A.A., 1949. The path of carbon in photosynthesis IV: the identity and sequence of the intermediates in sucrose synthesis. *Science*, 109(2824), pp.140-142.
- Cannell, G.R. and Dewar R.C., 1994. Carbon allocation in trees: a review of concepts for modelling. *Advances in Ecological Research*, 25, pp.59-104.

- Carbone, M.S., Richardson, A.D., Chen, M., Davidson, E.A., Hughes, H., Savage, K.E. and Hollinger, D.Y., 2016. Constrained partitioning of autotrophic and heterotrophic respiration reduces model uncertainties of forest ecosystem carbon fluxes but not stocks. *Journal of Geophysical Research: Biogeosciences*, 121(9), pp.2476-2492.
- Chen, Y.L., Brundrett, M.C. and Dell, B., 2000. Effects of ectomycorrhizas and vesicular–arbuscular mycorrhizas, alone or in competition, on root colonization and growth of *Eucalyptus globulus* and *E. urophylla*. *New Phytologist*, 146(3), pp.545-555.
- Chen, W., Koide, R.T., Adams, T.S., DeForest, J.L., Cheng, L. and Eissenstat, D.M., 2016. Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. *Proceedings of the National Academy of Sciences of the United States of America*, 113(31), pp.8741-8746.
- Claessens, H., Oosterbaan, A., Savill, P. and Rondeux, J., 2010. A review of the characteristics of black alder (*Alnus glutinosa* (L.) Gaertn.) and their implications for silvicultural practices. *Forestry*, 83(2), pp.163-175.
- Clements, F.E., 1916. *Plant succession: an analysis of the development of vegetation* (No. 242). Carnegie Institution of Washington.
- Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R.D., Wardle, D.A. and Lindahl, B.D., 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science*, 339(6127), pp.1615-1618.
- Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Deneff, K. and Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology*, 19(4), pp.988-995.
- Darwin, C., 1859. *On the Origin of Species by Means of Natural Selection Or the Preservation of Favoured Races in the Struggle for Life*. Oxford University Press, H. Milford.
- Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H. and Tu, K.P., 2002. Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics*, 33(1), pp.507-559.
- Douds, D.D., Pfeffer, P.E. and Shachar-Hill, Y., 2000. Carbon partitioning, cost, and metabolism of arbuscular mycorrhizas. In *Arbuscular mycorrhizas: physiology and function* (pp. 107-129). Springer, Dordrecht.

Dittert, K., Wötzel, J. and Sattelmacher, B., 2006. Responses of *Alnus glutinosa* to anaerobic conditions-Mechanisms and rate of oxygen flux into the roots. *Plant Biology*, 8(02), pp.212-223.

Eissenstat, D.M., Kucharski, J.M., Zadworny, M., Adams, T.S. and Koide, R.T., 2015. Linking root traits to nutrient foraging in arbuscular mycorrhizal trees in a temperate forest. *New Phytologist*, 208(1), pp.114-124.

Epron, D., Bahn, M., Derrien, D., Lattanzi, F.A., Pumpanen, J., Gessler, A., Högberg, P., Maillard, P., Dannoura, M., Gérant, D. and Buchmann, N., 2012. Pulse-labelling trees to study carbon allocation dynamics: a review of methods, current knowledge and future prospects. *Tree Physiology*, 32(6), pp.776-798.

Epron, D., Ngao, J., Dannoura, M., Bakker, M.R., Zeller, B., Bazot, S., Bosc, A., Plain, C., Lata, J.C., Priault, P. and Barthes, L., 2011. Seasonal variations of belowground carbon transfer assessed by in situ $^{13}\text{CO}_2$ pulse labelling of trees. *Biogeosciences*, 8(5), pp.1153-1168.

Fahey, T.J., Woodbury, P.B., Battles, J.J., Goodale, C.L., Hamburg, S.P., Ollinger, S.V. and Woodall, C.W., 2010. Forest carbon storage: ecology, management, and policy. *Frontiers in Ecology and the Environment*, 8(5), pp.245-252.

Fonseca, M.B., Dias, T., Carolino, M.M., França, M.G.C. and Cruz, C., 2017. Belowground microbes mitigate plant-plant competition. *Plant Science*, 262, pp.175-181.

Forrester, D.I., 2014. The spatial and temporal dynamics of species interactions in mixed-species forests: from pattern to process. *Forest Ecology and Management*, 312, pp.282-292.

Gaia, M., Da Cunha, V. and Forterre, P., 2018. The Tree of Life. In *Molecular Mechanisms of Microbial Evolution* (pp. 55-99). Springer, Cham.

Giardina, C.P., Ryan, M.G., Binkley, D. and Fownes, J.H., 2003. Primary production and carbon allocation in relation to nutrient supply in a tropical experimental forest. *Global Change Biology*, 9(10), pp.1438-1450.

Giardina, C.P. and Ryan, M.G., 2002. Total belowground carbon allocation in a fast-growing Eucalyptus plantation estimated using a carbon balance approach. *Ecosystems*, 5(5), pp.487-499.

Gleason, H.A., 1926. The individualistic concept of the plant association. *Bulletin of the Torrey Botanical Club*, pp.7-26.

- Gougoulas, C., Clark, J.M. and Shaw, L.J., 2014. The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. *Journal of the Science of Food and Agriculture*, 94(12), pp.2362-2371.
- Gower, S.T., Isebrands, J.G. and Sheriff, D.W., 1995. Carbon allocation and accumulation in conifers. In *Resource physiology of conifers* (eds WK Smith, TM Hinckley), pp. 217– 254. Academic Press, San Diego.
- Gower, S.T., Krankina, O., Olson, R.J., Apps, M., Linder, S. and Wang, C., 2001. Net primary production and carbon allocation patterns of boreal forest ecosystems. *Ecological Applications*, 11(5), pp.1395-1411.
- Gunina, A., Smith, A.R., Godbold, D.L., Jones, D.L. and Kuzyakov, Y., 2017. Response of soil microbial community to afforestation with pure and mixed species. *Plant and Soil*, 412(1-2), pp.357-368.
- Grime, J.P., 1974. Vegetation classification by reference to strategies. *Nature*, 250(5461), p.26.
- Hauri, H.P. and Schweizer, A., 1992. The endoplasmic reticulum—Golgi intermediate compartment. *Current Opinion in Cell Biology*, 4(4), pp.600-608.
- Hemery, G. and Simblet, S., 2014. *The new Sylva: A discourse of forest and orchard trees for the 21st century*. Bloomsbury Press, London.
- Hines, E., Cornelius, J., Hecker, M., Lowe, Q. and Kevin, P., 2016. *European atlas of forest tree species*. Publications Office of the European Union, Luxembourg.
- Hobbie, E.A., Hofmockel, K.S., Van Diepen, L.T., Lilleskov, E.A., Ouimette, A.P. and Finzi, A.C., 2014. Fungal carbon sources in a pine forest: evidence from a ¹³C-labeled global change experiment. *Fungal Ecology*, 10, pp.91-100.
- Högberg, M.N., Briones, M.J., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., Thornton, B., Hurry, V., Linder, S., Näsholm, T. and Högberg, P., 2010. Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. *New Phytologist*, 187(2), pp.485-493.
- Hubert, J. and Cundall, E., 2006. *Choosing Provenance in Broadleaved Trees*. Forestry Commission. Edinburgh.

- Hulvey, K.B., Hobbs, R.J., Standish, R.J., Lindenmayer, D.B., Lach, L. and Perring, M.P., 2013. Benefits of tree mixes in carbon plantings. *Nature Climate Change*, 3(10), pp.869-874.
- Iversen, J., 1944. *Viscum*, *Hedera* and *Ilex* as climate indicators: A contribution to the study of the post-glacial temperature climate. *Geologiska Föreningen i Stockholm Förhandlingar*, 66(3), pp.463-483.
- Jakobsen, I. and Rosendahl, L., 1990. Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. *New Phytologist*, 115(1), pp.77-83.
- Johnson, D., Leake, J.R., Ostle, N., Ineson, P. and Read, D.J., 2002. In situ $^{13}\text{CO}_2$ pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. *New Phytologist*, 153(2), pp.327-334.
- Johnson, D. W., Woodward, C. and Meadows, M.W., 2014. A Three-Dimensional View of Nutrient Hotspots in a Sierra Nevada Forest Soil. *Soil Science Society of America Journal*, 78, S225-S236.
- Kagawa, A., Sugimoto, A. and Maximov, T.C., 2006. $^{13}\text{CO}_2$ pulse-labelling of photoassimilates reveals carbon allocation within and between tree rings. *Plant, Cell and Environment*, 29(8), pp.1571-1584.
- Kaiser, C., Kilburn, M.R., Clode, P.L., Fuchslueger, L., Koranda, M., Cliff, J.B., Solaiman, Z.M. and Murphy, D.V., 2015. Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation. *New Phytologist*, 205(4), pp.1537-1551.
- Kaschuk, G., Kuyper, T.W., Leffelaar, P.A., Hungria, M. and Giller, K.E., 2009. Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? *Soil Biology and Biochemistry*, 41(6), pp.1233-1244.
- Keitel, C., Matzarakis, A., Rennenberg, H. and Gessler, A., 2006. Carbon isotopic composition and oxygen isotopic enrichment in phloem and total leaf organic matter of European beech (*Fagus sylvatica* L.) along a climate gradient. *Plant, Cell and Environment*, 29(8), pp.1492-1507.
- Knohl, A., Werner, R.A., Brand, W.A. and Buchmann, N., 2005. Short-term variations in $\delta^{13}\text{C}$ of ecosystem respiration reveals link between assimilation and respiration in a deciduous forest. *Oecologia*, 142(1), pp.70-82.

- Kodama, N., Barnard, R.L., Salmon, Y., Weston, C., Ferrio, J.P., Holst, J., Werner, R.A., Saurer, M., Rennenberg, H., Buchmann, N. and Gessler, A., 2008. Temporal dynamics of the carbon isotope composition in a *Pinus sylvestris* stand: from newly assimilated organic carbon to respired carbon dioxide. *Oecologia*, 156(4), p.737.
- Kwong, W.K., del Campo, J., Mathur, V., Vermeij, M.J. and Keeling, P.J., 2019. A widespread coral-infecting apicomplexan with chlorophyll biosynthesis genes. *Nature*, 568(7750), p.103.
- Lavorel, S., 2013. Plant functional effects on ecosystem services. *Journal of Ecology*, 101(1), pp.4-8.
- Lindahl, B.D. and Tunlid, A., 2015. Ectomycorrhizal fungi–potential organic matter decomposers, yet not saprotrophs. *New Phytologist*, 205(4), pp.1443-1447.
- Litton, C.M., Raich, J.W. and Ryan, M.G., 2007. Carbon allocation in forest ecosystems. *Global Change Biology*, 13(10), pp.2089-2109.
- Locke, G.M.L., 1978. The growing stock of regions. Dewar, J. The case for regional silviculture. *Forestry*, 51, pp.1-19.
- Lorrain-Smith, R. and Worrell, R., 1992. The commercial potential of birch in Scotland. *Scottish Forestry*, 46(1), pp.48-55.
- Margulis, L. and Barreno, E., 2003. Looking at lichens. *Bioscience*, 53, pp.776–778.
- Marron, N., Plain, C., Longdoz, B. and Epron, D., 2009. Seasonal and daily time course of the ¹³C composition in soil CO₂ efflux recorded with a tunable diode laser spectrophotometer (TDLS). *Plant and Soil*, 318(1-2), pp.137-151.
- McCormack, M.L., Dickie, I.A., Eissenstat, D.M., Fahey, T.J., Fernandez, C.W., Guo, D., Helmisaari, H.S., Hobbie, E.A., Iversen, C.M., Jackson, R.B. and Leppälammi-Kujansuu, J., 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytologist*, 207(3), pp.505-518.
- McIntire, E.J. and Fajardo, A., 2014. Facilitation as a ubiquitous driver of biodiversity. *New Phytologist*, 201(2), pp.403-416.
- McVean, D.N., 1956. Ecology of *Alnus Glutinosa* (L.) Gaertn: VI. Post-Glacial History. *Journal of Ecology*, 44, pp.331-333.

- Michalet, R. and Pugnaire, F.I., 2016. Facilitation in communities: underlying mechanisms, community and ecosystem implications. *Functional Ecology*, 30(1), pp.3-9.
- Miles, J., 1986. *What are the effects of trees on soils?* Trees and Wildlife in the Scottish Uplands (eds D. Jenkins), pp. 55-62. Institute of Terrestrial Ecology, Huntingdon.
- Mitchell, A., Dahlstrom, P., Sunesen, E. and Darter, C., 1974. *A field guide to the trees of Britain and northern Europe (Vol. 34)*. Collins, London.
- Nadelhoffer, K.J. and Raich, J.W., 1992. Fine root production estimates and belowground carbon allocation in forest ecosystems. *Ecology*, 73(4), pp.1139-1147.
- Navarro, M.N.V., Jourdan, C., Sileye, T., Braconnier, S., Mialet-Serra, I., Saint-Andre, L., Dauzat, J., Nouvellon, Y., Epron, D., Bonnefond, J.M. and Berbigier, P., 2008. Fruit development, not GPP, drives seasonal variation in NPP in a tropical palm plantation. *Tree Physiology*, 28(11), pp.1661-1674.
- Odum, E., Barrett, G. and Brewer, R., 2005. *Fundamentals of ecology*. 5th. Belmont, CA: Thomson Brooks/Cole.
- Olsson, P.A. and Johnson, N.C., 2005. Tracking carbon from the atmosphere to the rhizosphere. *Ecology Letters*, 8(12), pp.1264-1270.
- Ostle, N., Whiteley, A.S., Bailey, M.J., Sleep, D., Ineson, P. and Manefield, M., 2003. Active microbial RNA turnover in a grassland soil estimated using a ¹³CO₂ spike. *Soil Biology and Biochemistry*, 35(7), pp.877-885.
- Patterson, G.S., 1993. *Forestry Commission Bulletin 109: The Value of Birch in Upland Forests for Wildlife Conservation*. HMSO, London.
- Řezáčová, V., Konvalinková, T. and Jansa, J., 2017. Carbon fluxes in mycorrhizal plants. In *Mycorrhiza-eco-physiology, secondary metabolites, nanomaterials* (pp. 1-21). Springer, Cham.
- Richards, T.A. and McCutcheon, J.P., 2019. Coral symbiosis is a three-player game. *Nature*, 568, pp.41-42.
- Roossinck, M.J., 2005. Symbiosis versus competition in plant virus evolution. *Nature Reviews Microbiology*, 3(12), p.917.

- Ryan, M.G., Hubbard, R.M., Pongracic, S., Raison, R.J. and McMurtrie, R.E., 1996. Foliage, fine-root, woody-tissue and stand respiration in *Pinus radiata* in relation to nitrogen status. *Tree physiology*, 16(3), pp.333-343.
- Ryan, F., 2002. *Darwin's blind spot: evolution beyond natural selection*. Houghton Mifflin Harcourt.
- Sagan, L., 1967. On the origin of mitosing cells. *Journal of Theoretical Biology*, 14(3), pp.225-274.
- Savill, P.S., 2019. *The silviculture of trees used in British forestry*. CABI, Wallingford.
- Schuur, E.A.G., Carbone, M.S., Pries, C.H., Hopkins, F.M. and Natali, S.M., 2016. Radiocarbon in terrestrial systems. In *Radiocarbon and Climate Change* (pp. 167-220). Springer, Cham.
- Sinsabaugh, R.L., Moorhead, D.L., Xu, X. and Litvak, M.E., 2017. Plant, microbial and ecosystem carbon use efficiencies interact to stabilize microbial growth as a fraction of gross primary production. *New Phytologist*, 214(4), pp.1518-1526.
- Slavíková, R., Püschel, D., Janoušková, M., Hujslová, M., Konvalinková, T., Gryndlerová, H., Gryndler, M., Weiser, M. and Jansa, J., 2017. Monitoring CO₂ emissions to gain a dynamic view of carbon allocation to arbuscular mycorrhizal fungi. *Mycorrhiza*, 27(1), pp.35-51.
- Smith, A.R., Lukac, M., Hood, R., Healey, J.R., Miglietta, F. and Godbold, D.L., 2013. Elevated CO₂ enrichment induces a differential biomass response in a mixed species temperate forest plantation. *New Phytologist*, 198(1), pp.156-168.
- Smith, S.E. and Read, D.J., 2010. *Mycorrhizal Symbiosis*. Academic Press, London.
- Spribile, T., Tuovinen, V., Resl, P., Vanderpool, D., Wolinski, H., Aime, M.C., Schneider, K., Stabentheiner, E., Toome-Heller, M., Thor, G. and Mayrhofer, H., 2016. Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science*, 353(6298), pp.488-492.
- Tedersoo, L., Bahram, M., Toots, M., Diedhiou, A.G., Henkel, T.W., Kjølner, R., Morris, M.H., Nara, K., Nouhra, E., Peay, K.G. and Polme, S., 2012. Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. *Molecular Ecology*, 21(17), pp.4160-4170.
- Tilman, D., 1982. *Resource Competition and Community Structure*. Princeton University Press, Princeton.

- Tilman, D., 1994. Competition and biodiversity in spatially structured habitats. *Ecology*, 75(1), pp.2-16.
- Tomè, E., Tagliavini, M. and Scandellari, F., 2015. Recently fixed carbon allocation in strawberry plants and concurrent inorganic nitrogen uptake through arbuscular mycorrhizal fungi. *Journal of Plant Physiology*, 179, pp.83-89.
- Tuovinen, V., Ekman, S., Thor, G., Vanderpool, D., Spribille, T. and Johannesson, H., 2019. Two Basidiomycete fungi in the cortex of wolf lichens. *Current Biology*, 29(3), pp.476-483.
- Valentine, A.J., Mortimer, P.E., Kleinert, A., Kang, Y. and Benedito, V.A., 2013. Carbon metabolism and costs of arbuscular mycorrhizal associations to host roots. In *Symbiotic endophytes* (pp. 233-252). Springer, Berlin.
- Verheyen, K., Vanhellemont, M., Auge, H., Baeten, L., Baraloto, C., Barsoum, N., Bilodeau-Gauthier, S., Bruelheide, H., Castagneyrol, B., Godbold, D., Haase, J., Hector, A., Jactel, J., Koricheva, J., Loreau, M., Mereu, S., Messier, C., Muys, B., Nolet, P., Paquette, A., Parker, J., Perring, M., Ponette, Q., Potvin, C., Reich, P., Smith, A., Weih, M., Scherer-Lorenzen, M., 2016. Contributions of a global network of tree diversity experiments to sustainable forest plantations. *Ambio*, 45(1), pp.29-41.
- Vogel, C., Mueller, C.W., Höschen, C., Buegger, F., Heister, K., Schulz, S., Schlöter, M. and Kögel-Knabner, I., 2014. Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils. *Nature Communications*, 5, 2947.
- Whittaker, R.H., 1956. Vegetation of the great smoky mountains. *Ecological Monographs*, 26(1), pp.1-80.
- Wingate L, Ogée J, Burlett R, Bosc A, Devaux M, Grace J, Loustau D, Gessler A., 2010. Photosynthetic carbon isotope discrimination and its relationship to the carbon isotope signals of stem, soil and ecosystem respiration. *New Phytologist*, 188(2) pp.576-89.
- Wright, D.P., Read, D.J. and Scholes, J.D., 1998. Mycorrhizal sink strength influences whole plant carbon balance of *Trifolium repens* L. *Plant, Cell and Environment*, 21(9), pp.881-891.
- Yang, D., Oyaizu, Y., Oyaizu, H., Olsen, G.J. and Woese, C.R., 1985. Mitochondrial origins. *Proceedings of the National Academy of Sciences of the United States of America*, 82(13), p.4443.

Zablen, L.B., Kissil, M.S., Woese, C.R. and Buetow, D.E., 1975. Phylogenetic origin of the chloroplast and prokaryotic nature of its ribosomal RNA. *Proceedings of the National Academy of Sciences of the United States of America*, 72(6), pp.2418-2422.

Chapter 2

Literature review: Understanding the role of plant-microbe symbiosis in the cycling of carbon in temperate forest ecosystems

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1. Introduction

Since Darwin first proposed the theory of evolution it has been widely understood that intra- and interspecific competition is the driving force in structuring the world's complex and diverse plant communities (Tilman et al., 1982), whereas co-operation and facilitation has been seen to be of lesser importance (Bruno et al., 2003). The traditional view of inter- and intra-specific competition was that the availability of resources is a major factor in speciation and ultimately contributes to the vast biodiversity observed in the natural world (Wright et al., 2017). Ecological theory over the last few centuries has been dominated by the belief in the importance of competition, predation and perturbation as the primary drivers of community structure (Boucher, 1988). However more recent empirical data has elucidated the importance of positive interactions between species in both terrestrial and marine ecosystems (Boucher, 1985; Callaway, 1995; Bertness and Leonard, 1997; Jones et al., 1997, Selesse, 2006; Van der Heijden and Horton, 2009). Facilitation appears to be more widespread and important than

originally thought and it has been widely reported that facilitative relationships become more importance at times of stress (McIntire and Fajardo, 2014). Therefore, more studies are required to inform ecological theory and to improve predictions of how biological communities will respond to the climatic changes the world is currently experiencing (Bruno et al., 2003).

In this paper we review the current knowledge and recent advances in the field of plant: microbe symbiosis, focusing particularly on how they mediate and facilitate biogeochemical cycling of carbon (C). In the process of reviewing the literature we will also identify understudied areas which need elucidation by future research.

2. What is symbiosis?

The term symbiosis was first used by A Frank, in 1877 when describing the mutualistic relationship observed between algae and fungi in lichen. However, over time, symbiosis has been used in a broader context to describe any long-term relationship between two unlike species (Tariq et al., 2017). Symbiosis is generally considered to include three separate types of relationship known as mutualism, commensalism and parasitism, although these categories are not always mutually exclusive (Martin and Schwab, 2012; Saffo, 1992). Rather than thinking of these types of symbioses as distinct categories they are a continuous spectrum of symbiosis which exhibits plasticity depending on environmental pressures (Leung and Poulin, 2008).

2.1. Mutualism

Mutualism is a symbiotic relationship when both organisms involved benefit, this can also be termed as reciprocal altruism (Paracer and Ahmadjian, 2000). Mutualisms may be far more important than current ecological theory suggests as mutualism facilitates or mediates a vast array of ecosystem processes that include symbiosis in corals, lichens, plants, and the evolutionary development of eukaryotic cells (Margulis, 1981). Small-scale effects of mutually positive biological relationships have been studied and their importance is widely accepted. However, the feedbacks from small to large scale positive relationships and how these processes impact on species richness, diversity, ecology and evolution of communities, ecosystems and populations remain poorly understood (Stachowicz, 2001).

2.1.2. Commensalism

Commensalism is a relationship between two living organisms where one is benefitted and the other is not significantly affected either positively or negatively (Nair, 2004). In practice these types of relationship are extremely hard to distinguish from parasitic relationships that cause the host very low levels of harm (which may not yet be detectable by current methods) or relationships where the host has developed adaptive strategies to minimise the damage caused by the microorganism (Leung and Poulin, 2008).

2.1.3. Parasitism

Parasitism is where one of the organisms involved benefits and to the detriment of the other. The benefitting organism (or parasite) can either keep the other alive, in this case the relationship is a biotrophic parasitism, or can result in its death, known as necrotic parasitism (Paracer and Ahmadjian, 2000).

Including these three types of biological relationships into a broader modern definition of symbiosis includes many more relationships than the original meaning of the term (Wilkinson, 2001). In 1994, Douglas redefined symbiosis as associations which persist for long periods of time, the time period being relative to the lifespan of the interacting organisms. This definition therefore excludes most parasitic relationships as over longer time periods one of the partners is affected detrimentally and will eventually die, ending the relationship (Ogle and Brown, 1997). It is Douglas's definition which we will be referring to throughout this thesis.

3. What are soil microbes?

During this review we will refer to microbes or micro-organisms, but what are microbes? Microbes or micro-organisms are general terms that describe a diverse range of organisms with a vast range in sizes. Microbes are organisms that are too small to see without the use of a microscope during one or more of their lifecycle stages although they can often exist as single cells or in clusters or colonies (Jenkinson and Lamont, 2005). Currently the most studied and important plant: microbe symbioses include both bacterial and fungal microbionts, although Protista, methanogenic archaea and ammonia-oxidizing archaea that have symbiotic relationships with plants do also exist (Rai, 2018; Minamisawa et al., 2016; Moissl-Eichinger et al., 2018). Soils are known to contain the largest abundance of terrestrial biodiversity which is responsible for providing a significant proportion of ecosystem function (Baldrian et al.,

2012). Terrestrial microorganisms are thought to exceed 10^{29} and regulate C storage and respiration (Flemming and Wuertz, 2019) as well as providing essential macronutrients which ensure continued plant productivity (Bardgett and Van Der Putten, 2014).

4. Temperate forests and how they differ from other forests

The Earth's temperate zone is defined as occupying the land area between the polar and tropical regions (i.e. latitudes of 25 degrees north or 50 degrees south of the Equator) (Kottek et al., 2006). The Köppen climate classification system describes the temperate climatic zone as when the coldest month has a mean temperature above -3°C but below 18°C (Köppen, 1884). Temperate forests cover 1038 Mha of land and are occupied by both deciduous and coniferous tree species that contain between 60 to 130 Mg C ha^{-1} in aboveground biomass. The total C stock estimated to be stored in temperate forest ecosystems is between $119 \pm 6 \text{ Pg C}$ (14 % of estimated current C stock in the world's forests), of which approximately one-third is found in aboveground biomass and two-thirds belowground (Dixon et al., 1994; Lal, 2005; Pan et al., 2011; Bolin, 1977). The extent of temperate forests has increased in recent years with an increase in tree canopy cover estimated to have gone from 4681 km^2 in 1982 to 5540 km^2 in 2016 (Song et al., 2018). This has led to temperate forests being a net sink of C with net emissions between 2006 and 2015 being estimated at -0.3 Pg C/year (Houghton and Nassikas, 2018). Temperate regions experience seasonal variations normally with a hot dry season or summer followed by a colder wetter season, known as winter. Trees in this region have developed strategies to increase the chances of them surviving these prolonged cold spells including leaf abscission known as deciduousness. In the UK there are 32 native tree species of which 29 are broadleaf, although currently only accounts for 49 % of the UK's total forest cover (Forestry Commission, 2018).

Temperate forests are characterised by these seasonal variations in climate and vegetation dynamics as due to the deciduous nature of some of the trees in the temperate biome seasonal variations in C sequestration occurs with changes in photosynthesis linked to leaf phenology (Gond et al., 1999). Photosynthesis ultimately drives C assimilation and sequestration in temperate deciduous forests and the timing of budburst and leaf senescence and abscission is therefore critical in determining the photosynthetically active months of the year (Chen et al., 1999; Richardson et al., 2009; Stoy et al., 2014). The environmental factors which affect temperate phenology are temperature and photoperiod and vernalisation (Hodges, 1990).

5. Belowground C in temperate forests

Soil C accounts for the largest pool of terrestrial C, with more C stored below-ground than can be found in terrestrial plants and the atmospheric system combined (Jobbágy and Jackson, 2000; Schelesinger, 1977). The soil C content is determined by the processes of litter fall, exudation and decomposition rates (Jandl et al., 2007). Seasonal variations also occur in the soil microbial community as the microbial biomass is dependent on root turnover, exudation and their own temperature dependence (Voříšková et al., 2014). Winter abundance of soil microbes has been reported to be 26- 33 % lower than that of summer microbe abundance, whilst soil winter fungal abundance was 15 % lower than summer abundance (Žifčáková et al., 2016). The addition to the rhizosphere of C from fine roots and mycorrhizae is estimated to be from 2 to 5 times more than the C derived from above-ground biomass (Fogel and Hunt, 1983). Understanding the dynamic processes of the soil organic C balance for climate change mitigation is an area of increasing interest and importance to humanity (McGuire et al., 2001). However, uncertainty about the precise amounts of belowground C allocation by plants is still preventing accurate model development (Reich et al., 2014). Soil organic matter was historically thought of as either labile or recalcitrant, distinguished by different residency times and turnover rates within the soil (Ahmed et al., 2015).

5.1. Fast pool or labile C

This C within the soil structure is readily available and therefore easily degradable by soil organisms and consists of exudates such as carbohydrates, amino acids, monosaccharides and soluble sugars which can be cycled between eight to ten times per annum (Coleman et al., 1983). Wilkinson et al (2014), estimate that amino acid and peptide turnover rates in productive grassland soils could be as fast as 20 times a minute. Due to the fast turnover times of these nutrients they are far more easily utilised for plant growth (Lee and Pankhurst, 1992) and decomposition (Belay-Tedla et al., 2009).

5.2. Recalcitrant C

More recalcitrant forms of soil C such as humic materials are much slower to turnover (Ahmed et al., 2015) and estimates for this suggests that the cycling takes places every decade to every century. In the case of C molecules in most protected, lignified, forms it is thought they remain in the soil for up to thousands of years (Qiao et al., 2014; Dungait et al., 2012; Kellner et al., 2014).

However more recently doubt has been cast on this concept as being over simplistic and is being replaced by new ideas that have led to an emerging new perspective of soil organic carbon dynamics (Schmidt et al., 2011). The molecular structure which was previously thought to account for the persistence of C in soil has been shown to have a minimal effect of the longevity of C in the soil system (Marschner et al., 2008). Keiluweit et al., (2017) postulated that the unexplained persistence of soil C was as a result of anaerobic microsites. The suggestion is that even in freely drained soils anaerobic conditions exist in microsites, confirmed by the presence of anaerobic soil processes such as reduction of Fe or methanogenesis (Nico and Fendorf, 2016). These anaerobic microsites provide refuge for highly reduced, fine particle sized organic compounds such as lipids, cutin and waxes (Mikutta et al., 2006) allowing accumulation due to oxygen limitation reducing decomposition processes (Koven et al., 2013). This is a rapidly developing area of soil science which will no doubt continue to be developed into more pragmatic modelling of soil C (Luo et al., 2016).

A large range of other factors can influence the total belowground C stocks, these include climatic factors (particularly temperature and moisture), soil and, landscape factors (Lange et al., 2015). The site location, climate and vegetation of each location can drastically alter the ability of the soil to sequester C; this is known as the C carrying capacity (Liang et al., 2017). The site conditions (soil type, pH, and nutrient availability, water content and organic matter) can also have a huge influence on the microbial diversity and activity (Pickles and Anderson, 2016). The forest soil C stock is also affected by anthropogenic factors such as forest management regimes and disturbance perturbations (Seidl et al., 2014; Triviño et al., 2015).

Precipitation can play a major role in determining the C storage capacity of soils. Meta-analysis of soil C content in different land-use management scenarios revealed that areas with annual rainfall of 2000- 3000 mm sequestered 24 % more C than land with either higher or lower rainfall rates (Guo and Gifford, 2002). It has been postulated that although higher rainfall theoretically increases decomposition that this is offset by increased C leaching into the deep soil profiles with less microbial activity (Jenny 2012; Post et al., 1982). Water availability to plants is a major abiotic factor that influences net primary productivity and therefore the relative partitioning of C under all land-uses including forests. Water stress, however, can be difficult to evaluate at a stand scale due to the spatial heterogeneity of moisture within forest soils and differences in water use efficiency of tree species (Anderegg et al., 2015).

6. Plant-microbe symbiosis

Plant: microbe symbiotic relationships are a vital component of plant nutrient acquisition by mediating soil organic matter decomposition and nutrient cycles in forest soils (Van De Heijden et al., 2008). As microbial communities are generally limited by C (Oren et al., 2001) they are reliant on and stimulated by exudates within the rhizosphere (Van De Heijden et al., 2008). Microorganisms within the rhizosphere rapidly consume C-base exudates leading to expeditious belowground C turnover and respiration (Helal and Sauerbeck, 1989). The presence of symbiotic microbial communities within the rhizosphere has been found to increase the plants allocation of C to the root structures (Reid et al., 1983; Harris et al., 1985; Koch and Johnston, 1984) and to increase root exudation (Smith and Read, 2010). The mechanics of these processes are not fully understood, but microorganisms could be increasing plant exudation by producing root hormones that increase the roots leakiness (Bowen, 1994) or by physically damaging roots to facilitate leakage (Grayston et al., 1998). However, this can be confused by the fact that micro-organisms can also exude similar substances (Rambelli, 1973) and that plants respond to symbiosis by increasing photosynthesis (Rygiewicz and Anderson, 1994). Exudation is also extremely important in acquisition of nutrition by stimulating microbial activity through increasing availability of cations (Grayston et al., 1997) and in some cases through mineral weathering (Linehan et al., 1985). Root exudation is not uniformly dispersed over the root surface and generally the exudation activity is clustered towards the growing tip of the roots (Bowen and Rovira, 1991; Farrar et al., 2003). Experiments with stable C isotopes to map the roots regions of exudation revealed that the longitudinal cell junctions at the apical regions were the most important for amino and organic acid exudation (Bowen, 1979; Jones and Durrah, 1994; Hoffland, 1992).

Plant microbe symbiosis occurs as a range of intimacies from extremely loose to intercellular (Mendes et al., 2013). In order for a plant-microbe symbiosis to occur the two partners have to locate each other this involves a series of recently discovered signalling stages (Venturi and Keel, 2016). The mycorrhizosphere is awash with a mixture of high- and low-molecular weight compounds derived from root border cells shed from the root tip, dead root cap cells during decomposition processes. Additionally, primary metabolites, organic and amino acids, polymerised sugars (Holmer et al., 2017) and secondary metabolites such as phenols, terpinoids and alkaloids (Venturi and Keel, 2016) are also present. These compounds provide a food source not only for those microbes that are beneficial, but also those that could be potentially harmful to the plants (Jones et al., 2009). Beneficial microbes can promote plant health by

increasing nutrient availability or by increasing the plants resistance to biotic and abiotic perturbations (Coleman-Derr and Tringe, 2014). The parasitic or pathogenic components of the rhizosphere's microbiome will, however, have the opposite effect on the plant's health and in some cases eventually lead to death (Mendes et al., 2013). The plants have therefore evolved strategies to distinguish between those microbes that will harm and those that will facilitate good health in order to develop relationships with those which will be beneficial (Holmer et al., 2017). It has also been hypothesised that root exudation is used as a form of messaging, whereby plants can stimulate physical and biological responses from other plants and soil micro-organisms (Walker et al., 2003). Interactions can be either symbiotic or defensive depending on the organism encountered and communication can be root-to-root or root-to-microbe. This process starts with the plant signalling the microbes with attractant chemicals, mostly flavonoids but also some strigolactanes followed by the micro-organism signalling to the plant to notify it of its proximity (Holmer et al., 2017). Recent advances in genomics has revealed the mechanisms in plants for regulating and signalling the microbial symbionts are similar in both mycorrhizal associations and nitrogen fixing symbiosis (Delaux et al., 2015; Bravo et al., 2016; Kamel et al., 2017). Modern high throughput genetics has revealed a vast diversity of micro-organisms within the rhizosphere soil microbiome, which can be both attracted and repelled by complex chemical signals released by the plant root into the rhizosphere (Holmer et al., 2017). Without an understanding of how root exudation is regulated within the plant it is difficult to fully appreciate the significance of this process to plants or the rhizosphere (Jones et al., 2004).

The purpose and impact that plant and microbial exudates have in shaping the rhizomicrobiome is the focus of current research. The hope is that we can begin to understand more about these positive relationships and promote them, thus reducing the need for artificial fertilisers and pesticides (Bai et al., 2015; Bulgarelli et al., 2012; Lundberg et al., 2012). What is known is that signalling chemicals stimulate morphological changes in both the mycosymbiont and the plant that are essential for the formation of the symbiotic relationship (Martin et al., 2007; Kohler et al., 2015). Through novel techniques utilising cDNA arrays, signature proteins specific to mycorrhizal fungi and nitrogen-fixing bacteria have been isolated and are believed to be involved in stimulating the required morphological modifications required to allow successful plant- microbe symbiosis to occur (Plett et al., 2014; Kohler et al., 2015; Martin et al., 2016). Plant: microbe symbiosis is increasingly being found to be fundamental to many ecosystem functions and processes including the acquisition of nutrients by plants, the

formation of soils and biogeochemical cycling (Wagg et al., 2014; Van Elsa et al., 2012; Van der Heijden et al., 2015). Many of these symbionts are presently unculturable (Newman, 2016), which means that they remain unclassified and their functions are yet undiscovered (Van der Heijden, 2008; Drigo et al., 2010; Mendes et al., 2011; Bulgarelli et al., 2012; Sessitsch et al., 2012).

It is almost impossible to separate root exudates from root secretions and therefore all organic substances released by healthy plant roots are considered to be exudates (Uren and Reisenauer, 1988). Quantifying exudates is difficult and as a result estimates vary considerably. What is generally accepted is that perennial plant species exude more assimilated C than annuals (Mooney, 1972). The reason for this is unclear, but it has been hypothesised that perennials need to divert more assimilate into the rhizosphere in order to increase survival all-year around (Harris et al., 1980), or that annuals have less C to allocate as they need to invest more into the woody growth of stems (Mooney, 1972). Total exuded C as a percentage of total C photo-assimilated is frequently estimated as 40 % (Van Venn et al., 1991). However, experiments with some tree species have revealed much higher percentages. Scots pine (*Pinus sylvestris*) transferred 60 % of total C assimilated below-ground (Ågren et al., 1980; Persson, 1978) and 73 % for Douglas fir (*Pseudotsuga menziesii*) (Fogel and Hunt, 1983; Santantonio, 1979). Jones and Durrah, (1995) have shown that the flow of exudates is not just one way and that significant amounts of C-based exudate can be reabsorbed by the plant roots partially explaining why the quantification of exudation rates is difficult.

6.1. Endophytes

Endophytes are microbes which live inside a plant for at least part of their lifecycle without causing disease symptoms (Wallace and May, 2018). Endophytes can be either bacteria, fungi, Protista or Achaea and it is believed that they are ubiquitous across all plant groups and climatic zones (Hardoim et al., 2015). Endophytes were first described by the German botanist, Johann Heinrich Friedrich Link in 1809 and are sometimes referred to as endosymbionts. Plant endophytes are extremely diverse and only very few have been classified or studied but can include fungi, bacteria and viruses (Rodriguez et al., 2009). Bacterial endophytes belong to a broad range of taxa, including α -Proteobacteria, β -Proteobacteria, γ -Proteobacteria, Firmicutes, Actinobacteria (Miliute et al., 2015). It is believed that every plant contains an endophyte and some may contain more as a result it is estimated that the most endophyte diversity occurs in the tropics (Deshmukh et al., 2015). Discovery of this diverse range of micro-organisms has

led to an understanding that the plant biome is as important to the survival of a plant as the bacteria which mammals rely on to aid digestion (Hardoim et al., 2015). Genetic links have been found which suggests that bacterial and fungal endophytes evolved alongside each other and endophytes such as arbuscular mycorrhizal fungi (Gherbi et al., 2008). Although this group of micro-organisms have been known about and studied for over a century there is still some work to be done, in categorising them (Rodriguez et al., 2009). What is clearly understood is that endophytes have played a crucial role in the development, health, evolution and ecology of land plants and have been present within plants since they became terrestrial (Brundrett et al., 2006). Endophytic fungi will be discussed in more detail in section 3.2.4.1.

6.2. Plant-bacterial symbiosis

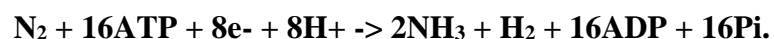
Plants normally defend themselves against bacterial infection by producing defence chemicals (Jones and Dangl, 2006), however in the case of plant: microbe symbiosis this does not occur. Our mechanistic understanding of plant: microbe symbiotic chemical signalling is in its infancy and it has been proposed that these plants have developed mechanisms to distinguish between pathogenic and mutualistic bacteria (Hirsch, 2004; Tellström et al., 2007). Alternatively, bacteria may suppress normal defence mechanisms (Bueno et al., 2001; Shaw and Long, 2003). In the upper litter layer of forest soils fungi dominates as the major decomposer, but in the mineral soil layer bacteria and fungi are more comparable in numbers (Baldrian, 2012). The importance of the bacterial component of soil generally increases with soil depth (Bååth and Anderson 2003). In the soils surface the relative bacterial to fungal composition is determined by pH, C: N ratio, climatic conditions and the identity of the plants located in the region (Högberg et al., 2007; Lladó, 2018; Van Der Linde, 2018). However, bacteria are less important for C storage than fungi within the soil as they are less efficient at C assimilation (Adu and Oades, 1978). The C that is stored by bacteria tends to be more labile and therefore shorter lived than that stored by fungi, this is because, in contrast to the soil fungi, the bacterial cell walls are comprised of phospholipids, which are rich in energy and can be readily degraded by other soil micro-organisms (Bailey et al., 2002). Plant-bacterial symbiosis is extremely species diverse and the development of modern genetic techniques has enabled the identification of previously unknown species involved in symbiosis. It is likely that our knowledge of the number of species involved in symbiosis will increase in future (Tilak et al., 2005). Some bacterial species are known to inhabit fungal fruiting bodies of both basidiomycetes (Dahm et al., 2005; Timonen and Hurek 2006; Pent et al., 2017) and ascomycetes (Barbieri et al., 2007, Quandt et al., 2015). The bacterial: fungal partnership may in some cases supply the fruiting

bodies with fixed atmospheric nitrogen (Barbieri et al., 2010) and in some fungal species may even be required for fungal fruiting to occur (Cho et al., 2003).

Generally, in plant: microbe symbiosis the fungal component microbial component utilises C which has been photosynthetically fixed by the photobiont and in return is provided with either nutrition, water or both which has been collected from the soil or bedrock by the mycorrhizal hyphae (Van der Heijden et al., 2015). The obvious exceptions to this rule are the mycoheterotrophic plants although some of these plants only require C from the mycobiont for the initial stages of germination and development (Dearnaley et al., 2016), after which they can photosynthesise and repay the debt to the fungi, known as the 'take now and pay later hypothesis' (Field et al., 2015). Normal plant: microbe resource exchange however has a cost to both partners and as a result both partners have evolved systems to withdraw rewards for partners not providing a service in return (Werner and Kiers, 2015). As a result of this partners that can provide more benefits are favoured over those that cannot (Selosse et al., 2016) and overtime this has led to the diversity of symbioses that currently exist. Upscaling to largescale from individual experimental data to model the importance of the C flow mediated by plant: microbe symbiosis is fraught with issues as many plant: microbe symbioses exhibit mixotrophy or plasticity of the partnership (Selosse et al., 2016).

6.2.1 Nitrogen-fixation

Although nitrogen constitutes over three-quarters of the Earth's atmosphere, it is inaccessible to plants in gaseous form (Geddes et al., 2015). In many terrestrial ecosystems plant productivity is limited by available nitrogen as it is one of the ineluctable building blocks of life, essential for the biosynthesis of amino acids, proteins and nucleic acids (Olge and Brown, 1997). The natural world has overcome this limitation with the process known as nitrogen fixation. The process of biological nitrogen fixation (discovered in the 19th century) is the conversion of atmospheric nitrogen gas to ammonia by an enzyme nitrogenase by the following reaction (Postgate, 1998):



Nitrogen-fixation has been described as the second most important biological process after photosynthesis (Gruber and Galloway, 2008). Specialist bacterium have evolved to populate the roots surface or/ and rhizosphere soil, or to pass through the cortex and into the plant as an endophyte to populate either the plants organs or specialised root nodules (Gray and Smith, 2005). Plants have reciprocated by simultaneously evolving strategies to; encourage these

symbiotic relationships and to become more hospitable hosts (Holmer et al., 2017). The genes required for the process are widespread in microbes inhabiting all environments (Gaby and Buckley, 2011). The diazotrophic bacteria responsible for biological nitrogen fixation can be divided into two distinct categories; Rhizobia, a group of paraphyletic bacteria and the diverse actinobacterial genus *Frankia* (Holmer et al., 2017). By far the most extensively studied plant: microbe symbiosis, is the relationship between plants and rhizobacteria (Tariq et al., 2017). The most important plant group to known contain nitrogen fixing bacteria are legumes, that fix 128 Tg. of N annually, globally (Cleveland et al., 1999; Galloway et al., 2004). Legumes fix nitrogen in a range of climatic vegetation zones from arid to tropical, as well as being extremely important in global agricultural systems (Van der Heijden, 2016).

Nitrogen- fixing plants have been found in an agricultural field experiments to increase the soils C storage by reducing the C dioxide emissions from the plants (Drinkwater et al., 1998). This is not unexpected as there is a well-documented link between the C and nitrogen biogeochemical cycles (Gruber and Galloway, 2008). However, the majority of these experiments have measured the effect of annual leguminous crops such as peas. Data from actinorhizal plants is sparse but research suggests that planting N-fixing trees has no direct significant effect on soil C (Guo and Gifford, 2002), although it has been found that as nitrogen fixers have a greater soil organic matter input that this results in faster decomposition rates (Conteh et al., 1997) and increases soil organic matter (Kasel et al., 2011; Nilsson and Schopfhauser, 1995). Nitrogen fixing endophytes (acetic acid bacteria) have recently been found on the needles of limber pine (*Pinus flexilis*) and form symbiotic relationships with the trees, exchanging carbohydrates for fixed nitrogen then made available to the plant as foliar feed (Moyes et al., 2016). Since this discovery it has been hypothesised that many more plant: bacterial symbioses may yet be undiscovered in the many more nitrogen limited biomes of the world (Wurzburger, 2016).

6.3. Other microbes forming symbioses with plants

Abundance in soils of archaea and viruses varies with soil temperature and moisture regime can range from levels below detection limits in arid ecosystems to over 1 billion per gram in soils with high moisture contents (Williamson et al., 2017). However, the frequency of these microbes within the soil is underestimated and the function still poorly understood.

6.3.1. Archaea

Until relatively recently archaea were still thought of as a type of bacteria, until with the advent of genomic classification the scientific community realised that these microbes although similar, have some different characteristics and were first reclassified a separate group in 1977 (Schleifer, 2009). Since then scientists have discovered that some archaea are linked to C assimilation through the oxidation of ammonia, although the precise role of these microbes in this process is still not completely understood (Pratscher et al., 2011). DNA analysis of soil micro-organisms suggests that archaea frequency in agricultural soils is higher than that of bacteria and that they are active at greater soil depths than bacteria (Leininger, 2006). Archaea found specifically in temperate forest soils been understudied but there appears to be a link between archaea diversity and low pH soils (Bates et al., 2011). It is also known that Archaea colonise fungal fruiting bodies and tree root which have symbiotic mycorrhizal associations, with populations found to be both more diverse and frequent than on non-mycorrhizal tree roots (Bomberg and Timonen 2009).

6.3.2. Protista

Recent advances in protistology have discovered that protists are far more frequent and diverse in soils than thought (Adl and Gupta, 2006). Many members of these group were previously classified as either protozoa or algae. It is thought that this group through its various functions within the soil are important for nutrient cycling particularly the bacterivores although many other functional traits are present and not fully understood or quantified. Forest soils contain 10^4 - 10^7 active protists per gram but these numbers have been found to fluctuate rapidly due to changes in environmental conditions such as availability of food, moisture and temperature (Adl and Coleman, 2005). During periods of less favourable conditions the protists form cysts which remain dormant until conditions alter but at any time both forms of protists will be present in the soil (Bamforth, 2001). The importance of protist biodiversity to nutrient cycling and decomposition is an area which will need further experimental investigation before being fully understood.

6.3.3. Viruses

Viruses are extremely abundant within soil (10^8 virus particles/g of soil) and are very poorly studied and therefore classified (Reavy et al., 2014). The diversity of soil viruses is now understood to be far higher than previously thought and virus species are thought to exceed all other cellular organisms by at least an order of magnitude (Casas and Rohwer, 2007). This amount of viral diversity within the soil is likely to affect plant growth and exudation in a number of unknown ways (Reavy et al., 2014). Plant viruses will directly affect the plants processes, but viruses also alter the lifecycle of other soil micro-organisms including bacteria and fungi which facilitate nutrients cycling including C.

6.4. Plant-fungal symbiosis

Plant: fungal symbiosis is now widely accepted to be a fundamental process which enabled the earliest rootless plants to adapt to the terrestrial environment (Simon et al., 1993; Heckman et al., 2001; Wang et al., 2010). Early terrestrial pioneers would have faced inhospitable conditions when they first left the water, with little organic matter, no soil formation and rocky mineral substrate. Fossilised plant remains have recently given us conclusive evidence that these early plants did benefit from fungal symbiosis (Remy et al., 1994; Redecker et al., 2000). Primitive plants alive today such as liverworts and ferns have recently been shown through novel genetic analysis to have symbiosis, not with arbuscular mycorrhizal fungi of the Glomeromycota, but with Endogone-like fungi known as Mucoromycotina and it seems likely that members of this group are responsible for colonisation of the land by plants (Bidartondo et al., 2011).

Fungi comprise a large percentage of soils biomass and are the most important microbial group in regulating biochemical cycles (Bailey et al., 2002; Buée et al., 2009). Some research suggests that fungal C storage is up to 26 times more than that of soil bacteria (Suberkropp and Weyers, 1996). However only a very small percentage of the estimated 1.5 million species of fungi have been described (Hawksworth, 2001) and the functional roles they play within nutrient cycles is still not resolved (McGuire and Treseder, 2010). Fungi have hyphal networks which enable them to colonise the upper leaf litter layers to which other forms of soil micro-organisms have little access (Holland and Coleman, 1987). The composition of fungal cell walls is comprised of chitin and melanin polymers, which are resistant to decomposition (Guggenberger et al., 1999), this means C stored by fungi tends to be more recalcitrant (Bailey et al., 2002) than forms sequestered by other microbial groups.

Fungal symbiotic associations are essential components of healthy forest ecosystems (Stamets, 2005). Symbiotic associations have long been known to increase plant growth by allowing the exchange of nutrients between the plants and fungal hyphae (Durrell et al., 1994). The photosynthetic rates of plants with mycorrhizal associations is increased to compensate for the loss of C to the rhizosphere (Dosskey et al., 1990; Rousseau and Reid, 1990). The carbohydrates are transported from the organs of photosynthesis (leaves) through branches and stems, then to root tissue where they are traded with fungi. In return the plants benefit from the far higher surface area and absorbance capacity for both essential nutrients, trace elements and water (Harrison, 2005). Forest belowground micro-organisms are often C limited (Grayston et al., 1998), whereas tree growth is often limited by nutrient availability (Krause et al., 1982). This is due in part to a lack of nutrient heterogeneity in uncultivated forest soils (Millard, 1996). Mycorrhizal fungal mycelium improves both nutrient solubility and spatial accessibility through their extensive hyphal networks (Sparling, 1994, Dighton and Mason, 1985). The fungal networks promote favourable conditions for beneficial bacteria to help the trees regulate nitrogen (Tornberg et al., 2003) but the bacteria are phosphorus limited (Sundareshwar et al., 2003) and rely on the fungus to supply them with that phosphorus (Stamets, 2005). Many plant species are unable to uptake phosphorus directly, but the mycorrhizal fungal mycelium can and supplies it to the plants (Li et al., 2006).

The interactions between the fungal networks and the plant roots within the rhizosphere are extremely complicated and involve transport through the soil as well as across root and hyphal interfaces (Leadley et al., 1997; Tinker and Nye, 2000). It has also been found that trees with mycorrhizal associations are more resistant to drought (Lehto, 1992), salt damage (Porcel et al., 2012), infectious diseases (Pozo, 2007) and other forms of toxicity (Tam, 1995). Within the forestry sector it is common practice for nursery stock to be inoculated with generic soil micro-organisms, known as bio-inoculants, although this may be beneficial, more research is needed to inoculate with the correct fungal species for each tree species, suitable for the site conditions and location (Trappe, 1977). Research suggests that mycorrhizae are significant drivers of mineral weathering (Thorley et al., 2015). Both ectomycorrhizal and arbuscular mycorrhizal have been found to increase soil rock weathering rates (Koele et al., 2014), with particular rock types being favoured by the fungus (Leake et al., 2008; Quirk et al., 2012). In order to capitalise on this beneficial function of mycorrhizal symbiosis a newly emerging science known as mycoforestry is attempting to utilise beneficial associations to maximise forest productivity and assist with habitat restoration projects (Stamets, 2005). Traditional

forestry ground preparation techniques such as tillage are far more destructive to the soil fungal communities than other groups of soil micro-organisms (Frey et al., 1999) and mycoforestry management techniques would avoid some traditional soil preparation techniques (Philips, 2017).

6.4.1 Hyphal turnover

Hyphal shedding and turnover are processes which result in major belowground C fluxes (Simard et al., 2015), in fact belowground inputs are adding more C to the soil than leaf litter (Godbold et al., 2006; Clemmensen et al., 2013) at least in part due to the dominance of saprophytic fungi in the upper soil profile (Hatton et al., 2015). Turnover rates are estimated to be 46 days for hyphae, 11 months for rhizomorphs and between 1 and 6 years for the plant-mycorrhizal interface at hyphal tips (Bledsoe et al., 2014). Experimentally hyphal turnover has been measured using mini-rhizotrons, in-growth mesh bags and cores (Wallander et al., 2013). In growth bag experiments seem to suggest a turnover rate of 0.1 year which equates to a 10-year residency (Wallander et al., 2004). When compared to the root turnover rates this seems a long turnover time suggesting the methodology may be underestimating these rates (Hendricks et al., 2006). The fact that differing parts of the mycelium might turnover at differing rates confounds accurate quantification (Ekblad et al., 2013). Mini-rhizotron investigations have shown that rhizomorphs can persist in the soil for up to 22 months (Vargas and Allen, 2008), whereas it is thought that single hyphae turnover is far quicker (Pritchard et al., 2008). We know little about fungal decomposition, but it is likely to take place via three pathways; autolysis whereby the material is reused, through normal saprophytic decomposition and soil faunal grazing (Pass and Szucsich, 2011). There is an increasing amount of evidence that hyphal turnover is an important player in the forest belowground soil C budget, but limited field experiments have been conducted to confirm this (Ekblad et al., 2013).

6.4.2. Plant: fungal symbiosis temporal dynamics

Succession is defined as 'a directional change in the composition, relative abundance and spatial pattern of species comprising communities' (Frankland, 1992). This process occurs within fungal communities as well as plants and fungi replace each other as the environmental conditions alter over time (Frankland, 1998). Fungal succession can be divided into two types, seral succession and substratum succession.

6.4.2.1. Seral Succession

Seral succession is driven by the dominant species of plant and as the plants move through the successional process so then do the fungal associations. This has been observed in field experiments in temperate forests and as the trees develop in age so then do the mycorrhizal fungal communities (Dighton and Mason, 1985).

6.4.2.2. Substratum succession

Substratum succession is the process of succession when driven by changes in the substrate. Initially pathogenic fungi will colonise leaf litter, followed by primary saprophytes and finally decomposers of the most recalcitrant material within the leaf litter (Frankland, 1998).

In reality all these fungal succession processes are occurring simultaneously, and generally fungal species diversity increases until canopy closure and then decreases as litter increases in nitrogen content (Fleming, 1984). The presence or absence of the mycelium can in some cases only be determined non-destructively by identifying the fruiting bodies and as many species fruit irregularly, this becomes difficult to quantify (Frankland, 1998). It appears that in early forest successional stages generalist pioneer mycorrhizae that have associations with more than one tree species are present. Overtime and into the later successional stages more specialised species-specific mycorrhizae colonise and dominate the rhizosphere (Twieg et al., 2007) and are more diverse in number than the pioneer fungi (Hart et al., 2014). It has been found that the practice of clear-cutting alters the soil condition sufficiently to require re-colonisation by species of mycorrhizae not present before harvesting (Jones et al., 2003). Understanding this and alleviating the problem by inoculation after clearcutting could help to reduce some of the post-harvest soil C loss (Heinonsalo and Sen, 2007).

6.4.3. Non-mycorrhizal fungal symbiosis

Approximately 10 % of plants are non-mycorrhizal, given the importance of mycorrhizal associations for plant nutrition, this poses the question; how do these non-mycorrhizal plants supply themselves with sufficient nutrients without them? Brassicaceae have lost the ability to form meaningful relationships with mycorrhizae, however some members of this plant group successfully survive in extremely nutrient limited soils. Almario et al., (2017), analysed the root microbiome of *Arabis alpine* (Brassicaceae), a non-mycorrhizal alpine herb, that grows in extremely impoverished rocky mountain soil, in an attempt to answer this question. The results consistently showed 15 fungal taxa, including Helotiales taxon. These were found to penetrate

the roots of the plants and supply them with phosphorus, in a very similar way to mycorrhizae (Almario et al., 2017). The Helotiades order of fungi are usually associated with ericoid symbiosis (Van der Heijden et al., 2017).

6.4.4. Mycorrhizal symbiosis

The term mycorrhizae was first coined in 1885 by the German plant pathologist A. B. Frank after he had been commissioned by the then King of Prussia (Frank, 2005 (an English translation of AB Frank's classic paper of 1885)) to investigate the possibility of commercial truffle cultivation. He discovered that the truffles formed symbiotic associations with certain tree species. Mycorrhizae literally translated means "fungus root" and is now a term used to describe the symbiotic, usually mutualistic association between plant and fungi (Frank, 2005). Since the late 19th century we have discovered many more types of mycorrhizae and they are now generally accepted as being an important component of all global ecosystems with approximately 95 % of all known plants benefitting from this symbiotic relationship (Mehrotra, 2005).

Recent intergenomic phylogenetic studies have suggested that mycorrhizal fungal species have evolved polyphyletically from a range of saprophytes at several different times (Venturini and Delledonne, 2015; Hibbett and Matheny, 2009). The first wave is thought to have developed alongside primitive terrestrial plants over 450 million years ago (Brundrett and Tedersoo, 2018; Redecker et al., 2013) these early mycorrhizae evolved to become arbuscular mycorrhizal fungi. Due to limited specimens and major gaps in fossil records it remains unclear if the first plants on land developed mycorrhizal associations or non-mycorrhizal associations, although it is known that plant genes associated with fungal symbiosis pre-date the first land plants (Delaux et al., 2015; Martin et al., 2017).

Mycorrhizal symbiotic relationships are based on mutual trading of resources where-by the tree (photobiont) provides the fungus (mycobionts) with C and the fungal hyphae provide nutrients in return (Buscot, 2015) normally nitrogen and phosphorus (Smith and Read, 2010). The mycobiont has access to a greater volume of soil due to their far greater surface area and smaller diameter allowing them to gain access to a larger area of soil from which to acquire nutrients (Tinker et al., 1994; Philips, 2017). The fungal hyphae in some cases are involved in the mobilization of nutrients that would otherwise be locked into soil organic complexes (Read and Perez-Moreno, 2004) and also by direct mineral weathering (Landeweert et al., 2001). The nutrients obtained are significant, with some plants obtaining up to 80 % of their nitrogen and

phosphorus from their mycorrhizal symbionts (Van der Heijden et al., 2015). Mycorrhizal networks can also move water bi-directionally, this type of transport tends to be diurnal. During the day the water is moved from the soil to the transpiring plants and then during the night moved to the driest parts of the soil matrix (Egerton-Warburton et al., 2007; Querejeta et al., 2003), thus buffering mycorrhizal plants from the effects of drought.

The two main types of mycorrhizae are characterised and differentiated by the type of plant-fungal interface present, the endomycorrhizae penetrate the roots cell wall (Allen, 1991) whereas ectomycorrhizal hyphae do not (Smith and Read, 2010). Although mycorrhizal networks occur most frequently in temperate and boreal ecosystems (Roy et al., 2008; Beiler et al., 2010), they are also found in tropical forests (Onguene and Kuyper, 2002; Mangan et al., 2010) Mediterranean forests and chaparral (Richard et al., 2005; Tedersoo et al., 2008), arctic tundra (Deslippe and Simard, 2011), grasslands (Gai et al., 2009) and woodland savannah (Dickie et al., 2004). Tedersoo et al., (2014) found that the diversity of fungi belowground in forests was inversely proportional to the aboveground diversity. In the tropics where, aboveground plant diversity is high, belowground fungal diversity is relatively low and generally as one moves away from the equator the diversity of mycorrhizal fungi increases (Wardle and Lindahl, 2014). During the early stages of plant root fungal colonisation, metabolic changes in the plant, which allow successful colonisation, are triggered by the presence of the fungi (Harrison, 2005). By priming a plants defence hormone, mycorrhizas can increase a plants resistance to a range of perturbation including nematodes (De La Peña et al., 2006), pathogens (Pozo et al., 2009; Whipps, 2004) as well as abiotic disturbances (Smith and Read, 2010). Mycorrhizal fungi can also attract beneficial insects by stimulating the production of volatiles (Babikova et al., 2014) which then predate on pests, both herbivores (Schausberger et al., 2012) and parasites (Guerrieri et al., 2004).

Dependent on the species of plant involved between 10 and 15 % of the below-ground C is respired by roots and 15- 25 % is exuded from the roots to the soil (Kuzyakov, 2002). Estimates of the total C transferred belowground to mycorrhizal fungi varies greatly. Wallander et al., (2001), estimated that between 700 to 900 kg of C per hectare of forest is transferred to ectomycorrhizae in the Silvåkra forest, in south-west Sweden, a Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) forest. Whereas studies that investigated the extent of C transfer to arbuscular mycorrhizae in laboratory conditions estimate that anything from 4-14 % of the C fixed by the plant is diverted to the fungi symbiont (Durall et al., 1994, Harris and Paul, 1987, Lambers 1987; Smith and Gianinazzi-Pearson, 1988). Ectomycorrhizae

are also understood to be a major contributor to soil C and are mediators of C fluxes within forest soils (Hogberg and Hogberg, 2002; Godbold et al., 2006; Hobbie, 2006). Ectomycorrhizal studies report anywhere from 6- 36 % of fixed C being allocated to the rhizosphere in laboratory experiments (Reid, Kidd and Ekwebelum, 1983) and in field studies estimates suggest that the ectomycorrhizal fungi consume in the region of 15 % of the photosynthetically fixed C (Vogt et al., 1982; Fogel and Hunt, 1983). Mycelial fins of mycorrhizae can develop and die within weeks (Finlay and Read, 1986; Bending and Read, 1995), this suggests a rapid mycorrhizal mycelium turnover rate (Smith and Read, 2010).

In short fungi are the most important group of micro-organisms for mediating C cycling and for long term belowground C sequestration (Treseder and Holden, 2013). Mycorrhizal C trading is now considered to be of greater importance than that of the saprophytic influence in the breakdown of litter (Clemmensen et al., 2013) and the fungi aid C sequestration by stimulating plant growth, decomposition and deposition. Managing a forest to be favourable to the colonisation of fungal diversity will be beneficial to both the productivity of the forest stand and its belowground C storage (Bailey et al., 2002; Stamets, 2005).

The following section will try to describe the phylogenetics of mycorrhizal fungi, this is an area of taxonomy currently in development aided by the advent of DNA sequencing technology (Shenoy et al., 2007). Recent developments such as Loron et al.'s (2019) discovery of fungal microfossils which appear to indicate that fungi evolved more than half a billion years before previously thought, suggesting that our phylogenetic fungal trees are not yet legitimate.

6.4.4.1. Endomycorrhizae

Endomycorrhizae are a large group of mycorrhizal fungi that have evolved the ability to penetrate the root cells which allows for a less inhibited flow of nutrients and C between the two organisms (Bonfante and Genre, 2010). Types of endomycorrhizae include: arbuscular, vesicular arbuscular mycorrhiza (VAM), ericoid, arbutoid, monotropoid, and orchid ectendomycorrhizas.

6.4.4.1.1. Vesicular arbuscular mycorrhizae

The term vesicular arbuscular mycorrhizal (VAM) associations was used to describe mycorrhizal fungi Glomeromycota. It was then discovered that many members of this taxonomic group do not actually have vesicles and the term was modified to become arbuscular

mycorrhizae (AM). Although arbuscular mycorrhizae are now the accepted term for this group of fungi, some members of the group do not form arbuscles either (Smith and Read, 2010).

6.4.4.1.2. Arbuscular mycorrhizae

The most frequent form of mycorrhizal association is arbuscular, this category accounts for 72 % of all mycorrhizal associations (Brundrett and Tedersoo, 2018). These associations are formed by a single group of fungi known as Glomeromycota (Holmer et al., 2017), which contains an estimated 1000 species (Redecker et al., 2013). Arbuscular mycorrhizae colonise the roots by penetrating the root with their hyphae and producing structures known as vesicles or arbuscles within the root cortex cells (Anderson and Cairney, 2007). These structures increase the contact surface area between the hyphae and the cell cytoplasm allowing efficient nutrient transfer (Peterson et al., 2004). Some have even postured that this symbiosis is so integrated that it should be considered a superorganism (Azco'n-Aguilar and Bago, 1994) with the whole being greater than the sum of the parts (Jakobsen, 1995).

It has been hypothesised that an arbuscular mycorrhiza produces a glycoprotein referred to as Glomalin, which was discovered in 1996 (Wright and Upadhyaya, 1996.). It was believed that this sticky substance, changes the soil structural properties by binding particles together. It was widely accepted to be a major driver of long-term C storage in soils containing arbuscular fungi as it consists of between 30- 40 % C and persists in the soils for an estimated 7- 42 years, depending on soil temperature and moisture (Wright and Nichols, 2002). As a result of recalcitrance of the molecule it is estimated that it accounts for up to 27 % of stored soil C (Wright and Nichols, 2002). It could be of particular interest to modelling future C storage under increasing atmospheric C dioxide concentrations as experimentation has shown hyphae are longer and produce up to five times more glomalin than during current C dioxide concentrations (Rillig et al., 1999). However more recent studies (Rillig et al., 2004) have doubted the reliability of glomalin assays and glomalin has never been positively isolated, leading to the now less specific term glomalin-related soil protein (GRSP) (Singh et al., 2013). Although it was accepted that glomalin was an arbuscular mycorrhizal metabolite no conclusive evidence to support this has yet been published (Vlček and Pohanka, 2019).

6.4.4.1.3. Ericoid

Ericoid mycorrhizal symbiosis is thought to have evolved 140 million years ago (Cullings, 1996) and facilitated the *Ericaceae* colonising the acidic impoverished soil, that they are normally associated with, such as heathlands, bogs and boreal forests (Cairney and Meharg,

2003). The fungi involved in this form of mycorrhizal association are the Ascomycota and they have evolved the ability to supply their photobiont with both phosphorous and nitrogen despite the harsh acidic nutritionally poor soil they inhabit (Smith and Read, 2010). These specialised fungi can mediate the acquisition by the plant of metal such as iron, aluminium and manganese which are often occur in their highly plant-available forms in acidic soils (Meharg and Cairney, 1999). Rather than forming arbuscules, ericoid mycorrhizae colonise root hair epidermal cells using coil-like hyphal structures that can penetrate the walls of cortical cells, but not the plasma membranes (Smith and Read, 2010). The coil acts as the plant-microbe interface allowing nutrient transfer from one to the other. A colonised Ericaceae root can be up to 80 % fungi by volume and ericoid mycorrhizal hyphae have been found to release enzymes that allow the uptake of, otherwise unavailable, complex organic molecules, a semi-saprophytic characteristic (Cairney and Burke, 1998; Read et al., 2004). The importance and extent of this form of mycorrhizal symbiosis is not fully established as the identification is problematic (Smith and Read, 2010) although novel genetic analysis of the ericoid rhizosphere microbiome is beginning to suggest host specificity and geographical range of ericoid mycorrhizae (Walker et al., 2011).

6.4.4.1.4. Arbutoid

Plants that associate with arbutoid fungi are from both the *Arbutus* (twelve species of flowering Ericaceae plants) and *Arctostaphylos* (sixty species of small flowering shrubs from the genera Ericaceae), which includes evergreen shrub species such as strawberry tree (*Arbutus unedo*), madrone (*Arbutus menziesii*), bearberry (*Arctostaphylos* sp.) and manzanita (*Arctostaphylos* sp.) (Moore et al., 2011). Arbutoid fungi are a type of ectendomycorrhizae, more closely related to ectomycorrhizal, as they are also basidiomycetes and both share the feature of possessing a mantle, or sheath, which covers the host plant root (Smith and Read, 2010). The feature that distinguishes them from the first type of ectendomycorrhizae is that they penetrate the epidermal and not the cortical cells. The fungi also form a structure similar to a Hartig net and can use its sheath to store nutrients for release to the plant during lean times (Smith and Read, 2010). The most significant difference between ecto and arbutoid mycorrhizae are that the arbutoid hyphae enter the plants roots cortical cells.

6.4.4.1.5. Orchid mycorrhizas

Orchid mycorrhizal associations are found on plants of the *orchidaceae* which contains over 22,000 species, making it the largest group of flowering plants, although most are found in

tropical or sub-tropical environments rather than temperate forests (Moore et al., 2011). Unusually all orchids have a stage of their lifecycle where they cannot photosynthesise and as a result during this protocorm phase rely solely on their mycorrhizal association to supply them with C the association is therefore an obligate (for the plant but not the fungi) and without a suitable fungal partner is available seed germination and subsequent growth during the achlorophyllous stage will not occur (McCormick et al., 2018). We know from over a century's research that orchids can form relationships with an ecologically and taxonomically large range of fungi, which can include ectomycorrhizae, saprophytic fungi, root endophytes and fungi normally classified as pathogenic (Dearnaley et al., 2012).

6.4.5. Ectendomycorrhizas

Mycorrhizas which exhibit many of the features associated with ectomycorrhizal fungi but also show high levels of intracellular hyphal penetration of the epidermal and cortical cells of the plant roots (Smith and Read, 2010) and have dark septate hyphae which are mildly pathogenic to their plant hosts. Fungi that are ectendomycorrhizas are from the basidiomycetes the fungi normally associated with trees in ectomycorrhizal symbiosis. Although the ectendomycorrhizas are classified as endomycorrhizas, in evolutionary terms they are somewhere between endo and ectomycorrhizal (Moore et al., 2011).

6.4.6. Endophytic fungi

Endophytic fungi are fungi which are specialised in growing within the leaves, meristems, roots and reproductive tissues of plants (Rodriguez et al., 2009). The colonisation of the plants tissues by these fungi has been found to dramatically improve the plants chances of survival as a result of the secondary metabolites which the fungi produce which can protect the host from herbivory and aid nutrient acquisition particularly under poor light regimes (Davitt et al., 2010). Endophytic fungi are members of a broad range of fungal taxa including both Ascomycota and Basidiomycota (Unterseher, 2011) and are reliant on the photobiont to supply them with C (Miliute, 2015). They colonise their hosts in two distinctly different routes, the first is known as vertical transmission whereby they pass a clone of themselves from parent to offspring when a hypha penetrates the embryo in the seed (Suryanarayanan, 2013). The second horizontal transmission takes place when sexually reproduced spores are passed from one individual to another (Tadych, 2014). In temperate forest ecosystems approximately one hundred of over 1000 tree species have been investigated to establish if they had endophytic symbiosis all trees studied were found to have endophytes and many had multiple simultaneous colonisations

(Unterseher, 2011). Estimates of the amount of the total global species range from 500,000 (Sieber, 2007) or 1 million (Deshmukh et al., 2015) to 1.3 Mio endophytic fungi species (Dreyfuss and Chapela, 1994). Endophytic fungal hosts demonstrate no ill-health symptoms, however on a molecular level the plants are responding to the colonisation (Johnson et al., 2006) an assumption of mutualism prevails as non-endophytic trees are non-existent, so a controlled experiment is impossible (Sieber, 2007). The extent of the importance of this type of symbiotic relationship on C cycling is far from clear although it is known that many species will switch to saprophytic after the host plant senesces (Porrás-Alfaro and Bayman, 2011). Their ability to prime the decomposition of recalcitrant C within the soil matrix means they are significant in belowground C cycling (Dai et al., 2010).

6.4.7 Epiphytes

Epiphytic fungi are those species that live on the surface of (in this case) a tree or plant these tend to be primitive fungi such as yeasts and are unlikely to have a significant role in temperate forest ecosystems. Lichens will not be considered in this review as although they are symbioses they are not plant: microbe, rather microbe: microbe symbiosis.

6.4.8. Ectomycorrhizae

Ectomycorrhizal fungi (EMF) are characterised by the presence of a Hartig net and a mycelial mantle which forms around the host's lateral roots and occupy a space between the epidermal and cortical cells (Peterson et al., 2004). In evolutionary terms ectomycorrhizal fungi are the most advanced and have subsequently been around for the least amount of time (Brundrett and Tedersoo, 2018). Ectomycorrhizae are however the least abundant of all mycorrhizal fungi with only 2 % being part of the ectomycorrhizal category (Brundrett and Tedersoo, 2018). The majority of ectomycorrhizal associations are between a mycobiant and a tree species that are found in temperate, boreal and Mediterranean forests rather than tropical (Gerz et al., 2016; Soudzilovskaia et al., 2017; Brundrett and Tedersoo, 2018). Ectomycorrhizae are economically important in terms of supplying many important timber tree species with additional nutrition as well as some of the fungi themselves being valuable such as the truffles and porcini (Mello et al., 2015). Ectomycorrhizal fungi secrete a cocktail of enzymes which can mobilise nitrogen from the soil organic matter, AM fungi do not. As a result of this the EM fungi are able to acquire more nitrogen per surface area than the AM fungi (Averill et al., 2014). A theoretical model has predicted that soil with ectomycorrhizal fungi can store more C due to the uptake of

N decreasing rates of decomposition by limiting free-living decomposers, (Orwin et al., 2011) although no experimental evidence for this currently exists (Averill et al., 2014).

EMF consist of a large range of taxonomic groups the majority are basidiomycetes with fewer being part of the ascomycetes (Smith and Read, 2010), this variety leads to diverse morphological characteristics in colonisation techniques (Agerer, 1987-2002). There are over 20,000 known fungal ectomycorrhizal partners that form relationships with 6,000 trees including pines, beeches, oaks, eucalypts, dipterocarps and poplars (Brundrett, 2002; Van der Heijden, 2015; Martin et al., 2016). Studies comparing nutrient cycling in ectomycorrhizal fungi suggests that these fungi per unit of soil divert significantly more C below-ground than arbuscular mycorrhizae (Averill et al., 2014) particular in temperate forest ecosystems.

In addition to the structures that form in the rhizosphere during colonisation, the ectomycorrhizal fungi also form mycelia which grow into the bulk soil, the structure of these extramatrical mycelium also differs greater between the different taxonomic groups (Agerer, 2001). It is through these extrametrical mycelia that the fungal symbiont is able to forage for and transfer extra nutrients and water than the tree root alone would have access to and also in the cases of common mycorrhizal networks to transfer carbohydrates between trees (Selosse et al., 2006; Simard and Durall, 2004).

6.4.9. Monotropoid

Monotropoid mycorrhizal associations are unusual as they associate with plants with 10 genera of plants which are entirely achlorophyllous and are therefore unable to photosynthesise and produce their own carbohydrates. These plants not only rely on the mycorrhizas for minerals and nutrients as with other mycorrhizal plants, but use common mycorrhizal networks to utilise C supplies from nearby plants. This is therefore considered a parasitic symbiotic relationship and the plant is referred to as mycoheterotrophs (Moore et al., 2011). This type of mycorrhizal association is native in temperate conifer forests and are using common mycorrhizal networks to gain C photosynthesised by common temperate trees such as pine, spruce and fir species. Monotropoid mycorrhizae have many similar features to the arbutoid and until recently were considered part of the same group, when it was realised that there some fundamental morphological differences i.e. that the hyphae do not penetrate the cells (Smith and Read, 2010).

7. Common mycorrhizal networks (CMN)

Mycorrhizal infections are thought to take place when an uninfected root meets a mycelial hypha of a fungi which is already connected to a plant (Read et al., 1976) the mycorrhizae is therefore not reliant on a single plant species or individual to supply them with carbohydrates (Bücking, et al., 2016). Generally, mycorrhizae are not species specific and as a result this mycelial inter-tree connection can be either inter or intraspecific (Selosse et al., 2006) and are commonly referred to as the Wood Wide Web (Beiler et al., 2010). The mycorrhizae by connecting to the plants roots directly can guarantee a share of the photosynthetically fixed C which gives them a competitive advantage over those microbes that are free-living in the soil (Simard et al., 2015). The networks can include many plants with differing traits or various ages the magnitude and direction of the resource fluxes are mediated by the plant and fungal network (Simard et al., 2015).

7.1. Transfer between mycorrhizal plants via CMN

In the real world common mycorrhizal networks tend to be highly complex, often with more than one fungal species as well as more than one tree species (Beiler et al., 2010; Bahram et al., 2011; Horton and Bruns, 2001; Taylor, 2002; Dickie et al., 2004; Nara, 2006; Tweig et al., 2007). This makes the job of disentangling what has been transferred by whom an insurmountable problem with current methodologies (Simard et al., 2015). However, it is accepted that transfer of elements and molecules between multiple plants through mycorrhizal networks does occur and is distinct from transport through rhizomes and root grafts (Fraser et al., 2006; Philip et al., 2010). Indirect uptake of root exudates from the soil water and subsequent transfer through the hyphae is also taking place simultaneously (Perry et al., 1989; Rillig and Mummery; 2006; Philip et al., 2010; Jones et al., 2009). The elements and molecules that we know can and are transported via CMN are currently limited, but include lipids (Bago et al., 2002), amino acids (Jin et al., 2005), C (Francis and Read, 1984; Brownlee et al., 1983; Finlay and Read, 1986), nitrogen (Arnebrant et al., 1993; Bethlenfalvay et al., 1991), phosphorus (Newman and Eason, 1993; Whittingham and Read, 1982), water (Egerton-Warburton et al., 2007; Plamboeck et al., 2007; Querejeta et al., 2003) and other more nutrient analogues (rubidium, arsenic and caesium) (Meding and Zasoski, 2008; Gyuricza et al., 2010) as well as genetic material (Giovannetti et al., 2004; Giovannetti et al., 2006). Phosphorus transfer studies have been less frequent than nitrogen with only a small number looking at

either arbuscular mycorrhizae (Eason and Newman, 1990; Eissenstat, 1990; Wilson et al, 2006) or ectomycorrhizal networks (Finlay and Read, 1986; Perry et al., 1989).

Studies have shown that transfer of C between plants can take place via arbuscular (Hirrel, 1979; Francis and Read, 1984; Grime et al., 1987) or ectomycorrhizal (Brownlee et al., 1983; Simard et al., 1997) fungal symbioses. In arbuscular mycorrhizae however, C is not transferred from one plant to another the C remains in the roots and is not redistributed to the growing aboveground tips (Robinson and Fitter, 1999). The discovery that transfer of C, water and nutrients can be transferred between plants via CMN has been known for over five decades, but it has been found to be hard to quantify (Simard et al., 2015). Initially studies showed that plant seedlings could transfer C via common mycorrhizal networks (CMN) (Simard et al., 1997), but since then transfer has been shown to take place from mature trees to seedlings (Beiler et al., 2010) and between mature trees (Klein et al., 2016). Controversy remains however as experimentation has failed to date to prove net transfer via common arbuscular mycorrhizal networks (Francis and Read, 1984). Klein et al., estimate that transfer between trees via CMN can account for 280 kg of C per hectare, approximately equivalent to 4 % of the forests net primary production, although some of this may be C recaptured from exudation or root and hyphal turnover (Jones et al., 2009). C fluxes via CMN can account for up to 10 % for autotrophic and up to 85 % partially myco-heterotrophic and 100 % for fully myco-heterotrophic plants (Simard et al., 2015).

7.2. Pathways for C transfer

The precise mechanisms by which the resource transfer can take place are not understood (Simard et al., 2015), this problem is compounded by the fact that the mechanisms are likely to be different between plant species, type (deciduous or evergreen/ broadleaf or conifer) and mycobiont specific (Agerer, 2001). The literature does suggest that transfer generally takes place along source-sink, concentration gradients or energy potential gradients and the resource is then transported to the area in the plant where growth is occurring (Simard et al., 2015).

7.3. Signalling via CMN

Communication between plants through volatile compounds being released into the air and then “smelt” by their neighbours is now well established (Balwin and Schultz, 1983; Rhodes, 1983) and the ecological implications are well understood (Heil and Karban, 2010). Since the discovery of common mycorrhizal networks there has been interest in the possibility that CMN are also being used for communication between plants (Johnson and Gilbert, 2015), but the

extent and ecological implications of this is not yet clear. Many volatiles that are released during aerial communication are systemic and therefore found throughout the plant and potentially released belowground simultaneously (Chamberlain et al., 2001). More recently experimentation has revealed that these neighbouring plants produced a defence compound when connected via CMN to a plant under attack by herbivores (Babikova et al., 2013; Song et al., 2014) and pathogenic fungi (Song et al., 2010). We currently do not know if these chemicals are fungal species or stimuli specific (Johnson and Gilbert, 2015).

7.3.1. Allelopathy

The transfer of communication chemicals which have a positive outcome for both plants are in the majority however, allelopathic chemicals are also transferred through common networks (Barto et al., 2011). Allelopathic chemicals are released by some plants into the rhizosphere and give the releasing plant a competitive advantage by altering the soil chemistry to favour itself or negatively impact the competitors (Cipollini et al., 2012). One much studied temperate forest example of this is black walnut (*Juglans nigra* L.) (Willis, 2000), which famously produces the allelopathic aromatic phytotoxic compound juglone (5-hydroxy-1, 4 naphthoquinone) (Rice, 1974), the result of which is reduced plant competition around the walnut tree (Jose, 2002). It has been understood for some time that common mycorrhizal networks can act like ‘superhighways’ extending the region of influence of these allelopathic compounds in the soil (Barto et al., 2012). Arbuscular common mycorrhizal networks have experimentally been shown to not only increase the bioactive zone but also to increase the effectiveness of the compound by mediating the chemical interaction processes (Achatz et al., 2014). The subject is highly complex with research reporting that allelopathic compounds inhibit (Souto et al., 2000) or stimulate mycorrhizal hyphal growth (Rose et al., 1983). Some species of ectomycorrhizal fungi are intolerant to allelopathic compounds, a phenomenon in the UK known as ‘heather check’ is well documented and occurs as a result of allelopathic compounds exuded by heather roots. These compounds severely inhibit the fungi normally associated with Sitka spruce (*Picea stichensis*), leading in some cases severe chlorosis of the spruce trees (Taylor and Tabbush, 1990). It has also been reported that arbuscular mycorrhizae can protect native tree species from the effect of the allelopathic chemical released by invasive non-native plant species (Barto et al., 2010). This area of science would benefit from more research being undertaken to but what is clear is that these plants: fungal symbioses have significant implications for plant communities by altering interspecies chemical interactions (Barto et al., 2011).

7.4. Potential pathways for signalling chemicals

The physiological mechanisms and pathways of many of the signalling compounds are still not well understood. It is hypothesised that C and nutrients are transferred both apoplastically and symplastically across a source-sink gradient (Simard et al., 2015) and there are the following speculations as to the potential pathways that these chemicals could take

- a) Hyphae have the ability to modify the rhizosphere and it is possible that this modification could increase the soils potential as a transport pathway by either altering aggregation, electrical conductivity or by manipulating the microbial community. Signally speed through this potential pathway is likely to be slow however due to the increased path length and resistance (Hillel, 1998).
- b) The chemical could be transported either passively or actively through hyphal cell membranes and into the cytoplasm. This pathway seems improbable as the cell membranes are impermeable and hydrophobic. It transports through the membrane were to occur it would need transport mechanism, such as a membrane transporter chemical and these have not yet been observed in hyphae (Barto et al., 2012).
- c) Chemical transport could be apoplastic, although due to the hydrophobic nature of the hyphal cell walls this would likely only be possible for nonpolar chemicals (Allen, 2007).
- d) Chemical signals could be transported through the water found on the hyphal surface either by microbes or capillary action, however this would only be possible in those that are water soluble and is unlikely over long distances (Johnson and Gilbert, 2015; Barto et al., 2012; Allen, 1996).
- e) In some cases, mycorrhizal hyphae will curl around each other to form a rope-like structure. This structure provides the opportunity for small pockets of air or water to be trapped between the hyphal strands and allow the transport of water-soluble compounds or hydrophobic volatile chemicals respectively (Freise and Allen, 1991). The rope-like structures are not observed frequently and therefore the contribution of this pathway is unlikely to be substantial.
- f) Electrical signal may also be conducted through their CMN to enable plant to plant communication (Johnson and Gilbert, 2015), this has been experimentally observed in

both artificial leaf damage (Mousavi et al., 2013) and invertebrate herbivory (Salvador-Recatala, 2014).

Potential pathway a, d and e have the problem of coming into contact with the vast array of microbial inhabitants of the soil and therefore contamination in the form biofilm growth is more likely (Toljander, et al., 2005).

8. Plants with both ecto and arbuscular mycorrhizal associations

Some species of tree can form symbiotic relationship with multiple types of symbiosis, namely both ecto and arbuscular mycorrhizal species simultaneously. These trees known by horticulturalists as bridging trees and are added to species mixtures in the hopes that the nutritional benefits of the foraging of both fungal types can be utilised (Philips, 2017). Trees with AM and EM fungi have different functional traits to those without such as root architecture related to acquisition of nutrients (Comas and Eissenstat, 2004; Guo et al., 2008; Valverde-Barrantes et al., 2015). and leaf litter chemistry (Cornelissen et al., 2001; Midgley et al., 2015; Lin et al., 2016) Temperate trees which exhibit this characteristic include alder (*Alnus* sp.), aspen (*Populus tremula*), willow (*Salix* sp.) and poplar (*Populus* sp.). Although Jack and Lindsey Hartley (1987) list more temperate species as sharing AM and EM symbiosis, including hawthorn (*Crateagus monogyna*), crab-apple (*Malus sylvestris*), wild cherry (*Prunus avium*), wild pear (*Pyrus pycaster*) and rowan (*Sorbus aucuparia*). Studies have noted that in some trees the seedlings seem to develop AM symbiosis before switching to EM partners later in their lifecycle (Adjoud-Sadadou and Halli-Hargas, 2017).

9. Potential effect of increased atmospheric C dioxide on plant microbe symbiosis

As atmospheric C dioxide concentration increases so does productivity (Bazzaz, 1990), therefore we would expect root exudation to increase. Experiments have shown an increase in allocation of C into the roots and an increase in exudation in *Pinus* seedlings (Norby et al., 1987). An increase in exudation has been shown to increase mycorrhizal biomass (Lewis et al., 1994) and density (O'Neill et al., 1987).

Increased atmospheric C dioxide concentrations and their effect on plant physiology has been studied globally using a network of FACE experiments (Norby and Zak, 2011). Increases in C dioxide has been to increase tree water use efficiency by inducing stomatal closure (Field et al., 1995; Picon et al., 1996). Plants also respond to elevated C dioxide by allocating more C to root production and turnover, which allows the roots to penetrate further into the soil and access water located deeper in the soil (Wullschleger, 2002) and can increase above ground biomass (Hoosbeek et al., 2011).

6.1. Stoichiometry

Atmospheric C dioxide levels have increased over the last century (Schimel et al., 2015) and as a result rates of photosynthesis have increased with CO₂ following a Michaelis–Menton curve (Zaehle et al., 2014) however this may become limited by nitrogen availability, due to an imbalance in stoichiometric C:N ratio (Inselsbacher and Näsholm, 2012). Most of the nitrogen found in the soil is there as a result of decomposition (Aber and Melillo, 1980) and if this process does not increase as quickly as photosynthesis, the lack of nitrogen becomes the factor which can limit the plants rate of C sequestration (Chaplin et al., 2009; Inselsbacher and Näsholm, 2012). The N availability in the soil also has a direct impact on its ability to store C (Bonan, 2008; Janssens et al., 2010). As a result of this fact C models that account for nitrogen availability often differ greatly from those that do not (Wieder, et al., 2015). Other nutrients such as potassium, tend not to be so limiting, although may become more so in future this is at least in part due to the fact that for healthy plant growth a plant will need ten times as much nitrogen as potassium (Reed et al., 2015). Nutrient deficiency can be assessed either in terms of a single species or as plant communities and in mixed species stands tree species may coexist that are limited by nitrogen and potassium differentially (DiTomasso and Aarssen, 1989; Koerselman and Meuleman, 1996), however this is not yet fully understood (Aerts and Chapin, 2000).

10. Effect of tree/ plant species on C cycling

The tree species located in a particular area of forest affects the soil organic content and therefore the C in soil due to exudation, root turnover and litter fall (Vesterdal et al., 2013). It has been found that the dominant tree species within the forest also determines the species and composition of fungal and bacterial communities within the soil (Urbanová, 2015). The effect

of tree species on microbial communities is generally accepted and experimentally supported (Grayston et al., 1998, Hackl et al., 2004, Hobbie et al., 2006, Ushio et al., 2008, Aponte et al., 2013), but not quantified (Urbanová, 2015). The dominant tree species will also determine the storage and dynamics of C in the soil, as a result of litter inputs, but also differences in exudates (Leuschner et al., 2013; Jandl et al., 2007; Hagen-Thorn et al., 2004). Differences in NPP requirements (Hansen et al., 2009), varying quality of organic matter addition to detritus (Hagen-Thorn et al., 2004), differences in depth of root penetration into the soil (Lai et al., 2016) and by influencing populations of soil fauna (Hobbie et al., 2006; Lynch et al., 2012) will all change with dominant species and result in a change in belowground C dynamics.

The tree species present within a stand can significantly alter the soil C storage capacity, yet the mechanisms involved in this are not fully understood (Vesterdal et al., 2013). It has been observed that the quality of litter can determine decomposition rates and therefore effect soil C storage (Vesterdal et al., 2008; Berg et al., 2010), however the link remains uncertain (Prescott, 2010). This will be important as tree plantations globally are increasing in area (Paquette and Messier, 2009) and species choices are changing, especially in temperate and boreal forests (Iverson et al., 2008; Garbelotto and Pautasso, 2012). Mixed species stands are increasingly being favoured by European temperate foresters (Mason and Connolly, 2013), as both climatic change risk reduction and adaptation strategies (Forrester, 2014). The driver for this has been to ensure that forests continue to supply society with ecosystems services under the pressures of changing climate and non-native pests and diseases (Quine et al., 2011). In the UK, mixed species stand tend to be more common in England than either Wales or Scotland (Smith and Gilbert, 2003). In some cases, mixed species forests will produce more biomass than the average for those species as single species stands, this is known as over-yielding. In others the mixed stand will produce more biomass than the highest yield of that species alone, this is known as transgressive over-yielding (Harper, 1977; Pretzsch, 2009).

The ability to produce far more as mixtures is dependent on the species having differing ecological niches or functional traits thus reducing interspecies competition (Loreau and Hector, 2001; Kelty, 2006; Richards and Schmidt, 2010) and on the relative growth rates and lifespan of the species differing from each other (Pretzsch, 2009). Soil organic matter also accumulates at a different rate in mixed species stands when compared to that of monocultures (Six *et al.*, 2002; Steinbeiss *et al.*, 2008). This in turn will alter the rate of turnover and detrital decomposition (King *et al.*, 2002) as well as the composition of the microbial communities (Pollierer, 2007). Increased species diversity within an area of forest increases that forest's

resilience to perturbations (Mason, 2007; Bolte *et al.*, 2009). Forests need to increase diversity in all forms; species choices, genetic diversity and provenance diversity will all improve a forests resistance and resilience to perturbations (Noss, 2001; Doim et al., 2010). Diversity within forest plant communities will determine the microbial community's productivity and diversity (Kowalchuk et al., 2002; Wardle et al., 2003).

Research suggests a relationship between plant diversity and ecosystem function (Tilman et al., 1997; Loreau, 2002). This relationship is not a linear relationship, however as some so-called keystone species have more important roles within the ecosystem and over soil functioning (Tilman et al., 1997; Leps et al., 2001, Huston 1997). Experiments using $^{13}\text{CO}_2$ can determine how much a species alters the C allocation (Ladygina and Hedlund, 2010). The differences in rates of C sequestration between species can be attributed to differences in rates of growth (Pérez-Cruzado et al., 2012), litter quality and quantity (Berg, 2000; Vesterdal et al., 2008) and type of ground cover (Lemma et al., 2006; Kasel et al., 2011) and other functional traits.

10.1. Functional traits

The functional trait of a plant is the particular ecological niche which it fulfils within an ecosystem (Mayfield et al., 2010). The range of ecosystem services a plant community provides is believed to be based on the number of functional traits within that community rather than the number of species (Díaz and Cabido, 2001; Prinzing et al., 2008) although the relationship is complicated (Naeem and Wright, 2003). Plants functional traits are a controlling factor in below-ground C storage, by controlling assimilation and transfer as well a residency time (De Deyn et al., 2008). Due to the fact that the majority of research in this field has been conducted on conifer species (Berthrong et al., 2009) the true significance of functional traits in forest trees C sequestration is not fully understood (Pérez-Cruzado et al., 2012). Species composition is more important than total species number in determining the size and residency time of the belowground C pools (Hulvey et al., 2013). Plant traits which regulate C sequestration and storage can thought of as two distinct trait types. This is either by increased primary production or allocating more C resources below-ground or by reducing C loss from processes such as respiration and leaching (De Deyn et al., 2008).

11. Conclusions

Knowledge gaps certainly exist in this field and the disconnect between those doing mycorrhizal ecological forest research and those who actually manage forests needs to be addressed in order to maximise the mitigation ability of temperate forests (Lindner et al., 2014). CMN may be far more important to the understanding of forest ecology than previously understood and is likely to determine seedling recruitment, survival and growth, mediate inter and intra specific facilitation and competition and species composition and succession (Fraser et al., 2006; Teste et al., 2009; Deslippe and Simard, 2011). Threats to fungal diversity from modern agricultural techniques, habitat fragmentation and nitrogen deposition are growing and have led to a series of academic discussions on how best to manage these micro-organisms. Given the fundamental importance of plant: microbe symbiosis humanity should be concerned by the decrease in abundance and diversity of these essential components of all ecosystems and the underlying services that they provide humanity. The challenge for scientists will be to be honest about the many uncertainties whilst communicating the need for planning for possible future scenarios (Millar et al., 2007; Yousefpour et al., 2012; Spittlehouse and Stewart, 2003). The importance of rhizodeposition of recently captured C into the rhizosphere for uptake by soil microbes has historically been underestimated (Högberg and Read, 2006), as it was always assumed that the main driver of soil microbial activity was litter decomposition (Wardle et al., 2004). It is widely accepted therefore that understanding the role of temperate forests in C cycling and sequestration is essential in the science of climatic change mitigation (Mason et al., 2013). The current models of C cycling and dynamics fail to take full account of plant: microbe symbiosis (Brzostek et al., 2016). This is as a result of a lack of understanding of the mechanisms behind these processes as well as a lack of reliable quantification and data to support the addition of this to models (Cheeke et al., 2017). Only by the development of a mycorrhizal driven, trait-based model, will we begin to reliably predict how climatic changes and shifts in the processes underlying biogeochemical cycles and subsequent shifts in species range.

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13. References

- Aber, J. D., and Melillo, J. M., 1980. Litter decomposition: measuring relative contributions of organic matter and nitrogen to forest soils. *Canadian Journal of Botany*, 58(4), pp.416-421.
- Achatz, M., Morris, E.K., Müller, F., Hilker, M. and Rillig, M.C., 2014. Soil hypha-mediated movement of allelochemicals: arbuscular mycorrhizae extend the bioactive zone of juglone. *Functional Ecology*, 28(4), pp.1020-1029.
- Adjoud-Sadadou, D. and Halli-Hargas, R., 2017. Dual mycorrhizal symbiosis: an asset for eucalypts out of Australia?. *Canadian Journal of Forest Research*, 47(4), pp.500-505.
- Adl, S.M. and Coleman, D.C., 2005. Dynamics of soil protozoa using a direct count method. *Biology and fertility of soils*, 42(2), pp.168-171.
- Adl, M.S. and Gupta, V.S., 2006. Protists in soil ecology and forest nutrient cycling. *Canadian Journal of Forest Research*, 36(7), pp.1805-1817.
- Adu, J. K., and Oades, J. M., 1978. Utilization of organic materials in soil aggregates by bacteria and fungi. *Soil Biology and Biochemistry*, 10(2), pp.117-122.
- Aerts, R., and Chapin Iii, F. S., 2000. The mineral nutrition of wild plants revisited. *Advances in Ecological Research*, 30, pp 1-67.
- Ågren, G. I., Axelsson, B., Flower-Ellis, J. G. K., Linder, S., Persson, H., Staaf, H., and Troeng, E., 1980. Annual carbon budget for a young Scots pine. *Ecological Bulletins*, 32 pp.307-313.
- Agerer, R., 1987–2002. *Colour atlas of ectomycorrhizae*. Einhorn-Verlag, Schwäbisch Gmünd, Germany.
- Agerer, R., 2001. Exploration types of ectomycorrhizae. *Mycorrhiza*, 11(2), pp.107-114.
- Aponte, C., García, L. V., and Marañón, T., 2013. Tree species effects on nutrient cycling and soil biota: a feedback mechanism favouring species coexistence. *Forest Ecology and Management*, 309, pp.36-46.
- Ahmed, I. U., Smith, A. R., Jones, D. L., and Godbold, D. L., 2015. Tree species identity influences the vertical distribution of labile and recalcitrant carbon in a temperate deciduous forest soil. *Forest Ecology and Management*, 359, pp.352-360.
- Allen, M.F., 1991. *The ecology of mycorrhizae*. Cambridge University Press.

Allen, M.F., 1996. The ecology of arbuscular mycorrhizas: a look back into the 20th century and a peek into the 21st. *Mycological research*, 100(7), pp.769-782.

Allen, M.F., 2007. Mycorrhizal fungi: highways for water and nutrients in arid soils. *Vadose Zone Journal*, 6(2), pp.291-297.

Almario, J., Jeena, G., Wunder, J., Langen, G., Zuccaro, A., Coupland, G. and Bucher, M., 2017. Root-associated fungal microbiota of nonmycorrhizal *Arabidopsis thaliana* and its contribution to plant phosphorus nutrition. *Proceedings of the National Academy of Sciences*, 114(44), pp. E9403-E9412.

Anderegg, W.R., Schwalm, C., Biondi, F., Camarero, J.J., Koch, G., Litvak, M., Ogle, K., Shaw, J.D., Shevliakova, E., Williams, A.P. and Wolf, A., 2015. Pervasive drought legacies in forest ecosystems and their implications for carbon cycle models. *Science*, 349(6247), pp.528-532.

Anderson, I.C. and Cairney, J.W., 2007. Ectomycorrhizal fungi: exploring the mycelial frontier. *FEMS Microbiology Reviews*, 31(4), pp.388-406.

Arnebrant, K., Ek, H., Finlay, R.D. and Söderström, B., 1993. Nitrogen translocation between *Alnus glutinosa* (L.) Gaertn. seedlings inoculated with *Frankia* sp. and *Pinus contorta* Dougl. ex Loud seedlings connected by a common ectomycorrhizal mycelium. *New Phytologist*, 124(2), pp.231-242.

Averill, C., Turner, B.L. and Finzi, A.C., 2014. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature*, 505(7484), p.543.

Azcó'n-Aguilar, C. and Bago, B., 1994. Physiological characteristics of the host plant promoting an undisturbed functioning of the mycorrhizal symbiosis. In S Gianinazzi, H Schuepp, eds, *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems*. Birkhäuser Verlag, Basel, pp. 47–60

Bååth, E., and Anderson, T. H., 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biology and Biochemistry*, 35(7), pp.955-963.

Babikova, Z., Johnson, D., Bruce, T., Pickett, J. and Gilbert, L., 2014. Underground allies: How and why do mycelial networks help plants defend themselves? What are the fitness,

regulatory, and practical implications of defence-related signaling between plants via common mycelial networks?. *BioEssays*, 36(1), pp.21-26.

Bago, B., Zipfel, W., Williams, R.M., Jun, J., Arreola, R., Lammers, P.J., Pfeffer, P.E. and Shachar-Hill, Y., 2002. Translocation and utilization of fungal storage lipid in the arbuscular mycorrhizal symbiosis. *Plant Physiology*, 128(1), pp.108-124.

Bahram, M., Pölme, S., Kõljalg, U. and Tedersoo, L., 2011. A single European aspen (*Populus tremula*) tree individual may potentially harbour dozens of *Cenococcum geophilum* ITS genotypes and hundreds of species of ectomycorrhizal fungi. *FEMS Microbiology Ecology*, 75(2), pp.313-320.

Bai, Y., Müller, D.B., Srinivas, G., Garrido-Oter, R., Potthoff, E., Rott, M., Dombrowski, N., Münch, P.C., Spaepen, S., Remus-Emsermann, M. and Hüttel, B., 2015. Functional overlap of the *Arabidopsis* leaf and root microbiota. *Nature*, 528(7582), p.364.

Bailey, V. L., Smith, J. L., and Bolton, H., 2002. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. *Soil Biology and Biochemistry*, 34(7), pp.997-1007.

Baldrian, P., Kolařík, M., Štursová, M., Kopecký, J., Valášková, V., Větrovský, T., Žifčáková, L., Šnajdr, J., Řídl, J., Vlček, Č. and Voříšková, J., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *The ISME journal*, 6 (2), p.248.

Baldwin, I.T. and Schultz, J.C., 1983. Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants. *Science*, 221(4607), pp.277-279.

Bamforth, S.S., 2001. Proportions of active ciliate taxa in soils. *Biology and fertility of soils*, 33(3), pp.197-203.

Barbieri, E., Guidi, C., Bertaux, J., Frey-Klett, P., Garbaye, J., Ceccaroli, P., Saltarelli, R., Zambonelli, A. and Stocchi, V., 2007. Occurrence and diversity of bacterial communities in *Tuber magnatum* during truffle maturation. *Environmental Microbiology*, 9(9), pp.2234-2246.

Barbieri, E., Ceccaroli, P., Saltarelli, R., Guidi, C., Potenza, L., Basaglia, M., Fontana, F., Baldan, E., Casella, S., Ryahi, O. and Zambonelli, A., 2010. New evidence for nitrogen fixation within the Italian white truffle *Tuber magnatum*. *Fungal biology*, 114(11-12), pp.936-942.

Bardgett, R.D. and Van Der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. *Nature*, 515(7528), p.505.

- Barto, E.K., Hilker, M., Müller, F., Mohny, B.K., Weidenhamer, J.D. and Rillig, M.C., 2011. The fungal fast lane: common mycorrhizal networks extend bioactive zones of allelochemicals in soils. *PLoS One*, 6(11), p.e27195.
- Barto, K., Friese, C. and Cipollini, D., 2010. Arbuscular mycorrhizal fungi protect a native plant from allelopathic effects of an invader. *Journal of chemical ecology*, 36(4), pp.351-360.
- Barto, E.K., Weidenhamer, J.D., Cipollini, D. and Rillig, M.C., 2012. Fungal superhighways: do common mycorrhizal networks enhance below ground communication?. *Trends in plant science*, 17(11), pp.633-637.
- Bates, S.T., Berg-Lyons, D., Caporaso, J.G., Walters, W.A., Knight, R. and Fierer, N., 2011. Examining the global distribution of dominant archaeal populations in soil. *The ISME journal*, 5(5), p.908.
- Bazzaz, F.A., 1990. The response of natural ecosystems to the rising global CO₂ levels. *Annual review of ecology and systematics*, 21(1), pp.167-196.
- Beiler, K.J., Durall, D.M., Simard, S.W., Maxwell, S.A. and Kretzer, A.M., 2010. Architecture of the wood-wide web: *Rhizopogon* spp. genets link multiple Douglas-fir cohorts. *New Phytologist*, 185(2), pp.543-553.
- Belay-Tedla, A., Zhou, X., Su, B., Wan, S., and Luo, Y., 2009. Labile, recalcitrant, and microbial carbon and nitrogen pools of a tallgrass prairie soil in the US Great Plains subjected to experimental warming and clipping. *Soil Biology and Biochemistry*, 41(1), pp.110-116.
- Bending, G.D. and Read, D.J., 1995. The structure and function of the vegetative mycelium of ectomycorrhizal plants: V. Foraging behaviour and translocation of nutrients from exploited litter. *New Phytologist*, 130(3), pp.401-409.
- Berg, B., 2000. Litter decomposition and organic matter turnover in northern forest soils. *Forest ecology and Management*, 133(1), pp.13-22.
- Berthrong, S. T., Jobbágy, E. G., and Jackson, R. B., 2009. A global meta-analysis of soil exchangeable cations, pH, carbon, and nitrogen with afforestation. *Ecological Applications*, 19(8), pp.2228-2241.
- Bertness, M.D. and Leonard, G.H., 1997. The role of positive interactions in communities: lessons from intertidal habitats. *Ecology*, 78(7), pp.1976-1989.

- Bethlenfalvay, G.J., Reyes-Solis, M.G., Camel, S.B. and Ferrera-Cerrato, R., 1991. Nutrient transfer between the root zones of soybean and maize plants connected by a common mycorrhizal mycelium. *Physiologia Plantarum*, 82(3), pp.423-432.
- Bidartondo, M.I., Read, D.J., Trappe, J.M., Merckx, V., Ligrone, R. and Duckett, J.G., 2011. The dawn of symbiosis between plants and fungi. *Biology letters*, 7(4), pp.574-577.
- Bledsoe, C.S., Allen, M.F. and Southworth, D., 2014. Beyond mutualism: complex mycorrhizal interactions. In *Progress in botany*, pp. 311-334. Springer, Berlin, Heidelberg.
- Bolin, B., 1977. Changes of land biota and their importance for the carbon cycle. *Science*, 196(4290), pp.613-615.
- Bolte, A., Ammer, C., Löf, M., Madsen, P., Nabuurs, G. J., Schall, P., Spathelf, P., and Rock, J., 2009. Adaptive forest management in central Europe: climate change impacts, strategies and integrative concept. *Scandinavian Journal of Forest Research*, 24(6), pp.473-482.
- Bomberg, M. and Timonen, S., 2009. Effect of tree species and mycorrhizal colonization on the archaeal population of boreal forest rhizospheres. *Applied Environmental Microbiology*, 75(2), pp.308-315.
- Bonan, G., 2008. Carbon cycle: Fertilizing change. *Nature Geoscience*, 1(10), pp.645-646.
- Bonfante, P. and Genre, A., 2010. Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. *Nature communications*, 1, p.48.
- Boucher, D.H., 1985. The idea of mutualism, past and future. In: Boucher D (ed) *The Biology of Mutualism*, pp 1–28. Oxford University Press, New York.
- Boucher, D. H., ed. 1985. *The Biology of Mutualisms: Ecology and Evolution*. Croom and Helm, London.
- Boucher, D.H. ed., 1988. *The biology of mutualism: ecology and evolution*. Oxford University Press on Demand.
- Bowen, G. D., 1979. Integrated and experimental approaches to the study of growth of organisms around roots. In *Soil-Borne Plant Pathogens*, pp. 209-227. Academic Press New York.
- Bowen, G. D., 1994. The ecology of ectomycorrhiza formation and functioning. *Plant and soil*, 159(1), 61-67.

- Bowen, G. D., and Rovira, A. D., 1991. The rhizosphere, the hidden half of the hidden half. *Plant Roots: The hidden half*. (Y. Waisel A. Eshel and U. Kafkafi. eds.) pp, 641-669.
- Bravo, A., York, T., Pumplun, N., Mueller, L.A. and Harrison, M.J., 2016. Genes conserved for arbuscular mycorrhizal symbiosis identified through phylogenomics. *Nature Plants*, 2(2), p.15208.
- Brownlee, C., Duddridge, J.A., Malibari, A. and Read, D.J., 1983. The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. In *Tree Root Systems and Their Mycorrhizas* (pp. 433-443). Springer, Dordrecht.
- Brundrett, M.C., 2002. Coevolution of roots and mycorrhizas of land plants. *New Phytologist*, 154(2), pp.275-304.
- Brundrett, M.C., Schulz, B.J.E., Boyle, C.J.C. and Sieber, T., 2006. *Microbial root endophytes*. Berlin. Germany, Springer-Verlag, 2006, 281-293.
- Brundrett, M.C. and Tedersoo, L., 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist*, 220(4), pp.1108-1115.
- Bruno, J.F., Stachowicz, J.J. and Bertness, M.D., 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution*, 18(3), pp.119-125.
- Brzostek, E.R., Dragoni, D., Brown, Z.A. and Phillips, R.P., 2015. Mycorrhizal type determines the magnitude and direction of root-induced changes in decomposition in a temperate forest. *New Phytologist*, 206(4), pp.1274-1282.
- Bücking, H., Mensah, J.A. and Fellbaum, C.R., 2016. Common mycorrhizal networks and their effect on the bargaining power of the fungal partner in the arbuscular mycorrhizal symbiosis. *Communicative and integrative biology*, 9(1), p.e1107684.
- Buée, M., Reich, M., Murat, C., Morin, E., Nilsson, R.H., Uroz, S. and Martin, F., 2009. 454 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytologist*, 184(2), pp.449-456.
- Bueno, P., Soto, M.J., Rodríguez-Rosales, M.P., Sanjuan, J., Olivares, J. and Donaire, J.P., 2001. Time-course of lipoxygenase, antioxidant enzyme activities and H₂O₂ accumulation during the early stages of *Rhizobium*–legume symbiosis. *New Phytologist*, 152(1), pp.91-96.

- Bulgarelli, D., Rott, M., Schlaeppi, K., van Themaat, E.V.L., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E. and Peplies, J., 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature*, 488(7409), p.91.
- Buscot, F., 2015. Implication of evolution and diversity in arbuscular and ectomycorrhizal symbioses. *Journal of plant physiology*, 172, pp.55-61.
- Cairney, J.W. and Meharg, A.A., 2003. Ericoid mycorrhiza: a partnership that exploits harsh edaphic conditions. *European Journal of Soil Science*, 54(4), pp.735-740.
- Cairney, J.W.G. and Burke, R.M., 1998. Extracellular enzyme activities of the ericoid mycorrhizal endophyte *Hymenoscyphus ericae* (Read) Korf and Kernan: their likely roles in decomposition of dead plant tissue in soil. *Plant and Soil*, 205(2), pp.181-192.
- Callaway, R.M., 1995. Positive interactions among plants. *The Botanical Review*, 61(4), pp.306-349.
- Casas, V., and Rohwer, F., 2007. Phage metagenomics. *Methods in enzymology*, 421, 259-268
- Chamberlain, K., Guerrieri, E., Pennacchio, F., Pettersson, J., Pickett, J.A., Poppy, G.M., Powell, W., Wadhams, L.J. and Woodcock, C.M., 2001. Can aphid-induced plant signals be transmitted aurally and through the rhizosphere?. *Biochemical Systematics and Ecology*, 29(10), pp.1063-1074.
- Chapin III, S., McFarland, J., David McGuire, A., Euskirchen, E. S., Ruess, R. W., and Kielland, K., 2009. The changing global carbon cycle: linking plant–soil carbon dynamics to global consequences. *Journal of Ecology*, 97(5), 840-850
- Cheeke, T.E., Phillips, R.P., Brzostek, E.R., Rosling, A., Bever, J.D. and Fransson, P., 2017. Dominant mycorrhizal association of trees alters carbon and nutrient cycling by selecting for microbial groups with distinct enzyme function. *New Phytologist*, 214(1), pp.432-442.
- Chen, J. M., Liu, J., Cihlar, J., and Goulden, M. L., 1999. Daily canopy photosynthesis model through temporal and spatial scaling for remote sensing applications. *Ecological modelling*, 124(2), 99-119.
- Cho, Y.S., Kim, J.S., Crowley, D.E. and Cho, B.G., 2003. Growth promotion of the edible fungus *Pleurotus ostreatus* by fluorescent pseudomonads. *FEMS microbiology letters*, 218(2), pp.271-276.

- Cipollini, D., Rigsby, C.M. and Barto, E.K., 2012. Microbes as targets and mediators of allelopathy in plants. *Journal of Chemical Ecology*, 38(6), pp.714-727.
- Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R. D., Wardle, D. A., and Lindahl, B. D., 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science*, 339(6127), 1615-1618.
- Cleveland, C.C., Townsend, A.R., Schimel, D.S., Fisher, H., Howarth, R.W., Hedin, L.O., Perakis, S.S., Latty, E.F., Von Fischer, J.C., Elseroad, A. and Wasson, M.F., 1999. Global patterns of terrestrial biological nitrogen (N₂) fixation in natural ecosystems. *Global biogeochemical cycles*, 13(2), pp.623-645.
- Coleman, D. C., Reid, C. P. P., and Cole, C. V., 1983. Biological strategies of nutrient cycling in soil systems. *Advances in ecological research*, 13, 1-55.
- Coleman-Derr, D. and Tringe, S.G., 2014. Building the crops of tomorrow: advantages of symbiont-based approaches to improving abiotic stress tolerance. *Frontiers in microbiology*, 5, p.283.
- Comas, L.H. and Eissenstat, D.M., 2004. Linking fine root traits to maximum potential growth rate among 11 mature temperate tree species. *Functional Ecology*, 18(3), pp.388-397.
- Conteh, A., Lefroy, R.D.B. and Blair, G.J., 1997. Dynamics of organic matter in soil as determined by variations in ¹³C/¹²C isotopic ratios and fractionation by ease of oxidation. *Australian Journal of Soil Research*, 35(4), pp.881-890.
- Cornelissen, J., Aerts, R., Cerabolini, B., Werger, M. and Van Der Heijden, M., 2001. Carbon cycling traits of plant species are linked with mycorrhizal strategy. *Oecologia*, 129(4), pp.611-619.
- Cullings, K. W., 1996. Single phylogenetic origin of ericoid mycorrhizae within the Ericaceae. *Canadian Journal of Botany* 74, pp.1896-1909.
- Dahm H., Wrotniak W., Strzelczyk E., Li C.-Y. and Bednarska E., 2005. Diversity of culturable bacteria associated with fruiting bodies of ectomycorrhizal fungi. *Phytopathologia Polonica* 38, pp.51-62.
- Dai, C.C., Chen, Y., Tian, L.S. and Shi, Y., 2010. Correlation between invasion by endophytic fungus *Phomopsis* sp. and enzyme production. *African Journal of Agricultural Research*, 5(11), pp.1324-1340.

- Davitt, A.J., Stansberry, M. and Rudgers, J.A., 2010. Do the costs and benefits of fungal endophyte symbiosis vary with light availability?. *New Phytologist*, 188(3), pp.824-834.
- Dearnaley, J.D., Martos, F. and Selosse, M.A., 2012. 12 Orchid mycorrhizas: molecular ecology, physiology, evolution and conservation aspects. In *Fungal associations*, pp. 207-230. Springer, Berlin, Heidelberg.
- Dearnaley J.W.D, Perotto S, Selosse M.A. 2016. Structure and development of orchid mycorrhizas. In: Martin F, ed. *Molecular mycorrhizal symbiosis*. Hoboken, NJ, USA: John Wiley and Sons, pp.63–86.
- De Deyn, G. B., Cornelissen, J. H., and Bardgett, R. D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecology letters*, 11(5), pp.516-531.
- De La Peña, E., Echeverría, S.R., Van Der Putten, W.H., Freitas, H. and Moens, M., 2006. Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*. *New Phytologist*, 169(4), pp.829-840.
- Delaux, P.M., 2017. Comparative phylogenomics of symbiotic associations. *New Phytologist*, 213(1), pp.89-94.
- Delaux, P.M., Radhakrishnan, G.V., Jayaraman, D., Cheema, J., Malbreil, M., Volkening, J.D., Sekimoto, H., Nishiyama, T., Melkonian, M., Pokorny, L. and Rothfels, C.J., 2015. Algal ancestor of land plants was preadapted for symbiosis. *Proceedings of the National Academy of Sciences*, 112(43), pp.13390-13395.
- Deshmukh, S.K., Verekar, S.A. and Bhave, S.V., 2015. Endophytic fungi: a reservoir of antibacterials. *Frontiers in microbiology*, 5, p.715.
- Deslippe, J.R. and Simard, S.W., 2011. Below-ground carbon transfer among *Betula nana* may increase with warming in Arctic tundra. *New Phytologist*, 192(3), pp.689-698.
- Díaz, S., and Cabido, M., 2001. Vive la difference: plant functional diversity matters to ecosystem processes. *Trends in Ecology and Evolution*, 16(11), pp.646-655.
- Dickie, I.A., Guza, R.C., Krazewski, S.E. and Reich, P.B., 2004. Shared ectomycorrhizal fungi between a herbaceous perennial (*Helianthemum bicknellii*) and oak (*Quercus*) seedlings. *New Phytologist*, 164(2), pp.375-382.

- Dighton, J., and Mason, P. A., 1985. Mycorrhizal dynamics during forest tree development, in: *Developmental Biology of Higher Fungi*, pp.117-139. Eds. D. Moore, L. Casselton, D. A. Wood and J. C. Frankland. British Mycological Society Symposium. Cambridge University Press, London.
- DiTommaso, A. and Aarssen, L.W., 1989. Resource manipulations in natural vegetation: a review. *Vegetatio*, 84(1), pp.9-29.
- Dixon, R. K., Solomon, A. M., Brown, S., Houghton, R. A., Trexler, M. C., and Wisniewski, J., 1994. Carbon pools and flux of global forest ecosystems. *Science*, 263(5144), pp.185-190.
- Doim, H., Takahashim, M., and Katanom, I., 2010. Genetic diversity increases regional variation in phenological dates in response to climate change. *Global Change Biology*, 16(1), pp.373-379.
- Douglas, A.E., 1994. *Symbiotic interactions* (No. 577.85 D733s). Oxon, GB: Oxford University Press, 1994.
- Dosskey, M. G., Linderman, R. G., and Boersma, L., 1990. Carbon-sink stimulation of photosynthesis in Douglas fir seedlings by some ectomycorrhizas. *New Phytologist*, 115(2), pp.269-274.
- Dreyfuss, M.M. and Chapela, I.H., 1994. Potential of fungi in the discovery of novel, low-molecular weight pharmaceuticals. In *Discovery of Novel Natural Products with Therapeutic Potential*, pp. 49-80. Newnes.
- Drigo, B., Pijl, A.S., Duyts, H., Kielak, A.M., Gamper, H.A., Houtekamer, M.J., Boschker, H.T., Bodelier, P.L., Whiteley, A.S., Van Veen, J.A. and Kowalchuk, G.A., 2010. Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO₂. *Proceedings of the National Academy of Sciences*, 107(24), pp.10938-10942.
- Drinkwater, L. E., Wagoner, P., and Sarrantonio, M., 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. *Nature*, 396(6708), pp.262-265.
- Dungait, J.A., Hopkins, D.W., Gregory, A.S. and Whitmore, A.P., 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. *Global Change Biology*, 18(6), pp.1781-1796.

- Durall, D.M., Jones, M.D. and Tinker, P.B., 1994. Allocation of ^{14}C -carbon in ectomycorrhizal willow. *New Phytologist*, 128(1), pp.109-114.
- Eason, W.R. and Newman, E.I., 1990. Rapid cycling of nitrogen and phosphorus from dying roots of *Lolium perenne*. *Oecologia*, 82(4), pp.432-436.
- Egerton-Warburton, L.M., Querejeta, J.I. and Allen, M.F., 2007. Common mycorrhizal networks provide a potential pathway for the transfer of hydraulically lifted water between plants. *Journal of Experimental Botany*, 58(6), pp.1473-1483.
- Eissenstat, D.M., 1990. A comparison of phosphorus and nitrogen transfer between plants of different phosphorus status. *Oecologia*, 82(3), pp.342-347.
- Ekblad, A., Wallander, H., Godbold, D.L., Cruz, C., Johnson, D., Baldrian, P., Björk, R.G., Epron, D., Kieliszewska-Rokicka, B., Kjoller, R. and Kraigher, H., 2013. The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling. *Plant and Soil*, 366(1-2), pp.1-27.
- Farrar, J., Hawes, M., Jones, D., and Lindow, S., 2003. How roots control the flux of carbon to the rhizosphere. *Ecology*, 84(4), 827-837.
- Field, C. B., Jackson, R. B., and Mooney, H. A., 1995. Stomatal responses to increased CO_2 : implications from the plant to the global scale. *Plant, Cell and Environment*, 18(10), 1214-1225.
- Field, K.J., Leake, J.R., Tille, S., Allinson, K.E., Rimington, W.R., Bidartondo, M.I., Beerling, D.J. and Cameron, D.D., 2015. From mycoheterotrophy to mutualism: mycorrhizal specificity and functioning in *Ophioglossum vulgatum* sporophytes. *New Phytologist*, 205(4), pp.1492-1502.
- Finlay, R.D. and Read, D.J., 1986. The structure and function of the vegetative mycelium of ectomycorrhizal plants: I. Translocation of ^{14}C -labelled carbon between plants interconnected by a common mycelium. *New Phytologist*, 103(1), pp.143-156.
- Finlay, R.D. and Read, D.J., 1986. The structure and function of the vegetative mycelium of ectomycorrhizal plants: II. The uptake and distribution of phosphorus by mycelial strands interconnecting host plants. *New Phytologist*, 103(1), pp.157-165.
- Fleming, L. V., 1984. Effects of soil trenching and coring on the formation of ectomycorrhizas on birch seedlings grown around mature trees. *New Phytologist*, 98(1), 143-153.

- Flemming, H.C. and Wuerztz, S., 2019. Bacteria and archaea on Earth and their abundance in biofilms. *Nature Reviews Microbiology*, 17(4), p.247.
- Fogel, R., and Hunt, G., 1983. Contribution of mycorrhizae and soil fungi to nutrient cycling in a Douglas-fir ecosystem. *Canadian Journal of Forest Research*, 13(2), 219-232.
- Forestry Commission. 2018. *Forestry Statistics 2018: A Compendium of Statistics about Woodland, Forestry and Primary. Wood Processing in the United Kingdom*. Dandy Booksellers Limited.
- Forrester, D. I., 2014. The spatial and temporal dynamics of species interactions in mixed-species forests: from pattern to process. *Forest Ecology and Management*, 312, 282-292.
- Francis, R. and Read, D.J., 1984. Direct transfer of carbon between plants connected by vesicular–arbuscular mycorrhizal mycelium. *Nature*, 307(5946), p.53.
- Frank, B., 2005. On the nutritional dependence of certain trees on root symbiosis with belowground fungi (an English translation of AB Frank’s classic paper of 1885). *Mycorrhiza*, 15(4), pp.267-275.
- Frankland, J. C. 1998. Fungal succession—unravelling the unpredictable. *Mycological Research*, 102(1), pp.1-15.
- Frankland, J. C., 1992. Mechanisms in fungal succession. *The Fungal Community: its organization and role in the ecosystem*, 2, pp.383-401.
- Fraser, E.C., Lieffers, V.J. and Landhäusser, S.M., 2006. Carbohydrate transfer through root grafts to support shaded trees. *Tree physiology*, 26(8), pp.1019-1023.
- Frey, S. D., Elliott, E. T., and Paustian, K. 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. *Soil Biology and Biochemistry*, 31(4), pp.573-585.
- Friese, C.F. and Allen, M.F., 1991. The spread of VA mycorrhizal fungal hyphae in the soil: inoculum types and external hyphal architecture. *Mycologia*, 83(4), pp.409-418.
- Gaby, J.C. and Buckley, D.H., 2011. A global census of nitrogenase diversity. *Environmental Microbiology*, 13(7), pp.1790-1799.

- Gai, J.P., Christie, P., Cai, X.B., Fan, J.Q., Zhang, J.L., Feng, G. and Li, X.L., 2009. Occurrence and distribution of arbuscular mycorrhizal fungal species in three types of grassland community of the Tibetan Plateau. *Ecological Research*, 24(6), p.1345.
- Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A. and Karl, D.M., 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry*, 70(2), pp.153-226.
- Garbelotto, M., and Pautasso, M., 2012. Impacts of exotic forest pathogens on Mediterranean ecosystems: four case studies. *European journal of plant pathology*, 133(1), pp.101-116.
- Geddes, B.A., Ryu, M.H., Mus, F., Costas, A.G., Peters, J.W., Voigt, C.A. and Poole, P., 2015. Use of plant colonizing bacteria as chassis for transfer of N₂-fixation to cereals. *Current opinion in biotechnology*, 32, pp.216-222.
- Gerz, M., Bueno, C.G., Zobel, M. and Moora, M., 2016. Plant community mycorrhization in temperate forests and grasslands: relations with edaphic properties and plant diversity. *Journal of vegetation science*, 27(1), pp.89-99.
- Gherbi, H., Markmann, K., Svistoonoff, S., Estevan, J., Autran, D., Giczey, G., Auguy, F., Péret, B., Laplaze, L., Franche, C. and Parniske, M., 2008. SymRK defines a common genetic basis for plant root endosymbioses with arbuscular mycorrhiza fungi, rhizobia, and Frankiabacteria. *Proceedings of the National Academy of Sciences*, 105(12), pp.4928-4932.
- Giovannetti, M., Avio, L., Fortuna, P., Pellegrino, E., Sbrana, C. and Strani, P., 2006. At the root of the wood wide web: self-recognition and nonself incompatibility in mycorrhizal networks. *Plant signaling and behavior*, 1(1), pp.1-5.
- Giovannetti, M., Sbrana, C., Avio, L. and Strani, P., 2004. Patterns of below-ground plant interconnections established by means of arbuscular mycorrhizal networks. *New Phytologist*, 164(1), pp.175-181.
- Godbold, D.L., Hoosbeek, M.R., Lukac, M., Cotrufo, M.F., Janssens, I.A., Ceulemans, R., Polle, A., Velthorst, E.J., Scarascia-Mugnozza, G., De Angelis, P. and Miglietta, F., 2006. Mycorrhizal hyphal turnover as a dominant process for carbon input into soil organic matter. *Plant and Soil*, 281(1-2), pp.15-24.

- Gond, V., de Pury, D.G., Veroustraete, F. and Ceulemans, R., 1999. Seasonal variations in leaf area index, leaf chlorophyll, and water content; scaling-up to estimate fAPAR and carbon balance in a multilayer, multispecies temperate forest. *Tree physiology*, 19(10), pp.673-679.
- Guggenberger, G., Frey, S. D., Six, J., Paustian, K., and Elliott, E. T., 1999. Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. *Soil Science Society of America Journal*, 63(5), pp.1188-1198.
- Guerrieri, E., Lingua, G., Digilio, M.C., Massa, N. and Berta, G., 2004. Do interactions between plant roots and the rhizosphere affect parasitoid behaviour?. *Ecological Entomology*, 29(6), pp.753-756.
- Guo, D., Xia, M., Wei, X., Chang, W., Liu, Y. and Wang, Z., 2008. Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. *New Phytologist*, 180(3), pp.673-683.
- Guo, L.B. and Gifford, R.M., 2002. Soil carbon stocks and land use change: a meta-analysis. *Global change biology*, 8(4), pp.345-360.
- Gray, E.J. and Smith, D.L., 2005. Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. *Soil Biology and Biochemistry*, 37(3), pp.395-412.
- Grayston, S. J., Wang, S., Campbell, C. D., and Edwards, A. C., 1998. Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biology and Biochemistry*, 30(3), pp.369-378.
- Grayston, S. J., Vaughan, D., and Jones, D., 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. *Applied soil ecology*, 5(1), pp.29-56.
- Grayston, S. J., Wang, S., Campbell, C. D., and Edwards, A. C., 1998. Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biology and Biochemistry*, 30(3), pp.369-378.
- Grime, J.P., Mackey, J.M.L., Hillier, S.H. and Read, D.J., 1987. Floristic diversity in a model system using experimental microcosms. *Nature*, 328(6129), p.420.
- Gruber, N. and Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen cycle. *Nature*, 451(7176), p.293-296.

- Gyuricza, V., Thiry, Y., Wannijn, J., Declerck, S. and Dupré de Boulois, H., 2010. Radiocesium transfer between *Medicago truncatula* plants via a common mycorrhizal network. *Environmental microbiology*, 12(8), pp.2180-2189.
- Hackl, E., Zechmeister-Boltenstern, S., Bodrossy, L., and Sessitsch, A., 2004. Comparison of diversities and compositions of bacterial populations inhabiting natural forest soils. *Applied and Environmental Microbiology*, 70(9), pp.5057-5065.
- Hagen-Thorn, A., Callesen, I., Armolaitis, K., and Nihlgård, B., 2004. The impact of six European tree species on the chemistry of mineral topsoil in forest plantations on former agricultural land. *Forest Ecology and Management*, 195(3), pp.373-384.
- Hansen, K., Vesterdal, L., Schmidt, I. K., Gundersen, P., Sevel, L., Bastrup-Birk, A., Pedersen, L. B., and Bille-Hansen, J., 2009. Litterfall and nutrient return in five tree species in a common garden experiment. *Forest Ecology and Management*, 257(10), pp.2133-2144.
- Hardoim, P.R., Van Overbeek, L.S., Berg, G., Pirttilä, A.M., Compant, S., Campisano, A., Döring, M. and Sessitsch, A., 2015. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews.*, 79(3), pp.293-320.
- Harley, J.L. and Harley, E.L., 1987. A check-list of mycorrhiza in the British flora. *New Phytologist*, 105, pp.1-102.
- Harper, J. L. 1977. *Population biology of plants*. Academic Press, London, UK.
- Harris, D., Pacovsky, R. S., and Paul, E. A., 1985. Carbon economy of soybean–Rhizobium–Glomus associations. *New Phytologist*, 101(3), pp.427-440.
- Harris, D. and Paul, E. A., 1987. Carbon requirements of vesicular-arbuscular mycorrhizas. In: *Ecophysiology of VA Mycorrhizal Plants* (Ed. by G. R. Safir), pp. 93–105. CRC Press. Boca Raton, Florida.
- Harris, W. F., Santantonio, D., and McGinty, D., 1980. The Dynamic Belowground Ecosystem1. In *Forests, Fresh Perspectives from Ecosystem Analysis: Proceedings of the 40th Annual Biology Colloquium, 1979*, 40, p. 119. Oregon State University Press.
- Harrison, M. J., 2005. Signaling in the arbuscular mycorrhizal symbiosis. *Annual Review Microbiology*, 59, pp.19-42.

- Hart, M. M., Gorzelak, M., Ragone, D., and Murch, S. J., 2014. Arbuscular mycorrhizal fungal succession in a long-lived perennial 1. *Botany*, 92(4), pp.313-320.
- Hatton, P.J., Castanha, C., Torn, M.S. and Bird, J.A., 2015. Litter type control on soil C and N stabilization dynamics in a temperate forest. *Global change biology*, 21(3), pp.1358-1367.
- Hawksworth, D. L., 2001. The magnitude of fungal diversity: the 1· 5 million species estimate revisited. *Mycological research*, 105(12), pp.1422-1432.
- Heckman, D.S., Geiser, D.M., Eidell, B.R., Stauffer, R.L., Kardos, N.L. and Hedges, S.B., 2001. Molecular evidence for the early colonization of land by fungi and plants. *Science*, 293(5532), pp.1129-1133.
- Heil, M. and Karban, R., 2010. Explaining evolution of plant communication by airborne signals. *Trends in ecology and evolution*, 25(3), pp.137-144.
- Heinonsalo, J., and Sen, R., 2007. Scots pine ectomycorrhizal fungal inoculum potential and dynamics in podzol-specific humus, eluvial and illuvial horizons one and four growth seasons after forest clear-cut logging. *Canadian journal of forest research*, 37(2), pp.404-414.
- Helal, H. M., and Sauerbeck, D., 1989. Carbon turnover in the rhizosphere. *Zeitschrift für Pflanzenernährung und Bodenkunde*, 152(2), pp.211-216.
- Hendricks, J. J., Mitchell, R. J., Kuehn, K. A., Pecot, S. D., and Sims, S. E., 2006. Measuring external mycelia production of ectomycorrhizal fungi in the field: the soil matrix matters. *New Phytologist*, 171(1), pp.179-186.
- Hibbett, D.S. and Matheny, P.B., 2009. The relative ages of ectomycorrhizal mushrooms and their plant hosts estimated using Bayesian relaxed molecular clock analyses. *BMC biology*, 7(1), p.13.
- Hillel, D., 1998. Environmental soil physics: Fundamentals, applications, and environmental considerations. Elsevier. London.
- Hirrel, M.C. and Gerdemann, J.W., 1979. Enhanced carbon transfer between onions infected with a vesicular-arbuscular mycorrhizal fungus. *New Phytologist*, 83(3), pp.731-738.
- Hirsch, A.M., 2004. Plant-microbe symbioses: a continuum from commensalism to parasitism. Pp. 345-363.

- Hobbie, E.A., 2006. Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. *Ecology*, 87(3), pp.563-569.
- Hobbie, S. E., Reich, P. B., Oleksyn, J., Ogdahl, M., Zytowskiak, R., Hale, C., and Karolewski, P., 2006. Tree species effects on decomposition and forest floor dynamics in a common garden. *Ecology*, 87(9), pp.2288-2297.
- Hodges, T., 1990. Predicting crop phenology. CRC Press, Boston, MA.
- Hoffland, E., 1992. Quantitative evaluation of the role of organic acid exudation in the mobilization of rock phosphate by rape. *Plant and Soil*, 140(2), pp.279-289.
- Högberg, M.N., Högberg, P. and Myrold, D.D., 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three?. *Oecologia*, 150(4), pp.590-601.
- Högberg, M.N. and Högberg, P., 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytologist*, 154(3), pp.791-795.
- Högberg, P., and Read, D. J., 2006. Towards a more plant physiological perspective on soil ecology. *Trends in Ecology and Evolution*, 21(10), pp.548-554.
- Holland, E.A. and Coleman, D.C., 1987. Litter placement effects on microbial and organic matter dynamics in an agroecosystem. *Ecology*, 68(2), pp.425-433.
- Holmer, R., Rutten, L., Kohlen, W., van Velzen, R. and Geurts, R., 2017. Commonalities in Symbiotic Plant-Microbe Signalling. In *Advances in Botanical Research* (Vol. 82, pp. 187-221). Academic Press. London.
- Hoosbeek, M. R., Lukac, M., Velthorst, E., Smith, A. R., and Godbold, D. L., 2011. Free atmospheric CO₂ enrichment increased above ground biomass but did not affect symbiotic N₂-fixation and soil carbon dynamics in a mixed deciduous stand in Wales. *Biogeosciences*, 8(2), pp.353-364.
- Horton, T.R. and Bruns, T.D., 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular ecology*, 10(8), pp.1855-1871.
- Houghton, R.A. and Nassikas, A.A., 2018. Negative emissions from stopping deforestation and forest degradation, globally. *Global change biology*, 24(1), pp.350-359.

- Hulvey, K. B., Hobbs, R. J., Standish, R. J., Lindenmayer, D. B., Lach, L., and Perring, M. P. 2013. Benefits of tree mixes in carbon plantings. *Nature Climate Change*, 3(10), pp.869-874.
- Huston, M. A., 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia*, 110(4), pp.449-460.
- Inselsbacher, E., and Näsholm, T., 2012. The below-ground perspective of forest plants: soil provides mainly organic nitrogen for plants and mycorrhizal fungi. *New Phytologist*, 195(2), pp.329-334.
- Iverson, L. R., Prasad, A. M., Matthews, S. N., and Peters, M. 2008. Estimating potential habitat for 134 eastern US tree species under six climate scenarios. *Forest Ecology and Management*, 254(3), pp.390-406
- Jakobsen, I. 1995. Transport of phosphorus and carbon in VA mycorrhizas. In A Varma, B Hock, eds, *Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology*. Springer Verlag, Berlin, pp 297–323.
- Jandl, R., Lindner, M., Vesterdal, L., Bauwens, B., Baritz, R., Hagedorn, F., Johnson, D.W., Minkinen, K. and Byrne, K. A., 2007. How strongly can forest management influence soil carbon sequestration?. *Geoderma*, 137(3), pp.253-268.
- Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G. and Papale, D., 2010. Reduction of forest soil respiration in response to nitrogen deposition. *Nature geoscience*, 3(5), pp.315-322.
- Jenkinson, H.F. and Lamont, R.J., 2005. Oral microbial communities in sickness and in health. *Trends in microbiology*, 13(12), pp.589-595.
- Jenny, H., 2012. *The soil resource: origin and behavior* (Vol. 37). Springer Science and Business Media.
- Jin, H., Pfeffer, P.E., Douds, D.D., Piotrowski, E., Lammers, P.J. and Shachar-Hill, Y., 2005. The uptake, metabolism, transport and transfer of nitrogen in an arbuscular mycorrhizal symbiosis. *New Phytologist*, 168(3), pp.687-696.
- Jobbágy, E. G., and Jackson, R. B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological applications*, 10(2), pp.423-436.

- Johnson, D. and Gilbert, L., 2015. Interplant signalling through hyphal networks. *New Phytologist*, 205(4), pp.1448-1453.
- Johnston, P.R., Sutherland, P.W. and Joshee, S., 2006. Visualising endophytic fungi within leaves by detection of (1→3)-β-d-glucans in fungal cell walls. *Mycologist*, 20(4), pp.159-162.
- Jones, C.G., Lawton, J.H. and Shachak, M., 1997. Positive and negative effects of organisms as physical ecosystem engineers. *Ecology*, 78(7), pp.1946-1957.
- Jones, D. L., and Darrah, P. R., 1994. Amino-acid influx at the soil-root interface of *Zea mays* L. and its implications in the rhizosphere. *Plant and Soil*, 163(1), pp.1-12.
- Jones, D. L., and Darrah, P. R., 1995. Influx and efflux of organic acids across the soil-root interface of *Zea mays* L. and its implications in rhizosphere C flow. *Plant and Soil*, 173(1), pp.103-109.
- Jones, D.L., Hodge, A. and Kuzyakov, Y., 2004. Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist*, 163(3), pp.459-480.
- Jones, M. D., Durall, D. M., and Cairney, J. W., 2003. Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. *New Phytologist*, 157(3), pp.399-422.
- Jones, D.L., Nguyen, C. and Finlay, R.D., 2009. Carbon flow in the rhizosphere: carbon trading at the soil–root interface. *Plant and Soil*, 321(1-2), pp.5-33.
- Jones, J.D. and Dangl, J.L., 2006. The plant immune system. *Nature*, 444(7117), p.323.
- Jose, S., 2002. Black walnut allelopathy: current state of the science. In *Chemical ecology of plants: Allelopathy in aquatic and terrestrial ecosystems* (pp. 149-172). Birkhäuser, Basel.
- Kamel, L., Keller-Pearson, M., Roux, C. and Ané, J.M., 2017. Biology and evolution of arbuscular mycorrhizal symbiosis in the light of genomics. *New Phytologist*, 213(2), pp.531-536.
- Kasel, S., Singh, S., Sanders, G. J., and Bennett, L. T., 2011. Species-specific effects of native trees on soil organic carbon in biodiverse plantings across north-central Victoria, Australia. *Geoderma*, 161(1), pp.95-106.
- Keiluweit, M., Wanzek, T., Kleber, M., Nico, P. and Fendorf, S., 2017. Anaerobic microsites have an unaccounted role in soil carbon stabilization. *Nature communications*, 8(1), p.1771.

- Kellner, H., Luis, P., Pecyna, M.J., Barbi, F., Kapturska, D., Krüger, D., Zak, D.R., Marmeisse, R., Vandenbol, M. and Hofrichter, M., 2014. Widespread occurrence of expressed fungal secretory peroxidases in forest soils. *PLoS One*, 9(4), p.e95557.
- Kelty, M. J., 2006. The role of species mixtures in plantation forestry. *Forest Ecology and Management*, 233(2), 195-204.
- King, R. F., Dromph, K. M., and Bardgett, R. D., 2002. Changes in species evenness of litter have no effect on decomposition processes. *Soil Biology and Biochemistry*, 34(12), 1959-1963.
- Klein, T., Siegwolf, R.T. and Körner, C., 2016. Belowground carbon trade among tall trees in a temperate forest. *Science*, 352(6283), pp.342-344.
- Koch, K. E., and Johnson, C. R., 1984. Photosynthate partitioning in split-root citrus seedlings with mycorrhizal and nonmycorrhizal root systems. *Plant Physiology*, 75(1), pp.26-30.
- Koele, N., Dickie, I. A., Blum, J. D., Gleason, J. D., and de Graaf, L., 2014. Ecological significance of mineral weathering in ectomycorrhizal and arbuscular mycorrhizal ecosystems from a field-based comparison. *Soil Biology and Biochemistry*, 69, pp.63-70.
- Koerselman, W., and Verhoeven, J. T. A., 1992. Nutrient dynamics in mires of various trophic status: nutrient inputs and outputs and the internal nutrient cycle. In *Fens and bogs in the Netherlands* (pp. 397-432). Springer Netherlands.
- Kohler, A., Kuo, A., Nagy, L.G., Morin, E., Barry, K.W., Buscot, F., Canbäck, B., Choi, C., Cichocki, N., Clum, A. and Colpaert, J., 2015. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature genetics*, 47(4), p.410.
- Köppen, W., 1884. Die Wärmezonen der Erde, nach der Dauer der heissen, gemässigten und kalten Zeit und nach der Wirkung der Wärme auf die organische Welt betrachtet. *Meteorologische Zeitschrift*, 1(21), pp.5-226.
- Kottek, M., Grieser, J., Beck, C., Rudolf, B. and Rubel, F., 2006. World map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift*, 15(3), pp.259-263.
- Koven, C.D., Riley, W.J., Subin, Z.M., Tang, J.Y., Torn, M.S., Collins, W.D., Bonan, G.B., Lawrence, D.M. and Swenson, S.C., 2013. The effect of vertically resolved soil biogeochemistry and alternate soil C and N models on C dynamics of CLM4. *Biogeosciences*, 10(11), pp.7109-7131.

- Kowalchuk, G. A., Buma, D. S., de Boer, W., Klinkhamer, P. G., and van Veen, J. A., 2002. Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. *Antonie van Leeuwenhoek*, 81(1-4), pp.509-520.
- Krause, H.H., Weetman, G.F., Koller, E. and Veilleux, J.M., 1982. Interprovincial forest fertilization program. Results of five-year growth remeasurements. *Information Report, Canadian Forestry Service*, (DPC-X-12), p.23.
- Kuzyakov, Y., 2002. Review: factors affecting rhizosphere priming effects. *Journal of Plant Nutrition and Soil Science*, 165(4), p.382.
- Ladygina, N., and Hedlund, K., 2010. Plant species influence microbial diversity and carbon allocation in the rhizosphere. *Soil Biology and Biochemistry*, 42(2), pp.162-168.
- Lai, Z., Zhang, Y., Liu, J., Wu, B., Qin, S. and Fa, K., 2016. Fine-root distribution, production, decomposition, and effect on soil organic carbon of three revegetation shrub species in northwest China. *Forest Ecology and Management*, 359, pp.381-388.
- Lal, R., 2005. Forest soils and carbon sequestration. *Forest ecology and management*, 220(1-3), pp.242-258.
- Lambers, H., 1987. Growth, respiration, exudation and symbiotic associations: The fate of carbon translocated to the roots. In: *Root Development and Function—Effects of the Physical Environment* (Ed. by P.J. Gregory, J.V. Lake and D.A. Rose), pp.125–145. Cambridge University Press, Cambridge.
- Landeweert, R., Hoffland, E., Finlay, R.D., Kuyper, T.W. and van Breemen, N., 2001. Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. *Trends in Ecology and Evolution*, 16(5), pp.248-254.
- Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., Mellado-Vázquez, P.G., Malik, A.A., Roy, J., Scheu, S. and Steinbeiss, S., 2015. Plant diversity increases soil microbial activity and soil carbon storage. *Nature communications*, 6, p.6707
- Leadley, P. W., Reynolds, J. F., and Chapin III, F. S., 1997. A model of nitrogen uptake by *Eriophorum vaginatum* roots in the field: ecological implications. *Ecological Monographs*, 67(1), pp.1-22.
- Leake, J. R., Duran, A. L., Hardy, K. E., Johnson, I., Beerling, D. J., Banwart, S. A., and Smits, M. M., 2008. Biological weathering in soil: the role of symbiotic root-associated fungi

biosensing minerals and directing photosynthate-energy into grain-scale mineral weathering. *Mineralogical Magazine*, 72(1), pp.85-89.

Lehto, T., 1992. Mycorrhizas and drought resistance of *Picea sitchensis* (Bong.) Carr. *New Phytologist*, 122(4), pp.661-668.

Lee, K. E., and Pankhurst, C. E., 1992. Soil organisms and sustainable productivity. *Soil Research*, 30(6), pp.855-892.

Leininger, S., Urich, T., Schlöter, M., Schwark, L., Qi, J., Nicol, G. W., Prosser, J. I., Schuster, S. C. and Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature*, 442(7104), pp.806-809.

Lemma, B., Kleja, D. B., Nilsson, I., and Olsson, M., 2006. Soil carbon sequestration under different exotic tree species in the southwestern highlands of Ethiopia. *Geoderma*, 136(3), pp.886-898.

Lepš, J., Brown, V.K., Len, D., Tomas, A., Gormsen, D., Hedlund, K., Kailová, J., Korthals, G.W., Mortimer, S.R., Rodriguez-Barrueco, C. and Roy, J., 2001. Separating the chance effect from other diversity effects in the functioning of plant communities. *Oikos*, 92(1), pp.123-134.

Leung, T.L.F. and Poulin, R., 2008. Parasitism, commensalism, and mutualism: exploring the many shades of symbioses. *Vie et Milieu*, 58(2), p.107.

Leuschner, C., Wulf, M., Bäuchler, P., and Hertel, D., 2013. Soil C and nutrient stores under Scots pine afforestations compared to ancient beech forests in the German Pleistocene: The role of tree species and forest history. *Forest Ecology and Management*, 310, pp.405-415.

Lewis, J., Thomas, R. B., and Strain, B. R., 1995. Effect of elevated CO₂ on mycorrhizal colonization of loblolly pine (*Pinus taeda* L.) seedlings. In *Belowground Responses to Rising Atmospheric CO₂: Implications for Plants, Soil Biota, and Ecosystem Processes* (pp. 81-88). Springer Netherlands.

Li, H., Smith, S. E., Holloway, R. E., Zhu, Y., and Smith, F. A., 2006. Arbuscular mycorrhizal fungi contribute to phosphorus uptake by wheat grown in a phosphorus-fixing soil even in the absence of positive growth responses. *New Phytologist*, 172(3), pp.536-543.

Liang, S., Hurteau, M.D. and Westerling, A.L., 2017. Potential decline in carbon carrying capacity under projected climate-wildfire interactions in the Sierra Nevada. *Scientific reports*, 7(1), p.2420.

- Lin, G., McCormack, M.L., Ma, C. and Guo, D., 2017. Similar below-ground carbon cycling dynamics but contrasting modes of nitrogen cycling between arbuscular mycorrhizal and ectomycorrhizal forests. *New Phytologist*, 213(3), pp.1440-1451.
- Lindner, M., Fitzgerald, J. B., Zimmermann, N. E., Reyer, C., Delzon, S., van der Maaten, E., Schelhass, M-J., Lasch, P., Eggers, J., van der Maaten-Theunissen, M., Suckow, F., Psomas, A., Poulter, B., and Hanewinkel, M., 2014. Climate change and European forests: What do we know, what are the uncertainties, and what are the implications for forest management? *Journal of environmental management*, 146, pp.69-83.
- Linehan, D. J., Sinclair, A. H., and Mitchell, M. C., 1985. Mobilisation of Cu, Mn and Zn in the soil solutions of barley rhizospheres. *Plant and Soil*, 86(1), pp.147-149.
- Lladó, S., López-Mondéjar, R. and Baldrian, P., 2018. Drivers of microbial community structure in forest soils. *Applied microbiology and biotechnology*, 102(10), pp.4331-4338.
- Loreau, M., and Hector, A., 2001. Partitioning selection and complementarity in biodiversity experiments. *Nature*, 412(6842), pp.72-76.
- Loreau, M., Naeem, S., and Inchausti, P., 2002. Biodiversity and ecosystem functioning. *Synthesis and Perspectives*. Oxford University Press on Demand.
- Loron, C.C., François, C., Rainbird, R.H., Turner, E.C., Borensztajn, S. and Javaux, E.J., 2019. Early fungi from the Proterozoic era in Arctic Canada. *Nature*, p.1.
- Lundberg, D.S., Lebeis, S.L., Paredes, S.H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., Del Rio, T.G. and Edgar, R.C., 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature*, 488(7409), p.86.
- Luo, Y., Ahlström, A., Allison, S.D., Batjes, N.H., Brovkin, V., Carvalhais, N., Chappell, A., Ciais, P., Davidson, E.A., Finzi, A. and Georgiou, K., 2016. Toward more realistic projections of soil carbon dynamics by Earth system models. *Global Biogeochemical Cycles*, 30(1), pp.40-56.
- Lynch, H. B., Epps, K. Y., Fukami, T., and Vitousek, P. M., 2012. Introduced canopy tree species effect on the soil microbial community in a montane tropical forest. *Pacific Science*, 66(2), pp.141-150.

- Mangan, S.A., Herre, E.A. and Bever, J.D., 2010. Specificity between Neotropical tree seedlings and their fungal mutualists leads to plant–soil feedback. *Ecology*, 91(9), pp.2594-2603.
- Marschner, B., Brodowski, S., Dreves, A., Gleixner, G., Gude, A., Grootes, P.M., Hamer, U., Heim, A., Jandl, G., Ji, R. and Kaiser, K., 2008. How relevant is recalcitrance for the stabilization of organic matter in soils?. *Journal of plant nutrition and soil science*, 171(1), pp.91-110.
- Margulis L. 1981. *Symbiosis in Cell Evolution: Life and Its Environment on the Early Earth*. San Francisco: Freeman
- Martin, B.D. and Schwab, E., 2012. Current usage of symbiosis and associated terminology. *International Journal of Biology*, 5(1), p.32.
- Martin, F., Kohler, A. and Duplessis, S., 2007. Living in harmony in the wood underground: ectomycorrhizal genomics. *Current Opinion in Plant Biology*, 10(2), pp.204-210.
- Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C. and Hibbett, D.S., 2016. Unearthing the roots of ectomycorrhizal symbioses. *Nature Reviews Microbiology*, 14(12), p.760.
- Martin, F.M., Uroz, S. and Barker, D.G., 2017. Ancestral alliances: plant mutualistic symbioses with fungi and bacteria. *Science*, 356(6340), p. eaad4501
- Mason, E. G., Manley, B. R. and Sands, R., 2013. Forestry and climate change. *Forestry in a global context*, (Ed. 2), pp.149-157.
- Mason, W.L. and Connolly, T., 2013. Mixtures with spruce species can be more productive than monocultures: evidence from the Gisburn experiment in Britain. *Forestry*, 87(2), pp.209-217.
- Mason, W. L., 2007. Changes in the management of British forests between 1945 and 2000 and possible future trends. *Ibis*, 149(s2), pp.41-52.
- Mayfield, M. M., Bonser, S. P., Morgan, J. W., Aubin, I., McNamara, S., and Vesk, P. A., 2010. What does species richness tell us about functional trait diversity? Predictions and evidence for responses of species and functional trait diversity to land-use change. *Global Ecology and Biogeography*, 19(4), pp.423-431.

- McCormick, M.K., Whigham, D.F. and Canchani-Viruet, A., 2018. Mycorrhizal fungi affect orchid distribution and population dynamics. *New Phytologist*, 219(4), pp.1207-1215.
- McGuire, A. D., Sitch, S., Clein J. S., Dargaville R., Esse, G., Foley, J., Heimann, M., Joos, F., Kaplan, J., Kicklighter, D. W., Meier, R. A., Melillo, J. M., Moore III, B., Prentice, I. C., Ramankutty, N., Reichenau, T., Schloss, A., Tian, H., Williams, L. J. and Wittenberg, U., 2001. Carbon balance of the terrestrial biosphere in the twentieth century: Analyses of CO₂, climate and land use effects with four process-based ecosystem models. *Global Biogeochemical Cycles*, 15(1), pp.183-206.
- McGuire, K. L., and Treseder, K. K., 2010. Microbial communities and their relevance for ecosystem models: decomposition as a case study. *Soil Biology and Biochemistry*, 42(4), pp.529-535.
- McIntire, E.J. and Fajardo, A., 2014. Facilitation as a ubiquitous driver of biodiversity. *New Phytologist*, 201(2), pp.403-416.
- Meding, S.M. and Zasoski, R.J., 2008. Hyphal-mediated transfer of nitrate, arsenic, cesium, rubidium, and strontium between arbuscular mycorrhizal forbs and grasses from a California oak woodland. *Soil Biology and Biochemistry*, 40(1), pp.126-134.
- Meharg, A.A. and Cairney, J.W., 1999. Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. In *Advances in Ecological Research* (Vol. 30, pp. 69-112). Academic Press. London.
- Mehrotra, V.S. ed., 2005. *Mycorrhiza: Role and Applications*/. Allied Publishers. New Delhi.
- Mello, A., Zampieri, E. and Balestrini, R., 2015. Ectomycorrhizal fungi and their applications. In *Plant Microbes Symbiosis: Applied Facets* (pp. 315-326). Springer, New Delhi.
- Mendes, R., Garbeva, P. and Raaijmakers, J.M., 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS microbiology reviews*, 37(5), pp.634-663.
- Mendes, R., Kruijt, M., De Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J.H., Piceno, Y.M., DeSantis, T.Z., Andersen, G.L., Bakker, P.A. and Raaijmakers, J.M., 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science*, p.1203980.

- Midgley, M.G. and Phillips, R.P., 2016. Resource stoichiometry and the biogeochemical consequences of nitrogen deposition in a mixed deciduous forest. *Ecology*, 97(12), pp.3369-3378.
- Mikutta, R., Kleber, M., Torn, M.S. and Jahn, R., 2006. Stabilization of soil organic matter: association with minerals or chemical recalcitrance?. *Biogeochemistry*, 77(1), pp.25-56.
- Miliute, I., Buzaitė, O., Baniulis, D. and Stanys, V., 2015. Bacterial endophytes in agricultural crops and their role in stress tolerance: a review. *Zemdirbyste-Agriculture*, 102(4), pp.465-478.
- Millar, C. I., Stephenson, N. L., and Stephens, S. L., 2007. Climate change and forests of the future: managing in the face of uncertainty. *Ecological applications*, 17(8), pp.2145-2151.
- Millard, P., 1996. Ecophysiology of the internal cycling of nitrogen for tree growth. *Zeitschrift für Pflanzenernährung und Bodenkunde*, pp.159 (1), 1-10.
- Minamisawa, K., Imaizumi-Anraku, H., Bao, Z., Shinoda, R., Okubo, T. and Ikeda, S., 2016. Are symbiotic methanotrophs key microbes for N acquisition in paddy rice root?. *Microbes and environments*, 31(1), pp.4-10.
- Moissl-Eichinger, C., Pausan, M., Taffner, J., Berg, G., Bang, C. and Schmitz, R.A., 2018. Archaea are interactive components of complex microbiomes. *Trends in microbiology*, 26(1), pp.70-85.
- Mooney, H. A., 1972. The carbon balance of plants. *Annual Review of Ecology and Systematics*, pp.315-346.
- Moore, D., Robson, G.D. and Trinci, A.P., 2011. *21st century guidebook to fungi with CD*. Cambridge University Press.
- Mousavi, S.A., Chauvin, A., Pascaud, F., Kellenberger, S. and Farmer, E.E., 2013. Glutamate receptor-like genes mediate leaf-to-leaf wound signalling. *Nature*, 500(7463), p.422.
- Moyes, A.B., Kueppers, L.M., Pett-Ridge, J., Carper, D.L., Vandehey, N., O'Neil, J. and Frank, A.C., 2016. Evidence for foliar endophytic nitrogen fixation in a widely distributed subalpine conifer. *New Phytologist*, 210(2), pp.657-668.
- Naeem, S., and Wright, J. P., 2003. Disentangling biodiversity effects on ecosystem functioning: deriving solutions to a seemingly insurmountable problem. *Ecology letters*, 6(6), pp.567-579.

- Nair, S., 2004. *Bacterial Associations: Antagonism to Symbiosis*. National Institute of Oceanography, Goa.
- Nara, K., 2006. Ectomycorrhizal networks and seedling establishment during early primary succession. *New Phytologist*, 169(1), pp.169-178.
- Newman, E.I. and Eason, W.R., 1993. Rates of phosphorus transfer within and between ryegrass (*Lolium perenne*) plants. *Functional Ecology*, pp.242-248.
- Newman, D.J., 2016. Predominately uncultured microbes as sources of bioactive agents. *Frontiers in microbiology*, 7, p.1832.
- Nico, M.K.P.S. and Fendorf, M.K.S., 2016. Are oxygen limitations under recognized regulators of organic carbon turnover in upland soils?. *Biogeochemistry*, 127(2-3), pp.157-171.
- Nilsson, S., and Schopfhauser, W., 1995. The carbon-sequestration potential of a global afforestation program. *Climatic change*, 30(3), pp.267-293.
- Norby, R. J., O'Neill, E. G., Hood, W. G., and Luxmoore, R. J., 1987. Carbon allocation, root exudation and mycorrhizal colonization of *Pinus echinata* seedlings grown under CO₂ enrichment. *Tree Physiology*, 3(3), pp.203-210.
- Norby, R.J. and Zak, D.R., 2011. Ecological lessons from free-air CO₂ enrichment (FACE) experiments. *Annual review of ecology, evolution, and systematics*, 42, pp.181-203.
- Noss, R. F., 2001. Beyond Kyoto: forest management in a time of rapid climate change. *Conservation Biology*, 15(3), pp.578-590.
- Ogle, H. and Brown, J., 1997. Plant-microbe symbioses. *Plant pathogens and plant diseases*. Rockvale Publications, pp.21-37.
- O'Neill, E. G., Luxmoore, R. J., and Norby, R. J., 1987. Increases in mycorrhizal colonization and seedling growth in *Pinus echinata* and *Quercus alba* in an enriched CO₂ atmosphere. *Canadian Journal of Forest Research*, 17(8), pp.878-883.
- Onguene, N. and Kuyper, T., 2002. Importance of the ectomycorrhizal network for seedling survival and ectomycorrhiza formation in rain forests of south Cameroon. *Mycorrhiza*, 12(1), pp.13-17.
- Oren, R., Ellsworth, D.S., Johnsen, K.H., Phillips, N., Ewers, B.E., Maier, C., Schäfer, K.V., McCarthy, H., Hendrey, G., McNulty, S.G. and Katul, G.G., 2001. Soil fertility limits carbon

sequestration by forest ecosystems in a CO₂-enriched atmosphere. *Nature*, 411(6836), pp.469-472.

Orwin, K.H., Kirschbaum, M.U., St John, M.G. and Dickie, I.A., 2011. Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: a model-based assessment. *Ecology Letters*, 14(5), pp.493-502.

Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G. and Ciais, P., 2011. A large and persistent carbon sink in the world's forests. *Science*, 333(6045), pp.988-993.

Paquette, A., and Messier, C., 2009. The role of plantations in managing the world's forests in the Anthropocene. *Frontiers in Ecology and the Environment*, 8(1), pp.27-34.

Paracer, S. and Ahmadjian, V., 2000. Symbiosis: an introduction to biological associations. Oxford University Press on Demand.

Pass, G., and Szucsich, N. U., 2011. 100 years of research on the Protura: many secrets still retained. *Soil Organisms*, 83(3), pp.309-334.

Pent, M., Pöldmaa, K. and Bahram, M., 2017. Bacterial communities in boreal forest mushrooms are shaped both by soil parameters and host identity. *Frontiers in microbiology*, 8, p.836.

Pérez-Cruzado, C., Mansilla-Salinero, P., Rodríguez-Soalleiro, R., and Merino, A., 2012. Influence of tree species on carbon sequestration in afforested pastures in a humid temperate region. *Plant and soil*, 353(1-2), pp.333-353.

Persson, H., 1978. Root dynamics in a young Scots pine stand in central Sweden. *Oikos*, pp.508-519.

Perry, D.A., Margolis, H., Choquette, C., Molina, R. and Trappe, J.M., 1989. Ectomycorrhizal mediation of competition between coniferous tree species. *New Phytologist*, 112(4), pp.501-511.

Peterson, R.L., Massicotte, H.B. and Melville, L.H., 2004. *Mycorrhizas: anatomy and cell biology*. NRC Research Press.

Prescott, C. E., 2005. Do rates of litter decomposition tell us anything we really need to know?. *Forest Ecology and Management*, 220(1), pp.66-74.

- Pretzsch, H., 2009. Forest dynamics, growth, and yield (pp. 1-39). Springer Berlin Heidelberg.
- Philip, L., Simard, S. and Jones, M., 2010. Pathways for below-ground carbon transfer between paper birch and Douglas-fir seedlings. *Plant Ecology and Diversity*, 3(3), pp.221-233.
- Phillips, F.J., 1909. A study of pinon pine. *Botanical Gazette*, 48(3), pp.216-223.
- Phillips, M., 2017. *Mycorrhizal Planet: How Symbiotic Fungi Work with Roots to Support Plant Health and Build Soil Fertility*. Chelsea Green Publishing.
- Pickles, B.J. and Anderson, I.C., 2016. Spatial ecology of ectomycorrhizal fungal communities. *Molecular Mycorrhizal Symbiosis*, pp.363-386.
- Picon, C., Guehl, J. M., and Aussenac, G., 1996. Growth dynamics, transpiration and water-use efficiency in *Quercus robur* plants submitted to elevated CO₂ and drought. In *Annales des Sciences Forestieres* (Vol. 53, No. 2-3, pp. 431-446). EDP Sciences.
- Plamboeck, A.H., Dawson, T.E., Egerton-Warburton, L.M., North, M., Bruns, T.D. and Querejeta, J.I., 2007. Water transfer via ectomycorrhizal fungal hyphae to conifer seedlings. *Mycorrhiza*, 17(5), p.439.
- Plett, J.M., Daguerre, Y., Wittulsky, S., Vayssières, A., Deveau, A., Melton, S.J., Kohler, A., Morrell-Falvey, J.L., Brun, A., Veneault-Fourrey, C. and Martin, F., 2014. Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 protein and represses jasmonic acid (JA) responsive genes. *Proceedings of the National Academy of Sciences*, 111(22), pp.8299-8304.
- Pollierer, M. M., Langel, R., Körner, C., Maraun, M., and Scheu, S., 2007. The underestimated importance of belowground carbon input for forest soil animal food webs. *Ecology Letters*, 10(8), pp.729-736.
- Post, W.M., Emanuel, W.R., Zinke, P.J. and Stangenberger, A.G., 1982. Soil carbon pools and world life zones. *Nature*, 298(5870), p.156.
- Postgate, J., 1998. The origins of the unit of nitrogen fixation at the University of Sussex. *Notes and Records of the Royal Society*, 52(2), pp.355-362.
- Porcel, R., Aroca, R., and Ruiz-Lozano, J. M., 2012. Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. *Agronomy for Sustainable Development*, 32(1), pp.181-200.

Porras-Alfaro, A. and Bayman, P., 2011. Hidden fungi, emergent properties: endophytes and microbiomes. *Annual review of phytopathology*, 49, pp.291-315.

Pozo, M.J. and Azcón-Aguilar, C., 2007. Unraveling mycorrhiza-induced resistance. *Current opinion in plant biology*, 10(4), pp.393-398.

Pozo, M.J., Verhage, A., García-Andrade, J., García, J.M. and Azcón-Aguilar, C., 2009. Priming plant defence against pathogens by arbuscular mycorrhizal fungi. In *Mycorrhizas-functional processes and ecological impact* (pp. 123-135). Springer, Berlin, Heidelberg.

Pratscher, J., Dumont, M. G., and Conrad, R., 2011. Ammonia oxidation coupled to CO₂ fixation by archaea and bacteria in an agricultural soil. *Proceedings of the National Academy of Sciences*, 108(10), pp.4170-4175.

Prinzing, A., Reiffers, R., Braakhekke, W.G., Hennekens, S.M., Tackenberg, O., Ozinga, W.A., Schaminée, J.H. and Van Groenendael, J.M., 2008. Less lineages—more trait variation: phylogenetically clustered plant communities are functionally more diverse. *Ecology Letters*, 11(8), pp.809-819.

Pritchard, S. G., Strand, A. E., McCormack, M. L., Davis, M. A., and Oren, R., 2008. Mycorrhizal and rhizomorph dynamics in a loblolly pine forest during 5 years of free-air-CO₂-enrichment. *Global Change Biology*, 14(6), pp.1252-1264.

Qiao, N.A., Schaefer, D., Blagodatskaya, E., Zou, X., Xu, X. and Kuzyakov, Y., 2014. Labile carbon retention compensates for CO₂ released by priming in forest soils. *Global Change Biology*, 20(6), pp.1943-1954.

Quandt, C.A., Kohler, A., Hesse, C.N., Sharpton, T.J., Martin, F. and Spatafora, J.W., 2015. Metagenome sequence of *Elaphomyces granulatus* from sporocarp tissue reveals Ascomycota ectomycorrhizal fingerprints of genome expansion and a Proteobacteria-rich microbiome. *Environmental microbiology*, 17(8), pp.2952-2968.

Querejeta, J., Egerton-Warburton, L.M. and Allen, M.F., 2003. Direct nocturnal water transfer from oaks to their mycorrhizal symbionts during severe soil drying. *Oecologia*, 134(1), pp.55-64.

Quine, C. P., Cahalan, C., Hester, A., Humphrey, J., Kirby, K., Moffat, A., and Valatin, G., 2011. Chapter 8 Woodlands in *UK National Ecosystem Assessment Technical Report*. UNEP-WCMC, Cambridge.

- Quirk, J., Beerling, D. J., Banwart, S. A., Kakonyi, G., Romero-Gonzalez, M. E., and Leake, J. R., 2012. Evolution of trees and mycorrhizal fungi intensifies silicate mineral weathering. *Biology letters*, 8(6), pp.1006-1011.
- Rai, A.N., 2018. *CRC Handbook of symbiotic cyanobacteria*. CRC Press.
- Rambelli, A., 1973. The rhizosphere of mycorrhizae. *Ectomycorrhizae. Their ecology and physiology*, pp.299-349.
- Ratnayake, M., Leonard, R.T. and Menge, J.A., 1978. Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation. *New Phytologist*, 81(3), pp.543-552.
- Read, D.J., Koucheki, H.K. and Hodgson, J., 1976. Vesicular-arbuscular mycorrhiza in natural vegetation systems. *New Phytologist*, 77(3), pp.641-653.
- Read, D.J., Leake, J.R. and Perez-Moreno, J., 2004. Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Canadian Journal of Botany*, 82(8), pp.1243-1263.
- Reavy, B., Swanson, M. M., and Talianky, M., 2014. Viruses in Soil. In *Interactions in Soil: Promoting Plant Growth* (pp. 163-180). Springer Netherlands.
- Redecker, D., Kodner, R. and Graham, L.E., 2000. Glomalean fungi from the Ordovician. *Science*, 289(5486), pp.1920-1921.
- Redecker, D., Schüßler, A., Stockinger, H., Stürmer, S.L., Morton, J.B. and Walker, C., 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza*, 23(7), pp.515-531.
- Reed, S.C., Yang, X. and Thornton, P.E., 2015. Incorporating phosphorus cycling into global modeling efforts: a worthwhile, tractable endeavor. *New Phytologist*, 208(2), pp.324-329.
- Reich, P. B., Luo, Y., Bradford, J. B., Poorter, H., Perry, C. H., and Oleksyn, J., 2014. Temperature drives global patterns in forest biomass distribution in leaves, stems, and roots. *Proceedings of the National Academy of Sciences*, 111(38), pp.13721-13726.
- Reid, C. P. P., Kidd, F. A., and Ekwebelam, S. A., 1983. Nitrogen nutrition, photosynthesis and carbon allocation in ectomycorrhizal pine. *Plant and soil*, 71(1-3), pp.415-431.

- Remy, W., Taylor, T.N., Hass, H. and Kerp, H., 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences*, 91(25), pp.11841-11843.
- Rhodes, D.F., 1983. Responses of alder and willow to attack by tent caterpillars and webworms: evidence for pheromonal sensitivity of willows. *Plant resistance to insects*, pp.55-68.
- Rice, E.L., 2012. *Allelopathy*. Academic press. London.
- Richard, F., Millot, S., Gardes, M. and Selosse, M.A., 2005. Diversity and specificity of ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated by *Quercus ilex*. *New Phytologist*, 166(3), pp.1011-1023.
- Richards, A. E., and Schmidt, S., 2010. Complementary resource use by tree species in a rain forest tree plantation. *Ecological Applications*, 20(5), pp.1237-1254.
- Richardson, A. D., Hollinger, D. Y., Dail, D. B., Lee, J. T., Munger, J. W., and O'Keefe, J., 2009. Influence of spring phenology on seasonal and annual carbon balance in two contrasting New England forests. *Tree physiology*, 29(3), pp.321-331.
- Rillig, M.C., 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. *Canadian Journal of Soil Science*, 84(4), pp.355-363.
- Rillig, M.C. and Mummey, D.L., 2006. Mycorrhizas and soil structure. *New Phytologist*, 171(1), pp.41-53.
- Rillig, M.C., Wright, S.F., Allen, M.F. and Field, C.B., 1999. Rise in carbon dioxide changes soil structure. *Nature*, 400(6745), p.628.
- Robinson, D. and Fitter, A., 1999. The magnitude and control of carbon transfer between plants linked by a common mycorrhizal network. *Journal of Experimental Botany*, 50(330), pp.9-13.
- Rodriguez, R.J., White Jr, J.F., Arnold, A.E. and Redman, R.S., 2009. Fungal endophytes: diversity and functional roles. *New Phytologist*, 182(2), pp.314-330.
- Rousseau, J. V. D., and Reid, C. P. P., 1990. Effects of phosphorus and ectomycorrhizas on the carbon balance of loblolly pine seedlings. *Forest Science*, 36(1), pp.101-112.

- Rose, S.L., Perry, D.A., Pilz, D. and Schoeneberger, M.M., 1983. Allelopathic effects of litter on the growth and colonization of mycorrhizal fungi. *Journal of Chemical Ecology*, 9(8), pp.1153-1162.
- Roy, M., Dubois, M.P., Proffit, M., Vincenot, L., Desmarais, E. and Selosse, M.A., 2008. Evidence from population genetics that the ectomycorrhizal basidiomycete *Laccaria amethystina* is an actual multihost symbiont. *Molecular Ecology*, 17(12), pp.2825-2838.
- Rygiewicz, P.T. and Andersen, C.P., 1994. Mycorrhizae alter quality and quantity of carbon allocated below ground. *Nature*, 369(6475), pp.58-60.
- Saffo, M.B., 1992. Coming to terms with a field: words and concepts in symbiosis. *Symbiosis*, 14(1-3), pp.17-31.
- Salvador-Recatalà, V., Tjallingii, W.F. and Farmer, E.E., 2014. Real-time, in vivo intracellular recordings of caterpillar-induced depolarization waves in sieve elements using aphid electrodes. *New Phytologist*, 203(2), pp.674-684.
- Santantonio, D., 1979. Seasonal dynamics of fine roots in mature stands of Douglas-fir of different water regimes: A preliminary report. *A Riedacker and J Gagnaire-Michard. CR des Reunions du Groupe d'Etude des Racines*, pp.190-203.
- Schausberger, P., Peneder, S., Jürschik, S. and Hoffmann, D., 2012. Mycorrhiza changes plant volatiles to attract spider mite enemies. *Functional Ecology*, 26(2), pp.441-449.
- Schleifer, K. H., 2009. Classification of Bacteria and Archaea: past, present and future. *Systematic and applied microbiology*, 32(8), pp.533-542.
- Schlesinger, W. H., 1977. Carbon balance in terrestrial detritus. *Annual review of ecology and systematics*, pp.51-81.
- Schmidt, M.W., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A. and Nannipieri, P., 2011. Persistence of soil organic matter as an ecosystem property. *Nature*, 478(7367), p.49.
- Seidl, R., Schelhaas, M.J., Rammer, W. and Verkerk, P.J., 2014. Increasing forest disturbances in Europe and their impact on carbon storage. *Nature climate change*, 4(9), p.806.

- Selosse, M.A., Bocayuva, M.F., Kasuya, M.C.M. and Courty, P.E., 2016. Mixotrophy in mycorrhizal plants: extracting carbon from mycorrhizal networks. *Molecular mycorrhizal symbiosis*, pp.451-471.
- Selosse, M.A., Richard, F., He, X. and Simard, S.W., 2006. Mycorrhizal networks: des liaisons dangereuses?. *Trends in Ecology and Evolution*, 21(11), pp.621-628.
- Sessitsch, A., Hardoim, P., Döring, J., Weilharter, A., Krause, A., Woyke, T., Mitter, B., Hauberg-Lotte, L., Friedrich, F., Rahalkar, M. and Hurek, T., 2012. Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. *Molecular Plant-Microbe Interactions*, 25(1), pp.28-36.
- Shenoy, B.D., Jeewon, R. and Hyde, K.D., 2007. Impact of DNA sequence-data on the taxonomy of anamorphic fungi. *Fungal Diversity*, 26, pp. 1-54
- Shaw, S.L. and Long, S.R., 2003. Nod factor inhibition of reactive oxygen efflux in a host legume. *Plant Physiology*, 132(4), pp.2196-2204
- Sieber, T.N., 2007. Endophytic fungi in forest trees: are they mutualists?. *Fungal biology reviews*, 21(2-3), pp.75-89.
- Simard, S.W., Perry, D.A., Jones, M.D., Myrold, D.D., Durall, D.M. and Molina, R., 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature*, 388(6642), p.579.
- Simard, S., Asay, A., Beiler, K., Bingham, M., Deslippe, J., He, X., Philip, L., Song, Y. and Teste, F., 2015. Resource transfer between plants through ectomycorrhizal fungal networks. In *Mycorrhizal networks* (pp. 133-176). Springer, Dordrecht.
- Simard, S.W. and Durall, D.M., 2004. Mycorrhizal networks: a review of their extent, function, and importance. *Canadian Journal of Botany*, 82(8), pp.1140-1165.
- Simon, L., Bousquet, J., Lévesque, R.C. and Lalonde, M., 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature*, 363(6424), p.67.
- Singh, P.K., Singh, M. and Tripathi, B.N., 2013. Glomalin: an arbuscular mycorrhizal fungal soil protein. *Protoplasma*, 250(3), pp.663-669.
- Six, J., Conant, R. T., Paul, E. A., and Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant and soil*, 241(2), pp.155-176.

- Smith, S.E. and Gianinazzi-Pearson, V., 1988. Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. *Annual review of plant physiology and plant molecular biology*, 39(1), pp.221-244.
- Smith, S., and Gilbert, J., 2003. National inventory of woodland and trees: Great Britain. *National inventory of woodland and trees: Great Britain*.
- Smith, S.E. and Read, D.J., 2010. *Mycorrhizal symbiosis*. Academic press. London.
- Song, X.P., Hansen, M.C., Stehman, S.V., Potapov, P.V., Tyukavina, A., Vermote, E.F. and Townshend, J.R., 2018. Global land change from 1982 to 2016. *Nature*, 560(7720), p.639.
- Song, Y.Y., Ye, M., Li, C., He, X., Zhu-Salzman, K., Wang, R.L., Su, Y.J., Luo, S.M. and Zeng, R.S., 2014. Hijacking common mycorrhizal networks for herbivore-induced defence signal transfer between tomato plants. *Scientific reports*, 4, p.3915.
- Song, Y.Y., Zeng, R.S., Xu, J.F., Li, J., Shen, X. and Yihdego, W.G., 2010. Interplant communication of tomato plants through underground common mycorrhizal networks. *PloS one*, 5(10), p.e13324.
- Soudzilovskaia, N.A., Vaessen, S., van't Zelfde, M. and Raes, N., 2017. Global Patterns of Mycorrhizal Distribution and Their Environmental Drivers. In *Biogeography of Mycorrhizal Symbiosis* (pp. 223-235). Springer, Cham.
- Souto, C., Pellissier, F. and Chiapusio, G., 2000. Allelopathic effects of humus phenolics on growth and respiration of mycorrhizal fungi. *Journal of Chemical Ecology*, 26(9), pp.2015-2023.
- Sparling, G.P., Hart, P.B.S., August, J.A. and Leslie, D.M., 1994. A comparison of soil and microbial carbon, nitrogen, and phosphorus contents, and macro-aggregate stability of a soil under native forest and after clearance for pastures and plantation forest. *Biology and Fertility of Soils*, 17(2), pp.91-100.
- Spittlehouse, D. L., and Stewart, R. B., 2004. Adaptation to climate change in forest management. *Journal of Ecosystems and Management*, 4(1), pp.1-11.
- Stachowicz, J.J., 2001. Mutualism, facilitation, and the structure of ecological communities: positive interactions play a critical, but underappreciated, role in ecological communities by reducing physical or biotic stresses in existing habitats and by creating new habitats on which many species depend. *AIBS Bulletin*, 51(3), pp.235-246.

Stamets, P., 2005. *Mycelium running: how mushrooms can help save the world*. Random House LLC.

Steinbeiss, S., Temperton, V. M., and Gleixner, G., 2008. Mechanisms of short-term soil carbon storage in experimental grasslands. *Soil Biology and Biochemistry*, 40(10), pp.2634-2642.

Stoy, P. C., Trowbridge, A. M., and Bauerle, W. L., 2014. Controls on seasonal patterns of maximum ecosystem carbon uptake and canopy-scale photosynthetic light response: contributions from both temperature and photoperiod. *Photosynthesis research*, 119(1-2), pp.49-64.

Suberkropp, K., and Weyers, H., 1996. Application of fungal and bacterial production methodologies to decomposing leaves in streams. *Applied and environmental microbiology*, 62(5), pp.1610-1615.

Sundareshwar, P. V., Morris, J. T., Koepfler, E. K., and Fornwalt, B., 2003. Phosphorus limitation of coastal ecosystem processes. *Science*, 299(5606), pp.563-565.

Suryanarayanan, T.S., 2013. Endophyte research: going beyond isolation and metabolite documentation. *Fungal ecology*, 6(6), pp.561-568.

Tadych, M., Bergen, M.S. and White Jr, J.F., 2014. *Epichloë* spp. associated with grasses: new insights on life cycles, dissemination and evolution. *Mycologia*, 106(2), pp.181-201.

Tam, P. C., 1995. Heavy metal tolerance by ectomycorrhizal fungi and metal amelioration by *Pisolithus tinctorius*. *Mycorrhiza*, 5(3), pp.181-187.

Tariq, M., Hameed, S., Khan, H.U., Munir, M.I. and Nushin, F., 2017. Role of microsymbionts in plant microbe symbiosis. *Journal of Applied Microbiology Biochemistry*, 1(2), p.6

Taylor, A.F., 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. *Plant and Soil*, 244(1-2), pp.19-28.

Taylor, C. M. A. and Tabbush, P. M. 1990. *Nitrogen deficiency in Sitka spruce plantations*. *Forestry Commission Bulletin*, 89. HMSO, London

Tedersoo, L., Bahram, M., Pölme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A. and Smith, M.E., 2014. Global diversity and geography of soil fungi. *Science*, 346(6213), p.1256688.

- Tedersoo, L., Jairus, T., Horton, B.M., Abarenkov, K., Suvi, T., Saar, I. and Kõljalg, U., 2008. Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. *New Phytologist*, 180(2), pp.479-490.
- Tellström, V., Usadel, B., Thimm, O., Stitt, M., Küster, H. and Niehaus, K., 2007. The lipopolysaccharide of *Sinorhizobium meliloti* suppresses defense-associated gene expression in cell cultures of the host plant *Medicago truncatula*. *Plant physiology*, 143(2), pp.825-837.
- Teste, F.P., Simard, S.W., Durall, D.M., Guy, R.D., Jones, M.D. and Schoonmaker, A.L., 2009. Access to mycorrhizal networks and roots of trees: importance for seedling survival and resource transfer. *Ecology*, 90(10), pp.2808-2822.
- Thorley, R.M., Taylor, L.L., Banwart, S.A., Leake, J.R. and Beerling, D.J., 2015. The role of forest trees and their mycorrhizal fungi in carbonate rock weathering and its significance for global carbon cycling. *Plant, Cell and Environment*, 38(9), pp.1947-1961.
- Tilak, K.V.B.R., Ranganayaki, N., Pal, K.K., De, R., Saxena, A.K., Nautiyal, C.S., Mittal, S., Tripathi, A.K. and Johri, B.N., 2005. Diversity of plant growth and soil health supporting bacteria. *Current science*, pp.136-150.
- Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M. and Siemann, E., 1997. The influence of functional diversity and composition on ecosystem processes. *Science*, 277(5330), pp.1300-1302.
- Tilman, D., 1982. *Resource competition and community structure*. Princeton University Press.
- Timonen, S. and Hurek, T., 2006. Characterization of culturable bacterial populations associating with *Pinus sylvestris*–*Suillus bovinus* mycorrhizospheres. *Canadian journal of microbiology*, 52(8), pp.769-778.
- Tinker, P.B., Durall, D.M. and Jones, M.D., 1994. Carbon use efficiency in mycorrhizas theory and sample calculations. *New Phytologist*, 128(1), pp.115-122.
- Tinker, P.B. and Nye, P.H., 2000. *Solute movement in the rhizosphere*. Oxford University Press.
- Toljander, J.F., Artursson, V., Paul, L.R., Jansson, J.K. and Finlay, R.D., 2005. Attachment of different soil bacteria to arbuscular mycorrhizal fungal extraradical hyphae is determined by hyphal vitality and fungal species. *FEMS Microbiology Letters*, 254(1), pp.34-40.

- Tornberg, K., Bååth, E. and Olsson, S., 2003. Fungal growth and effects of different wood decomposing fungi on the indigenous bacterial community of polluted and unpolluted soils. *Biology and Fertility of Soils*, 37(3), pp.190-197.
- Trappe, J.M., 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. *Annual Review of Phytopathology*, 15(1), pp.203-222.
- Treseder, K.K. and Holden, S.R., 2013. Fungal carbon sequestration. *Science*, 339(6127), pp.1528-1529.
- Triviño, M., Juutinen, A., Mazziotta, A., Miettinen, K., Podkopaev, D., Reunanen, P. and Mönkkönen, M., 2015. Managing a boreal forest landscape for providing timber, storing and sequestering carbon. *Ecosystem Services*, 14, pp.179-189.
- Twieg, B.D., Durall, D.M. and Simard, S.W., 2007. Ectomycorrhizal fungal succession in mixed temperate forests. *New Phytologist*, 176(2), pp.437-447.
- Unterseher, M., 2011. Diversity of fungal endophytes in temperate forest trees. In *Endophytes of forest trees* (pp. 31-46). Springer, Dordrecht.
- Urbanová, M., Šnajdr, J. and Baldrian, P., 2015. Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. *Soil Biology and Biochemistry*, 84, pp.53-64.
- Uren, N.C., 1998. The role of root exudates in nutrient acquisition. *Advances in plant nutrition*, pp.79-114.
- Uren, N.C. and Reisenauer, H.M., 1988. The role of root exudates in nutrient acquisition. In *Advances in Plant Nutrition*, Vol. 3 (PB Tinker and A. Lauchli, Eds.). Praeger, New York
- Ushio, M., Wagai, R., Balser, T.C. and Kitayama, K., 2008. Variations in the soil microbial community composition of a tropical montane forest ecosystem: does tree species matter?. *Soil Biology and Biochemistry*, 40(10), pp.2699-2702.
- Valverde-Barrantes, O.J., Smemo, K.A., Feinstein, L.M., Kershner, M.W. and Blackwood, C.B., 2015. Aggregated and complementary: symmetric proliferation, overyielding, and mass effects explain fine-root biomass in soil patches in a diverse temperate deciduous forest landscape. *New Phytologist*, 205(2), pp.731-742.

- Van Der Heijden, M.G., Bardgett, R.D. and Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters*, 11(3), pp.296-310.
- Van Der Heijden, M.G., De Bruin, S., Luckerhoff, L., Van Logtestijn, R.S. and Schlaeppli, K., 2016. A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. *The ISME journal*, 10(2), p.389.
- Van Der Heijden, M.G., Dombrowski, N. and Schlaeppli, K., 2017. Continuum of root–fungal symbioses for plant nutrition. *Proceedings of the National Academy of Sciences*, p.201716329.
- Van Der Heijden, M.G. and Horton, T.R., 2009. Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology*, 97(6), pp.1139-1150.
- Van Der Heijden, M.G., Martin, F.M., Selosse, M.A. and Sanders, I.R., 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist*, 205(4), pp.1406-1423.
- Van Der Linde, S., Suz, L.M., Orme, C.D.L., Cox, F., Andreae, H., Asi, E., Atkinson, B., Benham, S., Carroll, C., Cools, N. and De Vos, B., 2018. Environment and host as large-scale controls of ectomycorrhizal fungi. *Nature*, 558(7709), p.243.
- Van Elsas, J.D., Chiurazzi, M., Mallon, C.A., Elhottová, D., Křišťůfek, V. and Salles, J.F., 2012. Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proceedings of the National Academy of Sciences*, 109(4), pp.1159-1164.
- Van Veen, J.A., Liljeroth, E., Lekkerkerk, L.J.A. and Van de Geijn, S.C., 1991. Carbon fluxes in plant-soil systems at elevated atmospheric CO₂ levels. *Ecological applications*, 1(2), pp.175-181.
- Vargas, R. and Allen, M.F., 2008. Dynamics of fine root, fungal rhizomorphs, and soil respiration in a mixed temperate forest: integrating sensors and observations. *Vadose Zone Journal*, 7(3), pp.1055-1064.
- Venturi, V. and Keel, C., 2016. Signaling in the rhizosphere. *Trends in plant science*, 21(3), pp.187-198.
- Venturini, L. and Delledonne, M., 2015. Symbiotic plant-fungi interactions stripped down to the root. *Nature genetics*, 47(4), p.309.

Vesterdal, L., Clarke, N., Sigurdsson, B.D. and Gundersen, P., 2013. Do tree species influence soil carbon stocks in temperate and boreal forests?. *Forest Ecology and Management*, 309, pp.4-18.

Vesterdal, L., Schmidt, I.K., Callesen, I., Nilsson, L.O. and Gundersen, P., 2008. Carbon and nitrogen in forest floor and mineral soil under six common European tree species. *Forest Ecology and Management*, 255(1), pp.35-48.

Vlček, V. and Pohanka, M., 2019. Glomalin—an interesting protein part of the soil organic matter. *Soil and Water Research*, 29. (In press).

Vogt, K.A., Grier, C.C., Meier, C.E. and Edmonds, R.L., 1982. Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis* ecosystems in Western Washington. *Ecology*, 63(2), pp.370-380.

Voříšková, J., Brabcová, V., Cajthaml, T. and Baldrian, P., 2014. Seasonal dynamics of fungal communities in a temperate oak forest soil. *New Phytologist*, 201(1), pp.269-278.

Wagg, C., Bender, S.F., Widmer, F. and van der Heijden, M.G., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences*, 111(14), pp.5266-5270.

Walker, J.F., Aldrich-Wolfe, L., Riffel, A., Barbare, H., Simpson, N.B., Trowbridge, J. and Jumpponen, A., 2011. Diverse Helotiales associated with the roots of three species of Arctic Ericaceae provide no evidence for host specificity. *New Phytologist*, 191(2), pp.515-527.

Walker, T.S., Bais, H.P., Grotewold, E. and Vivanco, J.M., 2003. Root exudation and rhizosphere biology. *Plant physiology*, 132(1), pp.44-51.

Wallace, J.G. and May, G., 2018. Endophytes: The Other Maize Genome. In *The Maize Genome* (pp. 213-246). Springer, Cham.

Wallander, H., Ekblad, A., Godbold, D. L., Johnson, D., Bahr, A., Baldrian, P., Björk, R.G., Kieliszewska-Rokicka, B., Kjoller, R., Kraigher, H. and Plassard, C., 2013. Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal mycelium in forests soils—A review. *Soil Biology and Biochemistry*, 57, pp.1034-1047.

Wallander, H., Göransson, H., and Rosengren, U., 2004. Production, standing biomass and natural abundance of ^{15}N and ^{13}C in ectomycorrhizal mycelia collected at different soil depths in two forest types. *Oecologia*, 139(1), pp.89-97.

- Wallander, H., Nilsson, L.O., Hagerberg, D. and Bååth, E., 2001. Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. *New Phytologist*, 151(3), pp.753-760.
- Wang, B., Yeun, L.H., Xue, J.Y., Liu, Y., Ané, J.M. and Qiu, Y.L., 2010. Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. *New Phytologist*, 186(2), pp.514-525.
- Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., Van Der Putten, W. H., and Wall, D. H., 2004. Ecological linkages between aboveground and belowground biota. *Science*, 304(5677), pp.1629-1633.
- Wardle, D.A. and Lindahl, B.D., 2014. Disentangling global soil fungal diversity. *Science*, 346(6213), pp.1052-1053.
- Wardle, D. A., Yeates, G. W., Williamson, W., and Bonner, K. I., 2003. The response of a three trophic level soil food web to the identity and diversity of plant species and functional groups. *Oikos*, 102(1), pp.45-56.
- Werner, G.D. and Kiers, E.T., 2015. Partner selection in the mycorrhizal mutualism. *New Phytologist*, 205(4), pp.1437-1442.
- Wieder, W.R., Cleveland, C.C., Smith, W.K. and Todd-Brown, K., 2015. Future productivity and carbon storage limited by terrestrial nutrient availability. *Nature Geoscience*, 8(6), p.441.
- Wilkinson, A., Hill, P.W., Farrar, J.F., Jones, D.L. and Bardgett, R.D., 2014. Rapid microbial uptake and mineralization of amino acids and peptides along a grassland productivity gradient. *Soil Biology and Biochemistry*, 72, pp.75-83.
- Willis, R.J., 2000. Juglans spp., juglone and allelopathy. *Allelopathy J*, 7(1), pp.1-55.
- Whipps, J.M., 2004. Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Canadian journal of botany*, 82(8), pp.1198-1227.
- Whittingham, J. and Read, D.J., 1982. Vesicular-arbuscular mycorrhiza in natural vegetation systems. *New Phytologist*, 90(2), pp.277-284.
- Wilkinson, D.M., 2001. At cross purposes. *Nature*, 412(6846), p.485.

- Williamson, K.E., Fuhrmann, J.J., Wommack, K.E. and Radosevich, M., 2017. Viruses in soil ecosystems: an unknown quantity within an unexplored territory. *Annual review of virology*, 4, pp.201-219.
- Wilson, G.W.T., Hartnett, D.C. and Rice, C.W., 2006. Mycorrhizal-mediated phosphorus transfer between tallgrass prairie plants *Sorghastrum nutans* and *Artemisia ludoviciana*. *Functional Ecology*, pp.427-435.
- Wright, A.J., Wardle, D.A., Callaway, R. and Gaxiola, A., 2017. The overlooked role of facilitation in biodiversity experiments. *Trends in ecology and evolution*, 32(5), pp.383-390.
- Wright, S.F. and Upadhyaya, A., 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil science*, 161(9), pp.575-586.
- Wright, S., Nichols, K., Jawson, L., McKenna, L. and Almendras, A., 2001. Glomalin-A manageable soil glue. *Soil Science Society of America Special Publication Book*. Madison, USA.
- Wright, S.F. and a Nichols, K., 2002. Glomalin: hiding place for a third of the world's stored soil carbon. *Agricultural Research*, 50(9), p.4.
- Wullschlegel, S. D., Tschaplinski, T. J., and Norby, R. J., 2002. Plant water relations at elevated CO₂—implications for water-limited environments. *Plant, Cell and Environment*, 25(2), pp.319-331.
- Wurzburger, N., 2016. Old-growth temperate forests harbor hidden nitrogen-fixing bacteria. *New Phytologist*, 210(2), pp.374-376.
- Yousefpour, R., Jacobsen, J.B., Thorsen, B.J., Meilby, H., Hanewinkel, M. and Oehler, K., 2012. A review of decision-making approaches to handle uncertainty and risk in adaptive forest management under climate change. *Annals of forest science*, 69(1), pp.1-15.
- Zaehle, S., Medlyn, B.E., De Kauwe, M.G., Walker, A.P., Dietze, M.C., Hickler, T., Luo, Y., Wang, Y.P., El-Masri, B., Thornton, P. and Jain, A., 2014. Evaluation of 11 terrestrial carbon–nitrogen cycle models against observations from two temperate Free-Air CO₂ Enrichment studies. *New Phytologist*, 202(3), pp.803-822.

Žifčáková, L., Větrovský, T., Howe, A. and Baldrian, P., 2016. Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. *Environmental microbiology*, 18(1), pp.288-301.

Chapter 3

Carbon partitioning and temporal dynamics of three temperate tree species and associated microbial symbioses, estimated by ^{14}C pulse-labelling.

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1. Introduction

Forest ecosystems have a fundamental role in the global carbon (C) cycle and remove 30% of global anthropogenic CO₂ emissions annually (Le Quéré et al., 2016). The world's forests are not only absorbing atmospheric CO₂, but also adding C into the soil through a series of processes which can include decomposition of litter and woody debris, root turnover and rhizodeposition (Pausch and Kuzyakov, 2018). Rhizodeposition is the process by which, organic compounds (rhizodeposits) are released or shed from live roots into the rhizosphere or as a consequence of root and mycorrhizal death (Jones et al., 2009). The combined effect of rhizodeposition and litter-fall accounts for half the atmospheric C incorporated into terrestrial plants by photosynthesis (Lal, 2008; Paterson et al., 2009).

European forests are estimated to store 6.1 t C ha⁻¹ in litter and 113 t C ha⁻¹ in mineral soil (Sommer et al., 2017) and their responsiveness to management make them attractive candidates for C storage mitigation strategies (Nave et al., 2018). Despite developments in the quantification of C sinks and fluxes, many uncertainties in the role of forests to terrestrial C budgets persist (Tian et al., 2016; Pan et al., 2011), due to the relative difficulty in quantification of C allocation belowground (Leake et al., 2001). Specifically, C allocation into the

rhizospheric components of the C cycle (e.g. soils, micro-organisms and especially mycorrhizal hyphae) has often been underestimated or entirely overlooked in trees (Sommer et al., 2016; Sileshi, 2014) and when studied has largely focussed on arbuscular rather than ectomycorrhizae (Ekblad et al., 2013).

Within both coniferous and deciduous temperate forest trees, ectomycorrhizal associations are ubiquitous, and their biomass represents an important belowground C pool (Finlay and Söderström, 1992). Ectomycorrhizal hyphal networks in temperate soils are known to be extensive (Cairney, 2012), in some cases covering many square kilometres (Anderson and Cairney, 2007; Philips, 2017). Estimates of the extent of hyphae in forest soils range from 3–600 m g⁻¹ soil (Leake et al., 2004) or 30–8000 m hyphae m⁻¹ root (Smith and Read, 2010). Colonisation of roots by ectomycorrhizal fungi can positively influence tree root architecture (Ditengou et al., 2015), plant nutrient acquisition (Smith and Read, 2010) and water transport dynamics and repellency (Carminati et al., 2010; Moradi et al., 2012). These complex fungal symbionts not only benefit their tree hosts, but also perform fundamental ecosystem functions (Powell and Rillig, 2018) such as mediating the rate of decomposition within forest soils (Smith and Read, 2010), improving soil aggregation with fungal exudates (Wu et al., 2014) and increasing the abundance, diversity and activity of microbes in the rhizosphere (Paterson et al., 2009; Jones et al., 2009).

The largest flux of C from terrestrial ecosystems to the atmosphere is belowground autotrophic (e.g. root metabolism) and heterotrophic respiration (e.g. mineralisation of rhizodeposition) (Dilkes et al., 2004; Hill et al., 2007; Glanville et al., 2012). The soils capacity to store C through the process of rhizodeposition is therefore directly linked to the percentage of photosynthetically fixed C allocated belowground that is not rapidly respired (Hill et al., 2007) and which becomes stored in persistent, recalcitrant compounds in the soil. These compounds are typically associated with C which has been processed by the microbial biomass and is therefore secondary processed C. Predicting how increasing atmospheric CO₂ concentrations, temperatures, N deposition and changing precipitation patterns might alter the belowground C allocation and storage will be key to predicting future ecological C fluxes and feedbacks (Pausch and Kuzyakov, 2018).

The quantitative and qualitative investigation of rhizosphere importance to biogeochemical cycling is methodologically challenging (Oburger and Jones, 2018), due to a vast array of confounding issues. These include the fact that: (i) nutrients are simultaneously exuded from roots, hyphae whilst also being liberated from soil organic matter by microbes; (ii) nutrients

are continually removed from the rhizosphere by the microbial community; and (iii) sorption/desorption of nutrients and C by the soil's solid matrix (Oburger and Jones, 2018). Novel methodological developments, particularly in the use of isotopes are extending our understanding of rhizosphere processes (Oburger et al., 2013; Oburger and Schmidt, 2016). For example, the use of continuous or short-term labelling techniques (with stable and/or radioisotopes), are providing exciting opportunities to quantify forest C partitioning within the plant-microbe-soil compartments (Epron et al., 2012; Kuzyakov and Domanski, 2000). The short-term single $^{14}\text{CO}_2$ pulse technique allow us to distinguish between C that has been recently fixed from native C already present in the soil (Hill et al., 2007), as well as the separation of plant from soil organic matter derived C (Nguyen, 2003; Kuzyakov and Schneckenberger, 2004; Werth and Kuzyakov, 2008).

The aim of this study was to examine the spatiotemporal dynamics of recently plant captured C in three temperate tree species and to determine the relative allocation of C to belowground pools, using ^{14}C as a proxy. The mechanisms involved in determining C allocation to plant tissues are thought to be controlled by both root and shoot and these can differ between individuals, tree species, tree developmental stage (Farrar and Jones, 2000; Weiner, 2004), in response to climatic conditions (Sanaullah et al., 2012), or as a result of contrasting microbial symbiotic relationships (Brzostek et al., 2015). Here we used a ^{14}C pulse-tracing technique to estimate the relative importance of C pools and fluxes within the plant-fungal-soil system of three contrasting tree species.

Our hypotheses for this experiment were:

H₁: Belowground allocation of C will be greater in species with the largest diversity of microbial symbiotic associations.

H₂: The transfer of recently assimilated C to the soil microbial community will be greater in early (*B. pendula* and *A. glutinosa*), than late successional trees (*C. sativa*), because early successional stage trees need symbiotic partners to help with the poor nutritional status of the soil they colonise

2. Materials and methods

2.1. Experimental establishment

Twelve 2-year-old (1 + 1) bare rooted saplings of two temperate tree species namely; common alder (*Alnus glutinosa* (L.) Gaertn; Tree A (UK provenance zone 204)) and silver birch (*Betula pendula* Roth.; Tree B (UK provenance zone 204)) and twelve 1-year-old cell-grown sweet chestnut (*Castanea sativa* Mill.; Tree C (UK provenance zone 404)) were purchased from local forest nursery stock suppliers (Maelor Forest Nurseries Ltd., Bronington, UK) in November 2015. At the time of planting, the height of all tree saplings was between 0.2 – 0.4 m. The tree species were selected based on their contrasting traits, which was anticipated to maximise the positive effect on net productivity. Specifically, *B. pendula* is a relatively short-lived, light-demanding, early successional pioneer species, *C. sativa* is a late successional, shade tolerant, long lived species and *A. glutinosa* is intermediate in shade tolerance, tolerates waterlogged soils and is nitrogen fixing through an actinorhizal symbiotic association with *Frankia alni*. The saplings were planted into 10 L mesocosms (Ø 250 mm × 200 mm) that had been bisected into two equal-sized compartments using a 40 µm mesh (Normesh Limited, Lancashire, UK) (Plate 1) to allow the growth of root and hyphae in one compartment and the ingrowth of hyphae in the second compartment. Soil was collected from 0-10 cm depth plots under the trees species used in this experiment to avoid the common soil mishandling technique and potential subsequent incorrect estimation of soil biota (Reinhart and Rinella, 2016) and to ensure species-specific symbiotic soil microbes were present. Prior to planting, soil was air-dried, homogenised and sieved to pass 2 mm.

The soil was collected from the Henfaes experimental farm, Abergwyngregan, Gwynedd, Wales, (53°23 N, 4°01 W) (Ahmed et al., 2016) and is classified as a fine loamy textured Eutric Cambisol (Rheidol series) over alluvial gravel (Smith et al., 2013). The saplings were grown in a glasshouse at ambient conditions for 9 months to allow for the development of a root and hyphal network. A growth rate for the experimental establishment period was calculated by dividing the difference between the starting biomass (g) and the biomass prior to pulse-labelling and dividing this by the number of hours that the plants had to establish (274 days).

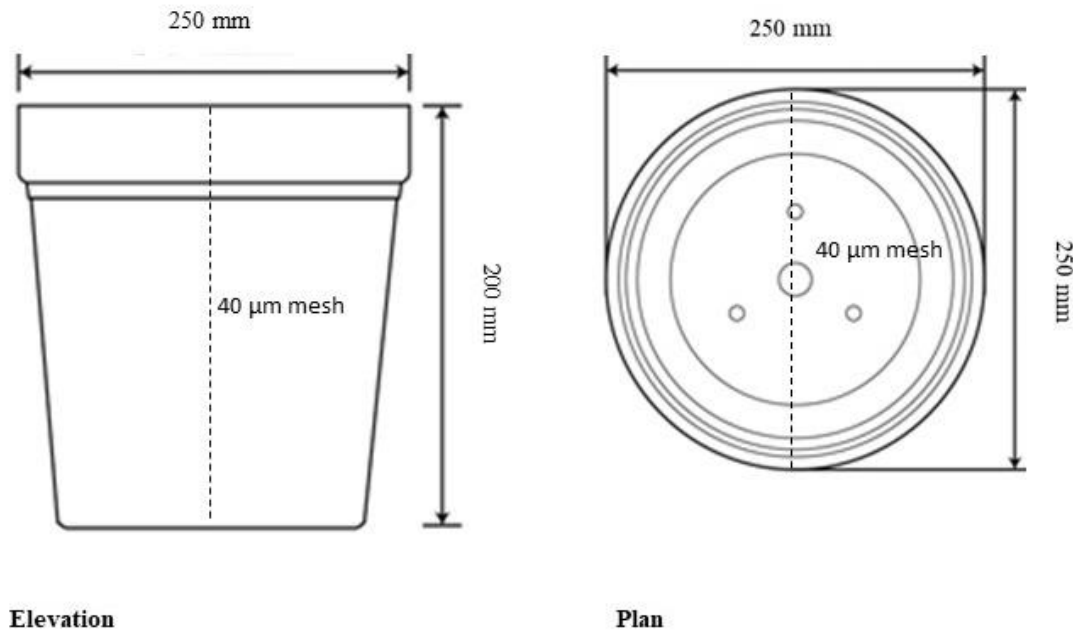


Figure 1: Diagram showing the experimental mesocosm set-up

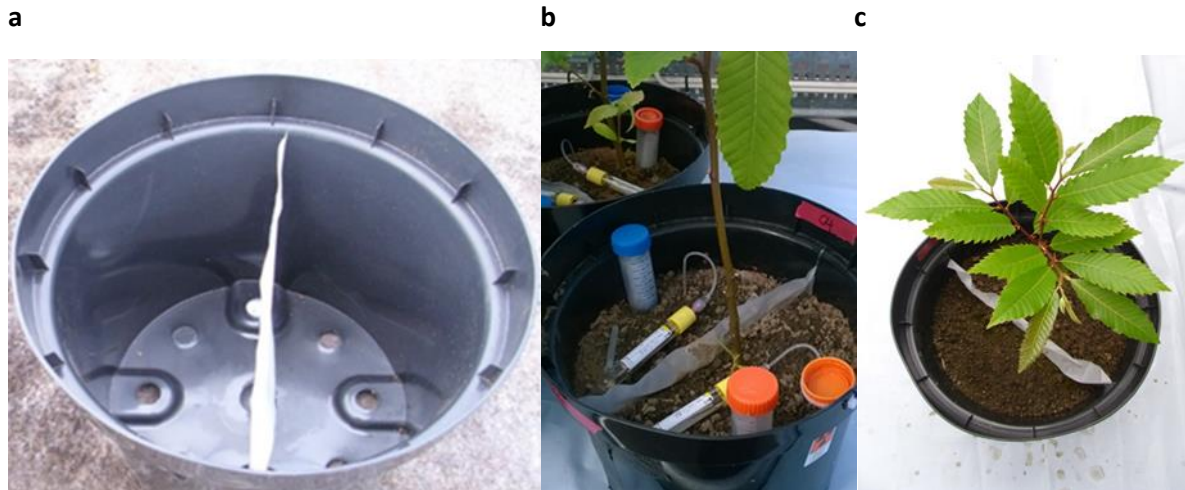


Plate 1: Photographs showing the experimental set-up (a) plant pot bisected with a nylon mesh with a 40-µm pore size to allow hyphal penetration but exclude the trees roots, (b) pot showing the soil solution collection tubes, and (c) showing *C (Castanea sativa)* tree established in the mesocosm.

2.2. Sampling procedure and isotope analysis

On 19th June 2016 each sapling was enclosed by 610 × 920 mm gas-proof bag (CP lab safety, Novato, CA, USA) and exposed to ¹⁴CO₂ generated by the addition of 200 µl of 2 MBq NaH¹⁴CO₃ (Amersham International, Amersham, UK) to 2 ml of 3 M HCl, in a 30 mm diameter vial fixed inside the plant mesocosm. Each sapling was exposed to the same activity (2 MBq)

for 2 h to allow for photo assimilation in full ambient light with a minimum photosynthetically active radiation level of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Different approaches were used to trace the ^{14}C pulse within the tree-soil system. Firstly, $^{14}\text{CO}_2$ evolved from the soil was collected by suspending two identical 3 M NaOH traps above the soil in both the plant and non-plant fraction. The traps allowed free passage of gases from the soil surface into a headspace chamber containing the NaOH traps. The surface area of the traps was calculated and scaled-up to the total area of half the pot. The traps were replaced at regular intervals (0, 24, 72, 168 and 336 h) after the end of the ^{14}C labelling period. Simultaneously, dissolved organic ^{14}C in soil solution was sampled following at 0, 24, 72, 168 and 336 h after the pulse-label was applied using Rhizon-MOM soil water samplers (Rhizosphere Research Products B.V., Wageningen, The Netherlands) (Jones et al., 2016). The ^{14}C content was determined by liquid scintillation counting, using a Wallac 1409 scintillation counter (Wallac EG and G, Milton Keynes, UK) with automatic quench correction and Optiphase Hisafe 3[®] (Perkin Elmer, UK) alkali-compatible scintillation fluid. After the defined time-periods (0, 24, 72, 168 and 336 hours after pulse labelling) the trees were destructively harvested and separated into old (present prior to pulse) and new foliage (unfurled during chase period), branch and stem, roots and soil samples. The plant material and soil were immediately oven dried at 105 °C to prevent loss of ^{14}C to microbial respiration. The roots were then carefully washed in sieves to remove the soil and stone before being re-oven dried at 105 °C. The plant biomass components (namely leaves since pulse, old leaves, branches and stem and roots) were weighed and recorded (Table 1) before being ground to a fine powder and homogenised using a MM200 laboratory mixer ball mill (Retsch GmbH, Hann, Germany). A subsample of 0.1 g was combusted using a Harvey Instruments Biological Oxidiser OX400 (R.J. Harvey Instrument Corp., Hillsdale, NJ, USA) and evolved $^{14}\text{CO}_2$ was dissolved into Oxysolve C-400 (Zinsser Analytic GmbH, Frankfurt, Germany) prior to quantification of ^{14}C using a Wallac 1409 liquid scintillation counter (Wallac EG and G, Milton Keynes, UK).

2.3. Soil Chemical Properties

To determine if the establishment of trees prior to pulse-labelling had modified the soil chemical properties, soil samples were collected using a 4 cm diameter stainless steel soil corer from the centre of each of the two mesocosm fractions immediately following the ^{14}C pulse-label. Soil was placed directly into gas-permeable polythene bags and stored at 5 °C in a cool box. For total elemental C and N measurement, a subsample of soil from each mesocosm was

dried at 105 °C and ball milled before analysis by dry combustion using a CN-2000 analyser (Leco Corp, St Joseph, MI, USA).

Soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were determined following extraction from soil in 0.5 M K_2SO_4 in a 1:5 (w/v) slurry and horizontal shaking for 1 hour at 200 rpm, followed by centrifugation at 4000 rpm for 10 min. Nitrate was determined colourimetrically by the vanadium chloride reduction method (Miranda et al., 2001). Ammonium was determined by the salicylate-hypochlorite photometric method of Mulvaney (1996). Plant-available phosphorus was extracted by horizontal shaking from fresh soil in 0.5 M acetic acid ($\text{CH}_3\text{CO}_2\text{H}$) at a ratio of 1:5 (w/v) and determined colourimetrically as molybdate-reactive P (Murphy and Riley, 1962). Readings obtained from fresh soil samples were adjusted for moisture content after oven drying at 105°C which was determined gravimetrically (Rowell, 1994). Soil pH and EC were measured in 1:2.5 v/v slurry of distilled water according to Smith and Doran (1996). Soil dissolved organic C (DOC) and total dissolved N (TDN) were determined using a Shimadzu TOC-V-TN analyser (Shimadzu Corp., Kyoto, Japan) following extraction (1:5 w/v) in 0.5 M K_2SO_4 (Jones and Willett, 2006). Soil chemical properties are shown in Table 2.

2.4. Root-to-shoot ratio

To assess differences in the source sink relationships between tree species we calculated the ^{14}C activity specific root: shoot ratio (Durall et al., 1994) and adjusted for fundamental differences in above/ belowground biomass using the following equation:

$$\frac{\text{kBq in root g}^{-1} \text{ root dry weight}}{\text{kBq in shoot tissue g}^{-1} \text{ shoot dry weight}}$$

2.5. Statistical analysis

Prior to analysis all data were tested for homogeneity of variance using Levene's test. Data was structured with two independent groups (factors): (i) species treatments comprised of the *A. glutinosa*, *B. pendula* and *C. sativa* as variables; and (ii) time. The dependant data (total plant biomass and partition biomass, soil chemical properties and ^{14}C activity of biomass pools, ^{14}C labelled respiration efflux) were tested for normality prior to analysis using the Shapiro-Wilk Test. The EC data were \log_{10} transformed to satisfy the assumption of normality. The statistical analysis was conducted using a two-way analysis of variance (ANOVA) and Tukey's post-hoc

test with SPSS® Statistics version 25.0 (IBM Corp, Chicago, 2017). Main and interactive effects were considered significant at $P \leq 0.05$.

3. Results

3.1. Experimental establishment

At the start of the pulse label, the total biomass of the three trees species were 57.4 g, 28.6 g and 26.9 g for *A. glutinosa*, *B. pendula* and *C. sativa* respectively (Table 1). At each of the sampling points, the *A. glutinosa* was consistently larger and by the end of the experiment its total biomass was twice that of the other two species. There was a statistically significant difference between the total biomass of the three tree species at the final harvesting time [$F_{(2, 9)} = 9.422$, $P = 0.01$] (Table 1). A post-hoc Tukey test also showed that the significant differences between the biomass partitions of the three species. Key findings included that all the biomass partitions of *A. glutinosa* were significantly larger than those of both *B. pendula* and *C. sativa*, with the exception of the woody branch and stem partitions. *A. glutinosa* was shown to have both larger leaves and both fine and coarse roots but had relatively less branches and stems. In contrast, the plant biomass partitioning for *B. pendula* and *C. sativa* were not significantly different from each other.

The growth rates during the establishment period of the experiment for the three species studied were $7.67 \times 10^{-3} (\pm 1.61 \times 10^{-3})$ g/h, 5.87×10^{-3} g/h ($\pm 0.20 \times 10^{-3}$), 4.08×10^{-3} g/h ($\pm 0.43 \times 10^{-3}$) dried weight for *A. glutinosa*, *B. pendula* and *C. sativa*, respectively. Each of the four biomass partitions measured for each tree species at each pulse-chase sampling time is shown in Table 1. The growth rates were found to be significantly different from each other [$F_{(2, 9)} = 7.25$, $P = 0.01$]. Overall, *A. glutinosa* had a growth rate that was significantly different to those of both *B. pendula* and *C. sativa*, whereas *B. pendula* and *C. sativa* had growth rates that were not significantly different from each other.

Table 1: Mean (g dwt) \pm SE biomass of four biomass partitions and total biomass of *A. glutinosa*, *B. pendula* and *C. sativa* determined by destructive harvesting at 0, 24, 72, 168 and 336 hours after the $^{14}\text{CO}_2$ pulse was applied after destructive harvesting ($n = 4$). Statistically significant results are given in bold and denoted by asterisks (*, $P < 0.1$; **, $P < 0.05$; ***, $P < 0.001$).

Tree species	Tree Partition	Hours after pulse					<i>P</i> -value	
		0	24	72	168	336	336 hours after pulse label completed	
<i>A. glutinosa</i>	New leaf	0	0	0	0.95 ± 0.12	1.75 ± 0.47	<i>B. pendula</i>	<i>C. sativa</i>
	Old leaf	30.60 ± 6.23	30.15 ± 0.89	27.39 ± 5.16	30.37 ± 6.73	34.34 ± 6.52	0.01*	0.02*
	Branch	12.05 ± 1.41	13.30 ± 0.30	11.09 ± 2.11	15.45 ± 2.36	22.02 ± 3.49	0.09	0.21
	Root	14.76 ± 3.03	14.67 ± 1.59	16.19 ± 2.59	13.43 ± 3.12	18.41 ± 5.63	0.00**	0.00**
	Total	57.41 ± 10.58	58.11 ± 10.58	54.67 ± 9.04	60.20 ± 11.45	76.52 ± 15.0	0.01*	0.02*
<i>B. pendula</i>	New leaf	0	0	0	1.28 ± 0.15	1.15 ± 0.48	<i>A. glutinosa</i>	<i>C. sativa</i>
	Old leaf	15.20 ± 0.74	15.24 ± 0.74	12.18 ± 1.58	11.61 ± 1.41	11.04 ± 1.55	0.01*	0.68
	Branch	6.08 ± 0.54	8.34 ± 0.43	2.96 ± 0.61	5.71 ± 0.15	7.32 ± 0.41	0.09	0.83
	Root	7.34 ± 0.62	7.30 ± 0.66	3.64 ± 0.62	6.97 ± 1.79	6.69 ± 0.80	0.00**	0.96
	Total	28.61 ± 1.28	30.88 ± 1.82	18.77 ± 2.56	25.57 ± 2.90	26.20 ± 2.47	0.01*	0.84
<i>C. sativa</i>	New leaf	0	0	0	0.88 ± 0.19	1.4 ± 0.42	<i>A. glutinosa</i>	<i>B. pendula</i>
	Old leaf	13.18 ± 1.60	13.87 ± 1.63	12.83 ± 0.99	13.43 ± 1.53	15.81 ± 1.36	0.02*	0.68
	Branch	5.67 ± 0.58	6.50 ± 0.18	4.79 ± 0.60	4.89 ± 0.71	6.55 ± 0.61	0.21	0.83
	Root	8.00 ± 0.90	7.32 ± 0.17	7.99 ± 0.59	8.95 ± 0.55	9.55 ± 1.38	0.00**	0.96
	Total	26.85 ± 2.85	27.69 ± 1.88	25.60 ± 0.95	28.15 ± 2.45	33.3 ± 1.73	0.02*	0.84

3.2. Soil chemical properties

Soil chemical properties for each of the species studied determined at the start of the experiment are shown in Table 2. Overall, there were few differences between treatments with the exception of the higher soil NO₃⁻ in the *A. glutinosa* treatments and lower pH under *C. sativa*.

Table 2: Soil chemical properties for soil collected from the mesocosms 0 hours after the ¹⁴C pulse was complete for all three species treatments (*A. glutinosa*, *B. pendula* and *C. sativa*). Data are mean ± SE (*n* = 4). Significant differences are highlighted in bold and the level of significance is denoted by **P* < 0.05.

Soil chemical properties								
Tree species	NO ₃ ⁻ (mg/kg)	NH ₄ ⁺ (mg/kg)	P (mg/kg)	pH	EC (μS/ cm)	C:N ratio	DON (mg/kg)	Total C (mg/kg)
<i>A. glutinosa</i>	12.52 ± 4.38 a	3.15 ± 0.95	6.94 ± 0.60	5.99 ± 0.23 b	112 ± 27	9.42 ± 0.70	6.57 ± 0.28	26.88 ± 0.98
<i>B. pendula</i>	3.73 ± 0.61 b	3.37 ± 0.25	7.88 ± 0.54	6.14 ± 0.10 b	124 ± 36	10.01 ± 0.23	5.92 ± 0.38	29.21 ± 1.39
<i>C. sativa</i>	4.61 ± 0.73 ab	3.00 ± 0.40	6.74 ± 1.52	5.65 ± 0.04 a	76 ± 4	9.48 ± 0.51	6.60 ± 0.88	35.29 ± 8.14
<i>Probability</i>	0.04*	0.42	0.68	0.01*	0.45	0.68	0.42	0.50

(EC: electrical conductivity, TC: total soluble C)

3.3. Allocation of ^{14}C to biomass compartments

^{14}C allocation to the different plant tissues (as a percentage of total ^{14}C detected in the entire tree), was significantly different between species (see Table 3) [$F_{(2, 537)} = 0.002$, $P = 0.027$]. A Tukey post-hoc test to see which plant partitions had significantly different ^{14}C allocation, revealed that ^{14}C allocation of *A. glutinosa* to leaves was significantly different to that of both *B. pendula* ($P = 0.01$) and *C. sativa* ($P = 0.02$). In contrast, *B. pendula* and *C. sativa* were found to not have differences in ^{14}C allocation to the leaf partition. The allocation of ^{14}C to the branch showed the same trend with *A. glutinosa* allocation being different to both *B. pendula* ($P < 0.001$) and *C. sativa* ($P < 0.001$). The belowground allocation of ^{14}C to the root partition contrarily did not show any differences between the results of the three species treatments.

Table 3: ^{14}C activity of each biomass plant partition determined after destructive harvesting expressed as a percentage of the total ^{14}C activity photo assimilated ^{14}C during the pulse labelling. Data are mean \pm SE ($n = 4$)

Tree species	Tree partition	Hours after $^{14}\text{CO}_2$ pulse to the tree canopy				
		0	24	72	168	336
<i>A. glutinosa</i>	New leaf	0	0	0	2.53 ± 0.28	1.05 ± 0.17
	Old leaf	93.11 ± 1.49	71.39 ± 3.20	44.29 ± 12.32	50.05 ± 3.99	38.75 ± 6.57
	Branch	3.43 ± 1.77	14.73 ± 1.49	12.32 ± 2.48	15.35 ± 3.65	8.49 ± 1.56
	Root	0.24 ± 0.06	4.40 ± 0.58	12.77 ± 0.66	9.85 ± 2.44	6.01 ± 2.13
	Root-free	2.30 ± 0.88	9.24 ± 2.09	28.54 ± 5.09	21.47 ± 7.29	19.61 ± 8.42
	Soil H_2O	$< 0.01 \pm 0.00$	$< 0.01 \pm 0.00$	$< 0.01 \pm 0.00$	$< 0.01 \pm 0.00$	$< 0.01 \pm 0.00$
<i>B. pendula</i>	New leaf	0	0	0	7.14 ± 1.26	1.13 ± 0.28
	Old leaf	92.75 ± 0.78	47.22 ± 7.05	45.17 ± 3.55	24.87 ± 1.36	16.42 ± 3.69
	Branch	3.09 ± 0.19	10.92 ± 2.30	8.06 ± 1.56	15.50 ± 3.10	7.48 ± 0.74
	Root	0.96 ± 0.41	5.40 ± 1.87	9.89 ± 1.10	14.36 ± 2.88	8.35 ± 0.50
	Root-free	2.69 ± 0.74	36.33 ± 11.61	36.03 ± 11.26	37.71 ± 7.75	26.42 ± 5.40
	Soil H_2O	$< 0.01 \pm 0.00$	$< 0.01 \pm 0.00$	$< 0.01 \pm 0.00$	$< 0.01 \pm 0.00$	$< 0.01 \pm 0.00$
<i>C. sativa</i>	New leaf	0	0	0	5.16 ± 1.50	
	Old leaf	88.27 ± 1.03	55.90 ± 11.27	46.85 ± 7.02	25.29 ± 4.70	
	Branch	5.28 ± 0.96	9.62 ± 2.32	11.03 ± 1.84	8.37 ± 1.97	
	Root	2.43 ± 0.39	5.46 ± 2.23	19.36 ± 2.39	13.20 ± 3.34	
	Root-free	3.63 ± 1.12	28.65 ± 12.37	21.10 ± 3.60	47.42 ± 12.85	
	Soil H_2O	$< 0.01 \pm 0.00$	$< 0.01 \pm 0.00$	$< 0.01 \pm 0.00$	$< 0.01 \pm 0.00$	

The initial harvesting interval directly after the pulse-labelling event showed that the pulse label technique resulted in consistent ^{14}C photo-assimilation across the replicates and the three-tree species. This experiment utilises ^{14}C as a surrogate for recently fixed C thus allowing estimations of the allocation of this C to each of the plant-fungal soil partitions and a comparison amongst the three studied species. This is summarised for the three species as schematic diagrams (Figure 2). The total plant ^{14}C activity determined at 0 hours (directly after the pulse labelling was complete) is assumed to be the total fixed ^{14}C for all the subsequent time treatments. The pools sizes shown in Figure 2, are shown as a percentage of the assumed ^{14}C fixed during the pulse that remained in each of the plant-soil partitions. C allocation as a percentage of the total C within the system is tabulated in Table 4. The ^{14}C remaining in the soil after the roots and soil solution had been recovered is assumed to be largely microbial biomass. The ^{14}C not recovered in any of the plant or soil partitions is therefore assumed to have been either respired by the tree roots or the soil microbial community. An estimation of aboveground respiration was calculated by using the ^{14}C recovered by destructively harvesting the mesocosm immediately after the pulse event as a proxy for the total amount fixed in all time treatments. Once the mass balance calculations for all the plant tissues, soil solution, soil and belowground respiration were combined and subtracted from the proxy for total ^{14}C fixed, then the resulting percentage was assumed to be aboveground respiration from leaves, stem and branch (Figure 2). Key findings include that on average $74.2 \pm 18.9 \%$, $59.9 \pm 10.6 \%$ and $83.8 \pm 18.0 \%$ in *A. glutinosa*, *B. pendula* and *C. sativa* respectively, of the total pulse labelled ^{14}C was recovered as plant or microbial biomass. We estimate therefore that respiration was on average $25.8 \pm 9.6 \%$ from the *A. glutinosa* treatments, $40.1 \pm 5.6 \%$ from the *B. pendula* treatments and $15.7 \pm 11.1 \%$ from the *C. sativa* microcosms. This was calculated by using the surface area of the respiration trap and scaling this up to the whole pots soil surface. However due to the sampling technique we were unable to definitively separate autotrophically or heterotrophically derived $^{14}\text{CO}_2$.

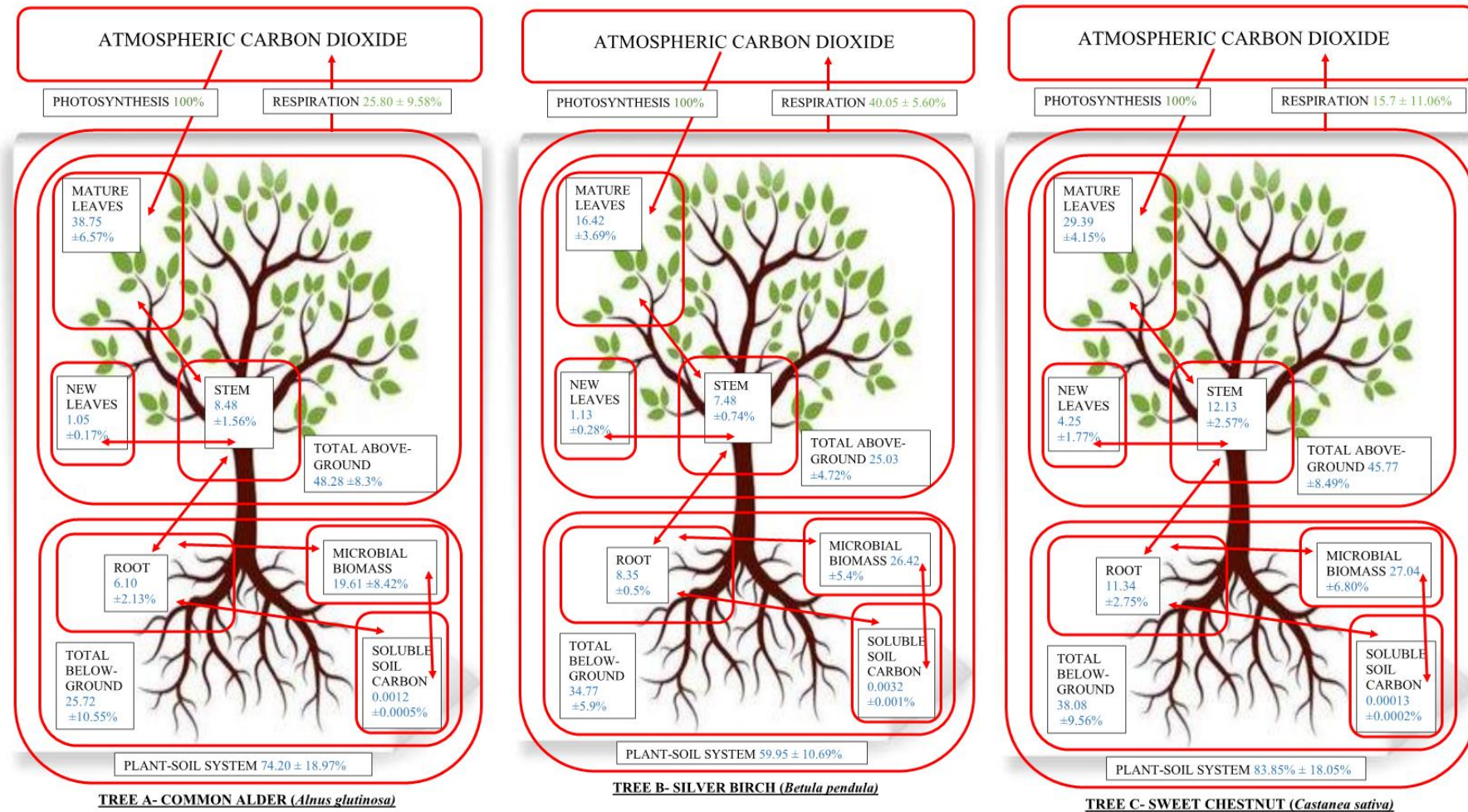


Figure 2: Systematic schematic diagrams of three tree species showing the ^{14}C fluxes and pools as a percentage of the activity assumed to have been fixed during the $^{14}\text{CO}_2$ pulse label applied to the tree canopy.

3.4. ^{14}C activity specific root-to-shoot ratio

The root-to-shoot specific activity can be seen to increase initially after the pulse event (Figure 3). Subsequently, as the newly photosynthesised ^{14}C is translocated into the tree roots it is either exuded or respired by the roots or mineralised by the soil microbes and therefore the root-to-shoot specific ^{14}C activity is seen to decrease. Allocation of assimilated ^{14}C was similar between all species for the first 72 hours with a root-to-shoot ratio ranging from 0.54 for *A. glutinosa* to 0.87 for *B. pendula*. After 72 hours, the allocation of ^{14}C between species diverged with the root-to-shoot ratio of *B. pendula* continuing to increase, whilst the root-to-shoot ratio of both *A. glutinosa* and *C. sativa* decreased by 19 and 23 % respectively. 360 hours after the pulse-labelling, the root-to-shoot ratio of *B. pendula* was 1.04, which was different to *A. glutinosa* ($F_{(2,9)} = 7.12$, $P = 0.01$), but not to *C. sativa* ($F_{(2,9)} = 7.12$, $P = 0.06$) (Figure 3).

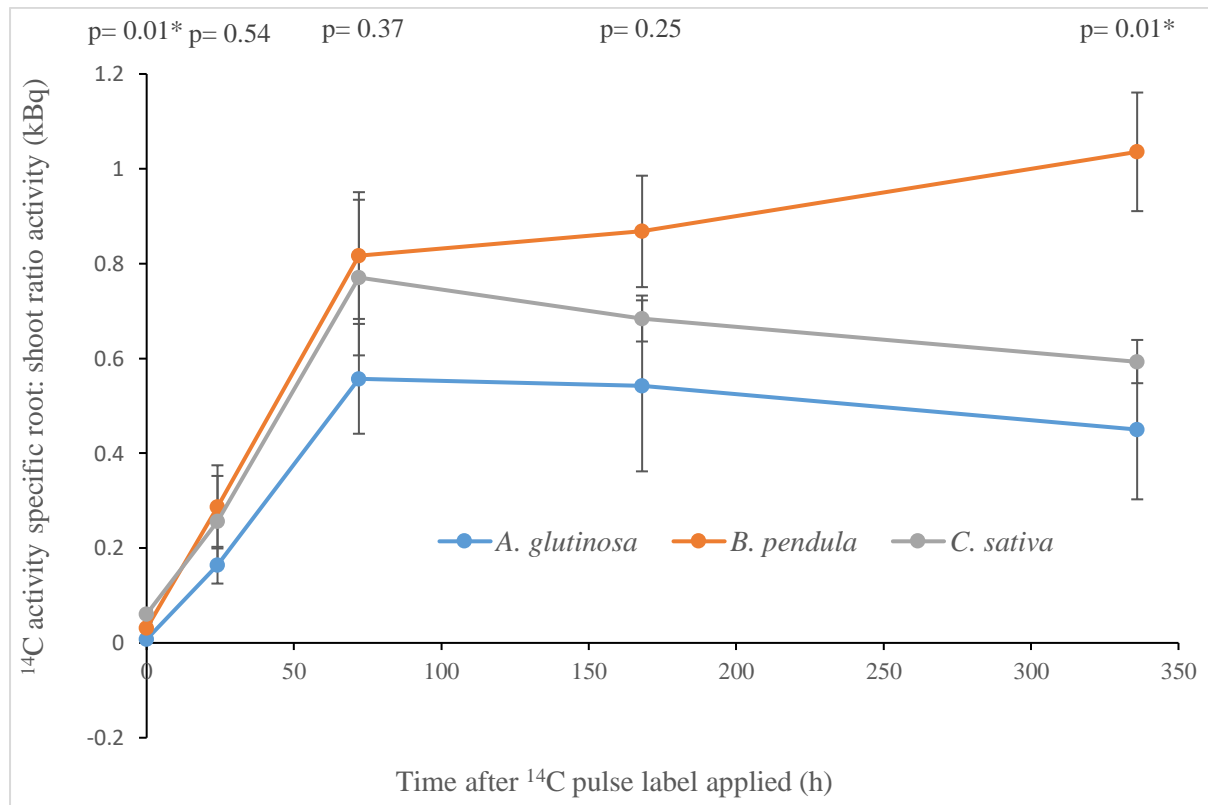


Figure 3: Root-to-shoot specific ^{14}C activity ratio over time of all three-tree species studied values represent mean \pm SE ($n = 4$).

3.5. Allocation of ^{14}C belowground

The percentage of ^{14}C detected in the belowground component of *A. glutinosa* was about ten percent less than that of the other two species although it does appear that the transfer takes place quicker in the case of *A. glutinosa* with the harvesting period 24 hours after the pulse was completed showing maximum belowground C (before respiration has started to reduce this) (Figure 4). In contrast, in *C. sativa* and *B. pendula* the mean maximum belowground allocation was delayed until after 168 and 336 hours after the pulse label was completed, respectively. The differences in timing of maximal belowground allocation was not found to be significant [$F_{(2, 9)} = 0.87$, $P = 0.45$].

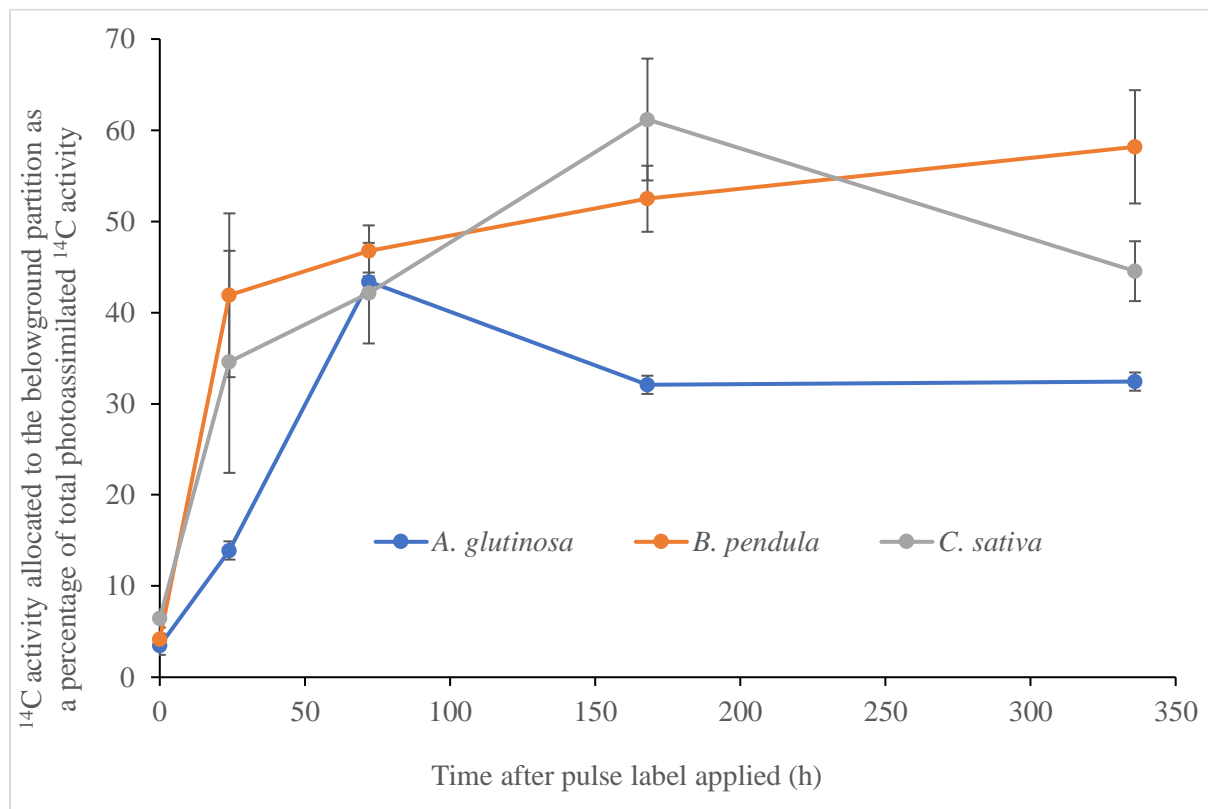


Figure 4: Mean total ^{14}C activity allocated to belowground plant partition determined after destructive harvesting expressed as a percentage of the total ^{14}C activity photo-assimilated during the pulse label. Data are mean \pm SE ($n = 4$).

Overall, there was a significant difference between the allocation of ^{14}C belowground as a percentage of total photoassimilated ^{14}C of the three species [$F_{(2,57)} = 3.32$, $P = 0.04$]. Examination of the differences at the end of the experiment (336 hours after the pulse was completed) (Figure 5) between species showed that belowground allocation of C in *A. glutinosa* was 25.8 % less than *B. pendula* [$F_{(2, 57)} = 3.32$, $P = 0.05$], but not different to *C. sativa* ($P =$

0.13), and that there was no difference in belowground C allocation between *B. pendula* and *C. sativa* [$F_{(2, 57)} = 3.32$, $P = 0.89$].

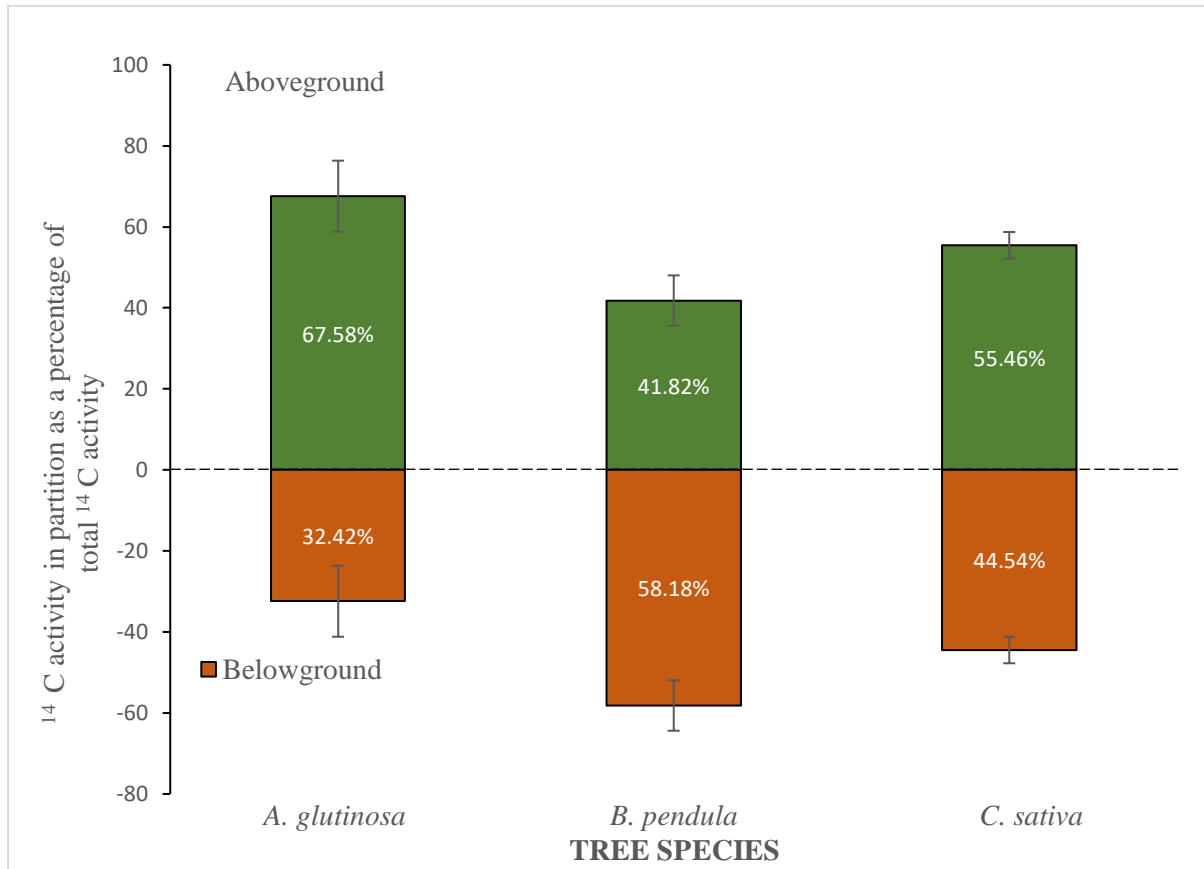


Figure 5: ^{14}C as a percentage of the total amount of ^{14}C fixed allocated to the above and belowground partitions in the three species (*A. glutinosa*, *B. pendula*, *C. sativa*). Data are mean \pm SE ($n = 4$).

3.6. Transfer of ^{14}C to microbial community (via mycorrhizal fungi)

The transfer of ^{14}C to the belowground partitions revealed some interesting differences between species. For example, the mean ^{14}C as a percentage of total fixed ^{14}C detected under *B. pendula* was approximately 10 times higher than under *A. glutinosa*. The ^{14}C activity located in the soil was shown to be significantly different under *A. glutinosa* when compared with *B. pendula* and *C. sativa* ($P = 0.04$).

The soil respiration from under the *C. sativa* was approximately four times higher than the $^{14}\text{CO}_2$ respired from under the other two species and was found to be significantly different to the soil respiration under *B. pendula* and *A. glutinosa* ($P = 0.02$). The ^{14}C activity in the soil solution was not found to be significantly different in any of the three species treatments. Total estimated transfer of C to micro-organisms, as a percentage of total fixed ^{14}C was 10 times less in *A. glutinosa* than the other two species and all three species were significantly different from each other as shown in Table 7 ($P < 0.001$). The ^{14}C activity measured in the soil solution was very low and therefore unlikely to be driving differences in ^{14}C transferred through the mesh suggesting that these results can largely be explained by differences in movement of ^{14}C via mycorrhizal symbionts.

Table 4: Estimated total ^{14}C activity transferred to microbial community including mycorrhizal fungi expressed as a percentage of total ^{14}C fixed (estimated by total ^{14}C measured immediately post-pulse (time 0 h). Data is a mean \pm SE ($n = 4$).

Mean accumulated ^{14}C activity as % of total fixed ^{14}C				
Tree Species	^{14}C activity in soil	^{14}C activity in soil pore H_2O	$^{14}\text{CO}_2$ respired	Estimated total transfer to microbial biomass
<i>A. glutinosa</i>	2.99 ± 0.66 a	$2.75 \times 10^{-4} \pm 9.87 \times 10^{-5}$	$2.00 \times 10^{-2} \pm 0.724 \times 10^{-2}$ a	3.01 ± 0.66 a
<i>B. pendula</i>	32.83 ± 10.29 b	$2.03 \times 10^{-4} \pm 4.18 \times 10^{-5}$	$2.77 \times 10^{-2} \pm 1.18 \times 10^{-2}$ a	32.9 ± 10.3 b
<i>C. sativa</i>	22.05 ± 6.44 b	$1.85 \times 10^{-4} \pm 4.65 \times 10^{-5}$	$7.78 \times 10^{-2} \pm 3.54 \times 10^{-2}$ b	22.1 ± 6.5 c
<i>P-value</i>	0.04	0.42	0.02	0.00

4. Discussion

^{14}C activity was detected in both the root biomass partition and Rhizon-extracted soil solution, immediately after the 2 h pulse label suggesting that ^{14}C was almost immediately exuded from the roots of all three species studied and that the $^{14}\text{CO}_2$ had been successfully absorbed during the pulse labelling event. Consequently, the ^{14}C activity measured in the destructively

harvested trees of all three species tree partitions was used as a proxy for the total ^{14}C activity assimilated for all subsequent percentage calculations.

4.1. Effect of establishment period on biomass and soil chemistry

To allow the tree saplings to establish and the roots to become colonised by mycorrhizal fungi, the trees were planted into mesocosms and grown for nine months in an unheated glasshouse in a randomised block design. As growth rates are species-specific, differences in plant biomass were apparent between the studied species at the start of the experiment. However, there was only a minimal amount of variation between the biomass compartments of each species studied therefore allowing comparison of C partitioning between and within species. Previous studies have shown that colonisation of roots by both AM and EM fungi can be suppressed in highly fertile soils, especially in the presence of high amounts of available N (Smith and Read, 2010; Kobae et al., 2016) and by long-term N deposition (Lilleskov et al., 2018). Fungal spore germination and viability can also be significantly reduced under high phosphorus conditions (Xavier and Germida, 1999). Suppression of colonisation has been explained by reduced requirement for nutrient acquisition via symbiosis i.e. that a tree that can support itself with its required nutrition will not need to invest photosynthetically derived carbohydrates into the development of microbial symbiosis (Johnson, 2010; Wagg et al., 2011; Johnson et al., 2015). The soils used in this work were Eutric Cambisols collected from a former agricultural field, however, our analysis only showed moderate amounts of nutrient enrichment (N, 6.95 ± 1.91 mg/kg; P, 7.19 ± 0.89 mg/kg P; mean of all species treatments \pm SE), and therefore it is possible that mycorrhizal colonisation was not greatly affected. Limited mycorrhizal colonisation due to high soil nutrient status would influence the translocation of C belowground and any attempt at extrapolation should be undertaken with caution. Oburger and Jones, (2018) highlighted that mesocosm based experiments are unlikely to accurately model real-life C dynamics in the field, due in part to faster microbial turnover and decomposition rates resulting from artificially increased temperatures and extreme variation in diurnal temperatures that can be a feature of glasshouse growing conditions.

At the start of the experiment prior to pulse labelling the soil chemistry was tested to determine if the species had significantly altered the belowground nutrient availability. It was found that the nitrate was significantly higher under *A. glutinosa* (see Table 2) than either of the other species studied. This is not surprising as *A. glutinosa* is actinorhizal, however, the biomass of *A. glutinosa* were significantly larger than the other species treatments and the nitrate levels

were still high, suggesting that if plant growth limitation occurred in *A. glutinosa* that nitrate was not the factor causing limitation. The only other chemical property which showed a significant difference between species treatments was pH where *C. sativa* was found to be significantly different to the other two and was slightly more acidic. This could be as a result of differences in chemical exudates released from the roots into the soil during the establishment period.

After the 9-month establishment period the *A. glutinosa* trees were larger, as can be seen in Table 2, and had a significantly larger total biomass. The growth rate of the *A. glutinosa*, *B. pendula* and *C. sativa* during the establishment period were 7.20, 3.07 and 2.84 g/h for, respectively. This was not unexpected as the literature suggests that *A. glutinosa* is a species with a growth rate which is fast relative to the other two species studied (Kupper et al., 2018). Differences in relative growth rates between tree species have been linked to the trees successional stage, with early-successional pioneer trees typically possessing small wind distributed seeds and high growth rates (Baltzer and Thomas, 2007; Modrzyński et al., 2015). In contrast, late-successional stage trees trade-off high growth rates in lighter conditions against persistence under low light levels (Walters and Reich, 2000, Modrzyński et al., 2015). *A. glutinosa* is actinorhizal and as the trees were potted up without additional fertilisation. It was anticipated that during the establishment period nutrients would become a limiting factor and that the *A. glutinosa* would invest carbohydrates into the developing a relationship with *Frankia alni* to negate this and after harvesting root nodules were observed. In the *A. glutinosa* treatments, this could explain the faster growth rate during the duration of the experiment (Sroka et al., 2018). *B. pendula* had the second largest biomass of three species, which again was expected as *B. pendula* is an early successional stage pioneer species which is short lived, and wind dispersed. As a result, *B. pendula* was expected to be faster growing than *C. sativa*. The growth period occurred in a glasshouse during an unexpectedly hot summer in north Wales and the *B. pendula* treatments seemed to suffer more than the other two species. This could explain why the biomass measured in the *B. pendula* was not as much as expected. *C. sativa*, however, is a Mediterranean species (Krebs et al., 2019) which is better adapted to cope with the elevated temperatures created by the glasshouse growing conditions described earlier. Although it is known that *B. pendula* has a large genotypic variation both within and among populations when compared to other temperate trees (Kasurinen et al., 2016), the *B. pendula* biomass data showed less variation (see Table 1) than *A. glutinosa* and slightly more than *C. sativa*.

4.2. Allocation of ^{14}C activity to biomass partitions

Allocation of ^{14}C to the plant partitions showed that *B. pendula* and *C. sativa* allocate a similar amount of C belowground, however, the *C. sativa* treatments retained C in their biomass and in the soil. In contrast, in the *B. pendula* treatments the C is returned to the atmosphere, as either autotrophic respiration by the plants or by heterotrophic respiration from the soil microbial community. This is consistent with the generally held view that plants at different successional stages adopt different nutrient acquisition strategies. Early successional species have higher growth rates, a higher demand for nutrients, are light demanding and have abundant fine rooting systems with long root hairs to facilitate microbial symbiosis (Rodina et al., 2014; Zangaro et al., 2013). Whereas later successional trees are tolerant of shade, have a slower growth rate, a lower nutrient demand and lower branching of fine roots with shorter root hairs (Rodina et al., 2019). In earlier successional stage trees a larger percentage of fixed C is invested belowground in the fine root system for utilisation by the roots for growth, repair or respiration, to facilitate root exudation and therefore initiate and maintain microbial and more specifically mycorrhizal symbiosis (Rodina et al., 2019). The soil micro-organisms are fundamental in mediating belowground biogeochemical cycles, in the transformation of organic matter and consequently plant nutritional acquisition (Blagodatskaya, 2014), which is a prerequisite of early successional tree growth and survival, but not late-stage trees as they tend to inhabit better formed, relatively fertile soils with higher organic matter.

When understanding the differences in allocation to partitions and belowground of ^{14}C activity as a proxy for C it is necessary to consider the differences in source (such as leaves) sink (parts of tree unable to photosynthesise but which demand carbohydrates) strength between species (Lacointe, 2000). The literature suggests that limited physical sink size may reduce photosynthesis as leaves accumulate leaf starch (Mahmud et al., 2018; Company et al., 2017). It is possible therefore that if the plants were root-bound during the establishment period that photosynthesis was limited by a lack of sink/ storage space for the carbohydrates. If this were the case, we would expect to see the tree growth rates declining and root biomass occupying the majority of soil volume. The mean root biomass for the three species studied measured after the final 336-hour pulse label was completed were 18.4 ± 5.6 g, 6.7 ± 0.8 g, 9.6 ± 1.4 g for *A. glutinosa*, *B. pendula* and *C. sativa*, respectively. Visual inspection of the root ball when removed from the 10-litre volume mesocosm suggested that the three species were not root-bound. The most productive species, *A. glutinosa*, had a mean root biomass 2- 3 times greater

than that of the other species and retained ^{14}C in old-leaves rather than translocating C to other plant partitions.

4.3. ^{14}C activity specific root-to-shoot ratio

The ^{14}C activity specific root-to-shoot ratio confirmed what had already been established by the allocation of ^{14}C belowground data. Specifically, it showed that the ^{14}C activity that was translocated from shoot to root was largest and quickest in *B. pendula* and least and slowest in *A. glutinosa* with *C. sativa* somewhere between the two. This is as we would expect as previously discussed early successional trees would inhabit poorer soils and are subsequently invest more C in facilitating symbiotic relationships with soil micro-organisms and would have to do this promptly in poor soil types to gain the nutrition needed for growth. Previous studies have shown that *B. pendula* has large fine root biomass and abundant short root tips relative to other temperate tree species (Priha et al., 1999). This will result in a stronger sink strength comparatively to *A. glutinosa* and *C. sativa* and therefore may go some way to explaining the speedier transfer belowground that is clearly demonstrated by these results. A larger number of shoot root tips are normally a functional trait associated with plants that need to ensure they develop mycorrhizal associations to fulfil their nutritional requirements (Kleinert et al., 2018).

C allocation belowground has also been shown to be affected by both biotic (e.g. herbivory) and abiotic (e.g. climate) variables and it is estimated that root production accounts for between 33–67 % of global terrestrial net primary productivity (Abramoff and Finzi, 2015). In particular, the storage of non-structural carbohydrates (NSC) belowground at the end of the temperate growing season is determined by climatic conditions and photoperiod, with some species reported as storing NSC belowground for more than ten years (Richardson et al., 2013). The ability of particular tree species in storing starch and soluble sugars belowground varies greatly (Richardson et al., 2015) and studies suggest that belowground allocation is phenologically asynchronous with aboveground allocation (Abramoff and Finzi, 2015). As air temperature warms faster than the soil temperature in the spring some studies report root growth lagging shoot growth by several weeks the reverse could be true in autumn (Steinaker and Wilson, 2008). The current model of plant allocation to partition is known as the Muench mass flow model (Thoms et al., 2017). It suggests a mass-flow system driven by continuous pressure allowing transport of carbohydrates along source sink gradients. This way plants maintain a concentration gradient that drives flows through osmoregulation (Ainsworth and Bush, 2011; Minchin and Thorpe, 1996).

Although we are beginning to understand the mechanisms which mediate the shoot to root allocation, our understanding is far from exhaustive at least in part to the multiple complex factors that can affect it including biotic (species, functional trait, successional stage, microbial symbiosis) and abiotic (soil chemical and physical properties, climate, elevation) (Qi et al., 2019). The optimal allocation hypothesis theorises that plants can modify their allocation strategies according to environmental conditions but that generally speaking those trees with greater belowground biomass will be better suited to sites with high competition or less than optimal water and nutrient regimes (Eziz et al., 2017; Ledo et al., 2018).

4.4. Allocation of ^{14}C activity belowground

In most of the tree species treatments, less than half of the total ^{14}C found in the mesocosm was allocated to the belowground partitions, with a mean of 32.4 % and 44.5 % for *A. glutinosa* and *C. sativa*, respectively. In the case of *B. pendula*, the transfer of C belowground was higher than the other two species with an average of 58.18 % of the ^{14}C activity located belowground at the last destructive harvest (360 hours after pulse-label was complete). It is known that mycorrhizal colonisation of tree roots plays an important role in facilitating primary succession (Brundrett, 2002) and the subsequent soil formation processes (Del Moral and Rozzell, 2005). Pioneer species involved in the initial colonisation of substrates will experience not only unfavourable nutrient regimes, but also extreme microclimatic conditions (e.g. drought), due to the lack of buffering capacity of young mineral soils with a low organic matter content. In these instances, mycorrhizal symbiotic relationships may have an important role in ensuring the persistence of early successional species by increasing water availability and therefore drought tolerance (Kikvidze et al., 2010).

The estimation of total belowground respiration revealed that the highest mean ^{14}C activity was respired from under the *B. pendula* treatments suggesting faster throughput of recently fixed C to soil solution where it was likely utilised by the microbial community as non-structural (not taken up by microbial biomass) and resulted in higher heterotrophic respiration. This could be as a result of the availability of the form of carbohydrate exuded by the roots of this particular species and relates to the early successional stage of *B. pendula*. *B. pendula* is known to contain more sugar in the sap than most UK native tree species (Ozolinčius et al., 2016) and in many European countries the sap was traditionally used to make *B. pendula* syrup (Enescu, 2017). *B. pendula* sap is approximately 98 % water, the other 2 % is fructose, glucose, acids, such as ascorbic acid (vitamin C), calcium, phosphorus, potassium, magnesium, manganese, zinc

sodium and iron (Korhonen and Maaranen, 2018). The rhizodeposits of *B. pendula* have also been found in studies to be of better nutritional value than those of other tree species such as conifers. The extra input of substrate resulting from the qualitative nature of the *B. pendula* rhizodeposits could have stimulated soil microbial growth, thus leading to the increased heterotrophic respiration in these treatments (Priha et al., 1999). Contrastingly *C. sativa* soil respiration ^{14}C activity and soil solution ^{14}C activity had very low readings, although the transfer of C to microbial biomass is similar to that seen in *B. pendula*. Under the *C. sativa* treatments, more ^{14}C activity was retained in the soil partitions, probably as structural microbial biomass then by the other two species in the following order *A. glutinosa* and *B. pendula*.

As *B. pendula* is an early successional pioneer species, exudation could be a mechanism to promote decomposition, to mineralise nutrients (i.e. organic matter priming) and to develop soil via accelerated weathering of primary minerals. *A. glutinosa* overcomes N being the limiting element in primary succession by N-fixation processes that reduce the need to allocate resources to soil mineralisation processes, whereas allocation of C to leaves maximises photo-assimilation and net primary productivity. Despite both *A. glutinosa* and *B. pendula* being early successional species *A. glutinosa* retained ~15 % more C in the plant-soil system with the majority being retained in aboveground biomass partitions with 26 % respired back to the atmosphere. Whereas in *C. sativa*, the partitioning of C above- and belowground was 46 % and 38 % respectively with only 16 % of the ^{14}C activity being respired.

It has been reported that as *A. glutinosa* is a species which tolerates waterlogged soil has the functional trait of depositing gaseous bubbles into its roots as it grows by a thermo-osmotic gas transport system (Schröder, 1989). These root bubbles are a morphological adaption that has evolved in response to extended periods of waterlogging and as *A. glutinosa* shares this trait with many aquatic plants, it has been suggested that it results in the species tolerance to waterlogging (Gill, 1975). The mechanism by which this is achieved is not entirely understood with Schröder suggesting that *A. glutinosa* can transport O_2 through its stems and therefore improve the supply to respiring roots under waterlogging (Schröder, 1989). However, a study that analysed the composition of the root gas bubbles and found the root gas was slightly lower in O_2 and richer in CO_2 than that of the atmospheric air and hypothesised that the root bubbles are enriched in CO_2 by root respirations (McVean, 1956). As these results were found in well-drained soil (below the soil water table) it is possible that the root bubbles are occupied by either O_2 absorbed by the stem and translocated to the roots or CO_2 respired by the root depending on the history of previous waterlogging and if the soil is anoxic or aerobic (McVean,

1956). Dittert et al., (2006) found that O₂ net flow was inversely correlated to O₂ concentration in the rooting media, suggesting that when root and microbial respiration increased the flux of O₂ from the stems into the root. In contrast to *A. glutinosa*, *B. pendula* allocates C belowground, but it would appear that more C is exuded into the soil solution and that a combination of microbial turnover and plant metabolism results in 40 % being respired back to the atmosphere. *B. pendula* as a species is not well suited to waterlogged soils and has likely contrasting root mechanisms as a result.

Methodological issues, such as the location of respiration traps could have compromised the reliability of both the microbial or tree root respiration data, particularly that heterogeneity may not have been captured by the use of one small respiration trap (which was 2.96 % of the total area of each half mesocosm) being used in each half of the bisected mesocosm. Differences in root architecture, as a result of different techniques used during propagation in the tree nursery, meant that the *A. glutinosa* and *B. pendula* seedlings were undercut whilst the *C. sativa* were cell-grown, which could have affected subsequent comparative root development. It is also interesting to note that although *B. pendula* is known to have more genetic diversity than most temperate tree both (Eriksson et al., 2003; Rusanen et al., 2003; Hynynen et al., 2009; Possen et al., 2014) that the standard error observed was smaller in this treatment than the other two.

The biomass of the three root partitions were 18.4 ± 5.6 g, 6.7 ± 0.8 g and 9.6 ± 1.4 g, for *A. glutinosa*, *B. pendula* and *C. sativa*, respectively. Of the three species, *B. pendula* is known to have the largest proportion of fine roots. Priha et al., (1998) found that *B. pendula* seedlings had a mean of 11,450 short root tips. However, root washing has been reported by others to be a potential source of inaccuracy for this experiment type because due to time constraints roots are often under sampled (Addo-Danso et al., 2016; Berhongaray et al., 2013; Levillain et al., 2011; Taylor et al., 2013). It is known that during washing procedures, fine roots and root hairs have the potential to be damaged and lost (Judd et al., 2015) even when utilising the small sieve size (0.2 mm²). Estimates of dry biomass losses from the standard root washing, drying and storage technique range from 20 % to 40 % (Aung, 1974; Maria do Rosário et al., 2000). Due to the fact that the *B. pendula* had most likely the highest proportion of fine to coarse roots when compared to the other two species, it seems likely that the quantification of belowground biomass is likely to be least accurate in *B. pendula*.

4.5. Transfer of ^{14}C activity to soil microbial community

The ^{14}C measured on the non-plant fraction is referred to as C transferred to the soil microbial community. Presumably, the majority of this has been transferred through the mesh by either the mycorrhizal hyphae or as exudates transported by the soil water solution before being utilised by the soil microbial community. Reported values for total C exuded belowground as a percentage of total fixed C from the gross primary production are variable and generally around 25– 63 % (Litton et al., 2007). However, the flow of rhizodeposition can be bi-directional and significant amounts of C-based exudate can be reabsorbed by the roots and (Jones and Darrah, 1994) potentially altering the net C flux to soil via exudation.

The results of this analysis were surprising as the total transfer was estimated to be in 3.01 ± 0.66 %, in *A. glutinosa*, 32.86 ± 10.30 % in *B. pendula* and 22.13 ± 6.47 % in *C. sativa*. It is known that the presence of mycorrhizal symbionts can increase the trees allocation of C belowground and the cost of photosynthetically fixed C of a mycorrhizal symbiosis has been reported to be between 4- 20 % for arbuscular mycorrhizal fungi (Řezáčová et al., 2017). Our estimate therefore seems plausible although it is interesting that the species show such a range of C allocated to mycobionts. A previous study quantified the number of viable N-fixing actinomycete *Frankia alni* by collecting a range of soils from under *B. pendula* and *A. glutinosa* and comparing the nodulation capacity by adding diluted soil solutions to potted *Alnus* plants, leaving for a predetermined time and then counting the root nodules (Smolander, 1990). The results found that the nodulation capacity of the soil under birch varied between 490- 6500 nodulation units (nu) cm^{-3} soil and was consistently as high as or higher than of *A. glutinosa*. This is surprising because if the *B. pendula* is using readily accessible carbohydrates as a medium for encouraging the development of free-living N fixing bacteria and as a strategy to overcome nitrogen limitation, then this may go some way to explaining the results we observed.

The total transfer of ^{14}C was problematic to quantify as a significant proportion of it will have been rapidly mineralised and then became difficult to disentangle from the autotrophic respiration of the tree roots. We can assume that the ^{14}C that was collected in the exclusively mycorrhizal partition originated as rhizodeposited C which was then assimilated into microbial biomass before being mineralised as $^{14}\text{CO}_2$. However, some of the respiration trapped on the plant half will have originated from the microbial community inhabiting the rhizosphere. To estimate the total transfer to soil microbes we will assume that the same amount of microbial

respiration occurs in both sides of the mesocosm, although there will likely be more microbial activity in closest proximity to the source of carbohydrates i.e. rhizosphere.

It is known from previous experimental results that rhizodeposition and subsequent transfer to soil micro-organisms and more specifically mycorrhizal fungi is ecologically significant (Godbold et al., 2006). Disentangling the importance of hyphal C from that of the roots they are connected in the field, is problematic. In this laboratory-based experiment we use a divided mesocosm to begin to understand the relative contribution of roots and hyphae to belowground C dynamics. The source of the ^{14}C measured in the mycorrhizal compartment can reasonably be assumed to have originated from roots via the process of exudation either as microbial or hyphal biomass or in soil solution. Visually the mycorrhizal fraction was not heavily colonised by mycelium of hyphae and this is supported by other fungal studies conducted using the same soils (Gunina et al., 2017). The nutrient regime of the soil used is known to have relatively high nitrogen status (Table 2) that will affect mycorrhizal development which could result in an underestimate of the importance of mycorrhizae to soil C storage.

4.6. Hypothesis discussion

Our first hypothesis was that the belowground allocation of C will be greater in species with the largest diversity of microbial symbiotic associations. The results of the comparison of the belowground allocation of ^{14}C activity, 336 hours after the pulse label was completed was 26 %, 35 % and 38 % for *A. glutinosa*, *B. pendula* and *C. sativa*, respectively. The lowest transfer was found in *A. glutinosa* which is actinorhizal, has arbuscular and ectomycorrhizal associations, whereas both *B. pendula* and *C. sativa* possess only ectomycorrhizal associations. Belowground allocation of C in *A. glutinosa* was 25.75 % less than *B. pendula* [$F(2, 57) = 3.32$, $P = 0.05$], but not different to *C. sativa* ($P = 0.13$), and that there was no difference in belowground C allocation between *B. pendula* and *C. sativa* [$F(2, 57) = 3.32$, $P = 0.89$]. The differences between the allocation of ^{14}C belowground as a percentage of total photo-assimilated ^{14}C , 336 hours after the pulse was completed, of the three species treatments was found to be significantly different [$F(2, 57) = 3.32$, $P = 0.04$] specifically and H_1 can therefore be accepted.

The second hypothesis was that the transfer of recently assimilated C to the soil microbial community will be greater in the early successional *B. pendula* and *A. glutinosa* than the late successional tree species *C. sativa*. However, the estimated total transfer to the microbial community (including the mycorrhizal component) using ^{14}C activity as a proxy for recently

fixed C was 3.01 ± 0.66 %, in *A. glutinosa*, 32.86 ± 10.30 % in *B. pendula* and 22.13 ± 6.47 % in *C. sativa*. Therefore although *A. glutinosa* is an early successional species it allocated the least ^{14}C activity to the microbial community, although as the *Frankia alni* are located in the root nodule and were homogenised with the roots some C transferred to internal microbes will not be included in this result. H_2 was therefore rejected.

5. Conclusions

C allocation in three temperate tree species was determined by the use of ^{14}C as a surrogate for recently fixed photosynthates to enable the approximate quantification of C allocated to different plant structures and soil under the three-tree species. The evidence from this study suggests that in the case of C offsetting or storage plantings it seems it would be best to plant *C. sativa* of the three species studied, to maximise belowground C storage. This study highlights the importance of the continuing focus on identifying and understanding the factors involved in belowground competition and facilitation in a range of ecological settings. In particular the fact that many current C accountancy models do not fully quantify the C sequestered belowground to fine roots and subsequently microbial symbionts. Anthropogenic modification of global C cycles is further increasing the need for more accurate C accountancy and global circulatory models. This will enable humanity to identify land-management changes that can best draw-down previously released anthropogenic greenhouse gas emissions and increase C sequestration rates.

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7. References

- Abramoff, R.Z. and Finzi, A.C., 2015. Are above-and below-ground phenology in sync? *New Phytologist*, 205(3), pp.1054-1061.
- Addo-Danso, S.D., Prescott, C.E. and Smith, A.R., 2016. Methods for estimating root biomass and production in forest and woodland ecosystem carbon studies: a review. *Forest Ecology and Management*, 359, pp.332-351.
- Ahmed, I.U., Smith, A.R., Jones, D.L. and Godbold, D.L., 2016. Tree species identity influences the vertical distribution of labile and recalcitrant carbon in a temperate deciduous forest soil. *Forest Ecology and Management*, 359, pp.352-360.
- Ainsworth, E.A. and Bush, D.R., 2011. Carbohydrate export from the leaf: a highly regulated process and target to enhance photosynthesis and productivity. *Plant Physiology*, 155(1), pp.64-69.
- Anderson, I.C. and Cairney, J.W., 2007. Ectomycorrhizal fungi: exploring the mycelial frontier. *FEMS Microbiology Reviews*, 31(4), pp.388-406.
- Aung, L.G., 1974. Root-shoot relationships. *The plant root and its environment*, pp.29-61.
- Baltzer, J.L. and Thomas, S.C., 2007. Physiological and morphological correlates of whole-plant light compensation point in temperate deciduous tree seedlings. *Oecologia*, 153(2), pp.209-223.
- Berhongaray, G., Janssens, I.A., King, J.S. and Ceulemans, R., 2013. Fine root biomass and turnover of two fast-growing poplar genotypes in a short-rotation coppice culture. *Plant and Soil*, 373(1-2), pp.269-283.
- Blagodatskaya, E., Blagodatsky, S., Anderson, T.H. and Kuzyakov, Y., 2014. Microbial growth and carbon use efficiency in the rhizosphere and root-free soil. *PloS one*, 9(4), .e93282.
- Brundrett, M.C., 2002. Coevolution of roots and mycorrhizas of land plants. *New Phytologist*, 154(2), pp.275-304.
- Brzostek, E.R., Dragoni, D., Brown, Z.A. and Phillips, R.P., 2015. Mycorrhizal type determines the magnitude and direction of root-induced changes in decomposition in a temperate forest. *New Phytologist*, 206(4), pp.1274-1282.

- Cairney, J.W., 2012. Extramatrical mycelia of ectomycorrhizal fungi as moderators of carbon dynamics in forest soil. *Soil Biology and Biochemistry*, 47, pp.198-208.
- Campany, C.E., Medlyn, B.E. and Duursma, R.A., 2017. Reduced growth due to belowground sink limitation is not fully explained by reduced photosynthesis. *Tree Physiology*, 37(8), pp.1042-1054.
- Carminati, A., Moradi, A.B., Vetterlein, D., Vontobel, P., Lehmann, E., Weller, U., Vogel, H.J. and Oswald, S.E., 2010. Dynamics of soil water content in the rhizosphere. *Plant and Soil*, 332(1-2), pp.163-176.
- Del Moral, R. and Rozzell, L.R., 2005. Long-term effects of *Lupinus lepidus* on vegetation dynamics at Mount St. Helens. *Plant Ecology*, 181(2), pp.203-215.
- Dilkes, N.B., Jones, D.L. and Farrar, J., 2004. Temporal dynamics of carbon partitioning and rhizodeposition in wheat. *Plant Physiology*, 134(2), pp.706-715.
- Ditengou, F.A., Müller, A., Rosenkranz, M., Felten, J., Lasok, H., Van Doorn, M.M., Legué, V., Palme, K., Schnitzler, J.P. and Polle, A., 2015. Volatile signalling by sesquiterpenes from ectomycorrhizal fungi reprogrammes root architecture. *Nature Communications*, 6, p.6279.
- Dittert, K., Wötzel, J. and Sattelmacher, B., 2006. Responses of *Alnus glutinosa* to anaerobic conditions-Mechanisms and rate of oxygen flux into the roots. *Plant Biology*, 8(02), pp.212-223.
- Durall, D.M., Jones, M.D. and Tinker, P.B., 1994. Allocation of ^{14}C -carbon in ectomycorrhizal willow. *New Phytologist*, 128(1), pp.109-114.
- Ekblad, A., Wallander, H., Godbold, D.L., Cruz, C., Johnson, D., Baldrian, P., Björk, R.G., Epron, D., Kieliszewska-Rokicka, B., Kjølner, R. and Kraigher, H., 2013. The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling. *Plant and Soil*, 366(1-2), pp.1-27.
- Enescu, C.M., 2017. Collection and use of birch sap, a less known non-wood forest product in Romania. *Scientific Papers: Management, Economic Engineering in Agriculture and Rural Development*, 17(1), pp.42-58.
- Epron, D., Bahn, M., Derrien, D., Lattanzi, F.A., Pumpanen, J., Gessler, A., Högberg, P., Maillard, P., Dannoura, M., Gérant, D. and Buchmann, N., 2012. Pulse-labelling trees to study

carbon allocation dynamics: a review of methods, current knowledge and future prospects. *Tree Physiology*, 32(6), pp.776-798.

Eriksson, G., Black-Samuelsson, S., Jensen, M., Myking, T., Rusanen, M., Skrøppa, T., Vakkari, P. and Westergaard, L., 2003. Genetic variability in two tree species, *Acer platanoides* L. and *Betula pendula* Roth, with contrasting life-history traits. *Scandinavian Journal of Forest Research*, 18(4), pp.320-331.

Eziz, A., Yan, Z., Tian, D., Han, W., Tang, Z. and Fang, J., 2017. Drought effect on plant biomass allocation: A meta-analysis. *Ecology and Evolution*, 7(24), pp.11002-11010.

Farrar, J.F. and Jones, D.L., 2000. The control of carbon acquisition by roots. *New Phytologist*, 147(1), pp.43-53.

Finlay, R. and Söderström, B., 1992. Mycorrhiza and carbon flow to the soil. *Mycorrhizal functioning: an integrative plant-fungal process*, pp.134-160.

Gill, C.J., 1975. The Ecological Significance of Adventitious Rooting as a Response to Flooding in Woody Species, with Special Reference to *Alnus glutinosa* (L.) Gaertn. *Flora*, 164(1), pp.85-97.

Glanville, H., Rousk, J., Golyshin, P. and Jones, D.L., 2012. Mineralization of low molecular weight carbon substrates in soil solution under laboratory and field conditions. *Soil Biology and Biochemistry*, 48, pp.88-95.

Godbold, D.L., Hoosbeek, M.R., Lukac, M., Cotrufo, M.F., Janssens, I.A., Ceulemans, R., Polle, A., Velthorst, E.J., Scarascia-Mugnozza, G., De Angelis, P. and Miglietta, F., 2006. Mycorrhizal hyphal turnover as a dominant process for carbon input into soil organic matter. *Plant and Soil*, 281(1-2), pp.15-24.

Gunina, A., Smith, A.R., Godbold, D.L., Jones, D.L. and Kuzyakov, Y., 2017. Response of soil microbial community to afforestation with pure and mixed species. *Plant and Soil*, 412(1-2), pp.357-368.

Hill, P.W., Marshall, C., Williams, G.G., Blum, H., Harmens, H., Jones, D.L. and Farrar, J.F., 2007. The fate of photosynthetically-fixed carbon in *Lolium perenne* grassland as modified by elevated CO₂ and sward management. *New Phytologist*, 173(4), pp.766-777.

- Hynynen, J., Niemistö, P., Viherä-Aarnio, A., Brunner, A., Hein, S. and Velling, P., 2009. Silviculture of birch (*Betula pendula* Roth and *Betula pubescens* Ehrh.) in northern Europe. *Forestry*, 83(1), pp.103-119.
- Johnson, N.C., 2010. Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New Phytologist*, 185(3), pp.631-647.
- Johnson, N.C., Wilson, G.W., Wilson, J.A., Miller, R.M. and Bowker, M.A., 2015. Mycorrhizal phenotypes and the Law of the Minimum. *New Phytologist*, 205(4), pp.1473-1484.
- Jones, D.L. and Darrah, P.R., 1994. Amino-acid influx at the soil-root interface of *Zea mays* L. and its implications in the rhizosphere. *Plant and Soil*, 163(1), pp.1-12.
- Jones, D.L., Nguyen, C. and Finlay, R.D., 2009. Carbon flow in the rhizosphere: carbon trading at the soil–root interface. *Plant and Soil*, 321(1-2), pp.5-33.
- Jones, D.L. and Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology and Biochemistry*, 38(5), pp.991-999.
- Jones, T.G., Evans, C.D., Jones, D.L., Hill, P.W. and Freeman, C., 2016. Transformations in DOC along a source to sea continuum; impacts of photo-degradation, biological processes and mixing. *Aquatic Sciences*, 78(3), pp.433-446.
- Judd, L.A., Jackson, B.E. and Fonteno, W.C., 2015. Advancements in root growth measurement technologies and observation capabilities for container-grown plants. *Plants*, 4(3), pp.369-392.
- Kasurinen, A., Koikkalainen, K., Anttonen, M.J., Possen, B., Oksanen, E., Rousi, M., Vapaavuori, E. and Holopainen, T., 2016. Root morphology, mycorrhizal roots and extramatrical mycelium growth in silver birch (*Betula pendula* Roth) genotypes exposed to experimental warming and soil moisture manipulations. *Plant and Soil*, 407(1-2), pp.341-353.
- Kikvidze, Z., Armas, C., Fukuda, K., Martínez-García, L.B., Miyata, M., Oda-Tanaka, A., Pugnaire, F.I. and Wu, B., 2010. The role of arbuscular mycorrhizae in primary succession: differences and similarities across habitats. *Web Ecology*, 10(1), pp.50-57.

- Kleinert, A., Benedito, V.A., Morcillo, R.J.L., Dames, J., Cornejo-Rivas, P., Zuniga-Feest, A., Delgado, M. and Muñoz, G., 2018. Morphological and symbiotic root modifications for mineral acquisition from nutrient-poor soils. In *Root Biology* (pp. 85-142). Springer, Cham.
- Kobae, Y., Ohmori, Y., Saito, C., Yano, K., Ohtomo, R. and Fujiwara, T., 2016. Phosphate treatment strongly inhibits new arbuscule development but not the maintenance of arbuscule in mycorrhizal rice roots. *Plant Physiology*, 171(1), pp.566-579.
- Korhonen, A. and Maaranen, S., Nordic Koivu Oy, 2018. *Method and apparatus for collecting sap*. U.S. Patent Application 15/574,900.
- Kupper, P., Ivanova, H., Söber, A., Rohula-Okunev, G. and Sellin, A., 2018. Night and daytime water relations in five fast-growing tree species: Effects of environmental and endogenous variables. *Ecohydrology*, 11(6), e1927.
- Kuzyakov, Y. and Domanski, G., 2000. Carbon input by plants into the soil. Review. *Journal of Plant Nutrition and Soil Science*, 163(4), pp.421-431.
- Kuzyakov, Y. and Schneckenberger, K., 2004. Review of estimation of plant rhizodeposition and their contribution to soil organic matter formation. *Archives of Agronomy and Soil Science*, 50(1), pp.115-132.
- Krebs, P., Pezzatti, G.B., Beffa, G., Tinner, W. and Conedera, M., 2019. Revising the sweet chestnut (*Castanea sativa* Mill.) refugia history of the last glacial period with extended pollen and macrofossil evidence. *Quaternary Science Reviews*, 206, pp.111-128.
- Lacointe, A., 2000. Carbon allocation among tree organs: a review of basic processes and representation in functional-structural tree models. *Annals of Forest Science*, 57(5), pp.521-533.
- Lal, R., 2008. Sequestration of atmospheric CO₂ in global carbon pools. *Energy and Environmental Science*, 1(1), pp.86-100.
- Leake, J.R., Donnelly, D.P., Saunders, E.M., Boddy, L. and Read, D.J., 2001. Rates and quantities of carbon flux to ectomycorrhizal mycelium following ¹⁴C pulse labeling of *Pinus sylvestris* seedlings: effects of litter patches and interaction with a wood-decomposer fungus. *Tree Physiology*, 21(2-3), pp.71-82.

- Leake, J., Johnson, D., Donnelly, D., Muckle, G., Boddy, L. and Read, D., 2004. Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. *Canadian Journal of Botany*, 82(8), pp.1016-1045.
- Ledo, A., Paul, K.I., Burslem, D.F., Ewel, J.J., Barton, C., Battaglia, M., Brooksbank, K., Carter, J., Eid, T.H., England, J.R. and Fitzgerald, A., 2018. Tree size and climatic water deficit control root to shoot ratio in individual trees globally. *New Phytologist*, 217(1), pp.8-11.
- Le Quéré, C., Andrew, R.M., Canadell, J.G., Sitch, S., Korsbakken, J.I., Peters, G.P., Manning, A.C., Boden, T.A., Tans, P.P., Houghton, R.A. and Keeling, R.F., 2016. Global carbon budget 2016. *Earth System Science Data*, 8(2), pp.605-649.
- Levillain, J., Thongo M'Bou, A., Deleporte, P., Saint-André, L. and Jourdan, C., 2011. Is the simple auger coring method reliable for below-ground standing biomass estimation in Eucalyptus forest plantations? *Annals of Botany*, 108(1), pp.221-230
- Lilleskov, E.A., Kuyper, T.W., Bidartondo, M.I. and Hobbie, E.A., 2019. Atmospheric nitrogen deposition impacts on the structure and function of forest mycorrhizal communities: a review. *Environmental Pollution*, 246, pp.148-162.
- Litton, C.M., Raich, J.W. and Ryan, M.G., 2007. Carbon allocation in forest ecosystems. *Global Change Biology*, 13(10), pp.2089-2109.
- Mahmud, K., Medlyn, B.E., Duursma, R.A., Campany, C. and Kauwe, M.G.D., 2018. Inferring the effects of sink strength on plant carbon balance processes from experimental measurements. *Biogeosciences*, 15(13), pp.4003-4018.
- Maria do Rosário, G.O., Van Noordwijk, M., Gaze, S.R., Brouwer, G., Bona, S., Mosca, G. and Hairiah, K., 2000. Auger sampling, ingrowth cores and pinboard methods. In *Root methods* (pp. 175-210). Springer, Berlin, Heidelberg.
- McVean, D.N., 1956. Ecology of *Alnus glutinosa* (L.) Gaertn: IV. root system. *The Journal of Ecology*, 84, pp.219-225.
- Minchin, P.E.H. and Thorpe, M.R., 1996. What determines carbon partitioning between competing sinks? *Journal of Experimental Botany*, 47, pp.1293-1296.
- Miranda, K.M., Espey, M.G. and Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*, 5(1), pp.62-71.

- Modrzyński, J., Chmura, D.J. and Tjoelker, M.G., 2015. Seedling growth and biomass allocation in relation to leaf habit and shade tolerance among 10 temperate tree species. *Tree Physiology*, 35(8), pp.879-893.
- Moradi, A.B., Carminati, A., Lamparter, A., Woche, S.K., Bachmann, J., Vetterlein, D., Vogel, H.J. and Oswald, S.E., 2012. Is the rhizosphere temporarily water repellent? *Vadose Zone Journal*, 11(3), p.3
- Mulvaney, R.L., 1996. Nitrogen—inorganic forms. *Methods of soil analysis part 3—Chemical methods*, (methodsofsoilan3), pp.1123-1184.
- Murphy, J. and Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27, pp.31-36.
- Nave, L.E., Domke, G.M., Hofmeister, K.L., Mishra, U., Perry, C.H., Walters, B.F. and Swanston, C.W., 2018. Reforestation can sequester two petagrams of carbon in US topsoils in a century. *Proceedings of the National Academy of Sciences of the United States of America*, 115(11), pp.2776-2781.
- Nguyen, C., 2003. Rhizodeposition of organic C by plants: mechanisms and controls. *Agronomie*, 23(5-6), pp.375-396.
- Oburger, E., Dell'mour, M., Hann, S., Wieshammer, G., Puschenreiter, M. and Wenzel, W.W., 2013. Evaluation of a novel tool for sampling root exudates from soil-grown plants compared to conventional techniques. *Environmental and Experimental Botany*, 87, pp.235-247.
- Oburger, E. and Jones, D.L., 2018. Sampling root exudates—mission impossible? *Rhizosphere*, 6, pp.116-133.
- Oburger, E. and Schmidt, H., 2016. New methods to unravel rhizosphere processes. *Trends in Plant Science*, 21(3), pp.243-255.
- Ozolinčius, R., Bareika, V., Rubinskienė, M., Viškelis, P., Mažeika, R. and Staugaitis, G. 2016. Chemical Composition of Silver Birch (*Betula pendula* Roth.) and Downy Birch (*Betula pubescens* Ehrh.) Sap. *Baltic Forestry*, 22(2), pp.222-229.
- Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G. and Ciais, P., 2011. A large and persistent carbon sink in the world's forests. *Science*, 333(6045), pp.988-993.

- Paterson, E., Midwood, A.J. and Millard, P., 2009. Through the eye of the needle: a review of isotope approaches to quantify microbial processes mediating soil carbon balance. *New Phytologist*, 184(1), pp.19-33.
- Pausch, J. and Kuzyakov, Y., 2018. Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale. *Global Change Biology*, 24(1), pp.1-12.
- Phillips, M., 2017. *Mycorrhizal Planet: How Symbiotic Fungi Work with Roots to Support Plant Health and Build Soil Fertility*, pp. 2017-2256. Chelsea Green Publishing, London.
- Possen, B.J., Rousi, M., Silfver, T., Anttonen, M.J., Ruotsalainen, S., Oksanen, E. and Vapaavuori, E., 2014. Within-stand variation in silver birch (*Betula pendula* Roth) phenology. *Trees*, 28(6), pp.1801-1812.
- Powell, J.R. and Rillig, M.C., 2018. Biodiversity of arbuscular mycorrhizal fungi and ecosystem function. *New Phytologist*, 220(4), pp.1059-1075.
- Priha, O., Grayston, S.J., Pennanen, T. and Smolander, A., 1999. Microbial activities related to C and N cycling and microbial community structure in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings in an organic and mineral soil. *FEMS Microbiology Ecology*, 30(2), pp.187-199.
- Qi, Y., Wei, W., Chen, C. and Chen, L., 2019. Plant root-shoot biomass allocation over diverse biomes: A global synthesis. *Global Ecology and Conservation*, 18, e00606.
- Reinhart, K.O. and Rinella, M.J., 2016. A common soil handling technique can generate incorrect estimates of soil biota effects on plants. *New Phytologist*, 210(3), pp.786-789.
- Řezáčová, V., Konvalinková, T. and Jansa, J., 2017. Carbon fluxes in mycorrhizal plants. In *Mycorrhiza-Eco-Physiology, Secondary Metabolites, Nanomaterials* (pp. 1-21). Springer, Cham
- Richardson, A.D., Carbone, M.S., Huggett, B.A., Furze, M.E., Czimczik, C.I., Walker, J.C., Xu, X., Schaberg, P.G. and Murakami, P., 2015. Distribution and mixing of old and new nonstructural carbon in two temperate trees. *New Phytologist*, 206(2), pp.590-597.
- Richardson, A.D., Carbone, M.S., Keenan, T.F., Czimczik, C.I., Hollinger, D.Y., Murakami, P., Schaberg, P.G. and Xu, X., 2013. Seasonal dynamics and age of stemwood nonstructural carbohydrates in temperate forest trees. *New Phytologist*, 197(3), pp.850-861.

- Rondina, A.B.L., Lescano, L.E.A.M., de Almeida Alves, R., Matsuura, E.M., Nogueira, M.A. and Zangaro, W., 2014. Arbuscular mycorrhizas increase survival, precocity and flowering of herbaceous and shrubby species of early stages of tropical succession in pot cultivation. *Journal of Tropical Ecology*, 30(6), pp.599-614.
- Rondina, A.B.L., Tonon, B.C., Lescano, L.E.A.M., Hungria, M., Nogueira, M.A. and Zangaro, W., 2019. Plants of Distinct Successional Stages Have Different Strategies for Nutrient Acquisition in an Atlantic Rain Forest Ecosystem. *International Journal of Plant Sciences*, 180(3), pp.186-199.
- Rowell, D.L., 1994. *Soil science: methods and applications*. Department of Soil Science, University of Reading.
- Rusanen, M., Vakkari, P. and Blom, A., 2003. Genetic structure of *Acer platanoides* and *Betula pendula* in northern Europe. *Canadian Journal of Forest Research*, 33(6), pp.1110-1115.
- Sanaullah, M., Chabbi, A., Rumpel, C. and Kuzyakov, Y., 2012. Carbon allocation in grassland communities under drought stress followed by ^{14}C pulse labeling. *Soil Biology and Biochemistry*, 55, pp.132-139.
- Schröder, P., 1989. Characterization of a thermo-osmotic gas transport mechanism in *Alnus glutinosa* (L.) Gaertn. *Trees*, 3(1), pp.38-44.
- Sileshi, G.W., 2014. A critical review of forest biomass estimation models, common mistakes and corrective measures. *Forest Ecology and Management*, 329, pp.237-254.
- Smith, A.R., Lukac, M., Hood, R., Healey, J.R., Miglietta, F. and Godbold, D.L., 2013. Elevated CO_2 enrichment induces a differential biomass response in a mixed species temperate forest plantation. *New Phytologist*, 198(1), pp.156-168.
- Smith, J.L. and Doran, J.W., 1996. Measurement and use of pH and electrical conductivity for soil quality analysis. In *Methods for assessing soil quality (Vol. 49)*. Soil Science Society of America Madison, WI.
- Smith, S.E. and Read, D.J., 2010. *Mycorrhizal Symbiosis*. Academic press. London.
- Smolander, A., 1990. *Frankia* populations in soils under different tree species—with special emphasis on soils under *Betula pendula*. *Plant and Soil*, 121(1), pp.1-10.

- Sommer, J., Dippold, M.A., Flessa, H. and Kuzyakov, Y., 2016. Allocation and dynamics of C and N within plant–soil system of ash and beech. *Journal of Plant Nutrition and Soil Science*, 179(3), pp.376-387.
- Sommer, J., Dippold, M.A., Zieger, S.L., Handke, A., Scheu, S. and Kuzyakov, Y., 2017. The tree species matters: Belowground carbon input and utilization in the myco-rhizosphere. *European Journal of Soil Biology*, 81, pp.100-107.
- Sroka, K., Chodak, M., Klimek, B. and Pietrzykowski, M., 2018. Effect of black alder (*Alnus glutinosa*) admixture to Scots pine (*Pinus sylvestris*) plantations on chemical and microbial properties of sandy mine soils. *Applied Soil Ecology*, 124, pp.62-68.
- Steinaker, D.F. and Wilson, S.D., 2008. Phenology of fine roots and leaves in forest and grassland. *Journal of Ecology*, 96(6), pp.1222-1229.
- Taylor, B.N., Beidler, K.V., Cooper, E.R., Strand, A.E. and Pritchard, S.G., 2013. Sampling volume in root studies: the pitfalls of under-sampling exposed using accumulation curves. *Ecology Letters*, 16(7), pp.862-869.
- Thoms, R., Köhler, M., Gessler, A. and Gleixner, G., 2017. Above and below ground carbohydrate allocation differs between ash (*Fraxinus excelsior* L.) and beech (*Fagus sylvatica* L.). *PloS one*, 12(9), p.e0184247.
- Tian, H., Lu, C., Ciais, P., Michalak, A.M., Canadell, J.G., Saikawa, E., Huntzinger, D.N., Gurney, K.R., Sitch, S., Zhang, B. and Yang, J., 2016. The terrestrial biosphere as a net source of greenhouse gases to the atmosphere. *Nature*, 531(7593), p.225.
- Wagg, C., Jansa, J., Stadler, M., Schmid, B. and Van Der Heijden, M.G., 2011. Mycorrhizal fungal identity and diversity relaxes plant–plant competition. *Ecology*, 92(6), pp.1303-1313.
- Walters, M.B. and Reich, P.B., 2000. Trade-offs in low-light CO₂ exchange: a component of variation in shade tolerance among cold temperate tree seedlings. *Functional Ecology*, 14(2), pp.155-165.
- Weiner, J., 2004. Allocation, plasticity and allometry in plants. *Perspectives in Plant Ecology, Evolution and Systematics*, 6(4), pp.207-215.
- Werth, M. and Kuzyakov, Y., 2008. Root-derived carbon in soil respiration and microbial biomass determined by ¹⁴C and ¹³C. *Soil Biology and Biochemistry*, 40(3), pp.625-637.

Wu, Q.S., Cao, M.Q., Zou, Y.N. and He, X.H., 2014. Direct and indirect effects of glomalin, mycorrhizal hyphae, and roots on aggregate stability in rhizosphere of trifoliate orange. *Scientific reports*, 4, p.5823.

Xavier, L.J.C. and Germida, J.J., 1999. Impact of human activities on mycorrhizae. In *Proceedings of the 8th International Symposium on Microbial Ecology* Bell CR, Brylinsky M, Johnson-Green P (eds) Atlantic Canada Society for Microbial Ecology, Halifax, Canada.

Zangaro, W., Rostirola, L.V., de Souza, P.B., de Almeida Alves, R., Lescano, L.E.A.M., Rondina, A.B.L., Nogueira, M.A. and Carrenho, R., 2013. Root colonization and spore abundance of arbuscular mycorrhizal fungi in distinct successional stages from an Atlantic rainforest biome in southern Brazil. *Mycorrhiza*, 23(3), pp.221-233.

8. Appendices/ Supplementary

Table S1: Root-to-shoot ratios with significance at each experimental time point. Values represent means \pm SE ($n = 4$).

Tree species	Hours after pulse				
	0	24	72	168	336
<i>A. glutinosa</i>	0.01 \pm 0.00 b	0.16 \pm 0.04	0.56 \pm 0.12	0.54 \pm 0.18	0.45 \pm 0.15 b
<i>B. pendula</i>	0.03 \pm 0.01 a	0.29 \pm 0.09	0.82 \pm 0.13	0.87 \pm 0.12	1.04 \pm 0.13 a
<i>C. sativa</i>	0.06 \pm 0.01 b	0.26 \pm 0.10	0.77 \pm 0.16	0.68 \pm 0.05	0.59 \pm 0.05 b
<i>Probability</i>	0.01	0.54	0.37	0.25	0.01

Table S2: mean total ^{14}C activity allocated to belowground plant partition determined after destructive harvesting expressed as a percentage of the total ^{14}C activity photo-assimilated during the pulse label. Data are mean \pm SE ($n = 4$)

Tree species	% of total ^{14}C assimilated/ Hours after pulse				
	0	24	72	168	336
<i>A. glutinosa</i>	3.46 \pm 0.93	13.91 \pm 1.89	43.39 \pm 4.84	32.07 \pm 4.13	32.43 \pm 8.77
<i>B. pendula</i>	4.16 \pm 0.63	41.90 \pm 8.98	46.77 \pm 2.80	52.48 \pm 3.62	58.18 \pm 6.21
<i>C. sativa</i>	6.46 \pm 1.01	34.60 \pm 12.18	42.13 \pm 5.52	61.18 \pm 6.68	44.54 \pm 3.28

Chapter 4

Investigating inter- and intra-specific carbon transfer between three temperate tree species via common mycorrhizal networks

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TDP conducted all empirical work with guidance on design, execution and data analysis from ARS, GWG and DLJ. TDP wrote the manuscript with all authors contributing to the final version.

1. Introduction

Plants act as a conduit between the abiotic and biotic fractions of the carbon (C) cycle by transforming atmospheric carbon dioxide (CO₂) to soil C through the process of photosynthesis (Pausch and Kuzyakov, 2018). Addition of photosynthetically derived carbohydrates to the soil comes in many forms including roots, root turnover, hyphal turnover, leaf and litter decomposition, mucilage and sloughed off cells, which then promotes microbial diversity and proliferation (Dennis et al., 2010; Neumann and Römheld, 2007). The soil micro-organisms present in the soil can be either free-living species that tend to concentrate within hot spots in the rhizosphere or those that form a more intimate relationships with plants and colonise large volumes of bulk soil. Mycorrhizal symbiotic relationships between a plant (photobiont) and mycorrhizal fungus (mycobiont) are ubiquitous amongst nearly all known terrestrial plant groups (Smith and Read, 2010; Van der Heijden, 2016). Mycorrhizal fungi can simultaneously colonise multiple plants at different developmental stages, intra- and inter-specifically (Selosse et al., 2006), and therefore are not exclusively reliant on a single partner for C or nutrient

acquisition (Bücking et al., 2016). This means that within forest ecosystems many trees can be connected underground by vast common mycorrhizal networks (CMNs) commonly referred to as the Wood Wide Web (Beiler et al., 2010).

These ecologically important plant-microbe partnerships allow the photobiont access to fungal-derived water and nutrients (C, nitrogen, phosphate or micronutrients) acquired from their extensive hyphal network, which they can trade for C with the plants (Smith and Read, 2010; Van der Heijden, 2009). The importance of mycorrhizal fungi as a pathway for photosynthetically derived C to be transferred belowground is considerable. Wallander et al., (2001) estimated that between 700 to 900 kg of C ha⁻¹ of net primary production in forests is assimilated by the mycorrhizal network, equivalent to 15 to 28% of net fixation (Vogt et al., 1982; Finlay 2008). Klein et al., (2016) estimated that transfer between trees via CMNs can account for 280 kg of C ha⁻¹, roughly equivalent to 4 % of the forests net primary production, although some of this may be C recaptured from exudation or root and hyphal turnover (Jones et al., 2009). It is also known that CMNs can connect plant species mediating the transport of plant signalling chemicals, stress chemicals, and allelochemicals over large distances, allowing multiple fungal and plant species to ‘communicate’ thus affecting the behaviour, fitness and survival of both partners by increasing the relative competitiveness of plants and fungi linked via these networks (Bücking, et al., 2016).

Mycorrhizal associations are classified into distinct groupings based on their morphology (Lukac and Godbold, 2011). In temperate forests, the commonest of these groupings are arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) associations. Studies have demonstrated that transfer of C occurs between plants via both AM (Hirrel, 1979; Francis and Read, 1984; Grime et al., 1987) and EM networks (Brownlee et al., 1983; Simard et al., 1997). Although it seems that the evidence of transfer between plants connected by EM fungi is more compelling in comparison to AM networks (Selosse, et al., 2006). Averill et al., (2014) found that EM networks can store up to 1.7 times more C than AM networks. Field experiments have observed transfer between seedlings (Simard et al., 1997), to seedlings from mature trees (Beiler et al., 2010) and between mature trees (Klein et al., 2016). AM transfer seems less clear, however, with some believing that the C remains in the hyphae that have colonised the plants root cell walls (Robinson and Fitter, 1999), and that the C is not nutritionally available to the plant (Pfeffer et al., 2004). The difficulty in quantifying this surreptitious belowground C transfer between mycorrhizal plants has kept the mechanics of CMN equivocal (Fellbaum et al., 2014), however, there is a growing consensus of the ecological importance of mycorrhizal

symbiosis (Johnson and Gilbert, 2015). The concept of a group of trees (often referred to as a guild), sharing resources mediated by CMNs is an ecological paradigm shift (Simard et al., 1997), which undermines our understanding the dynamics of plant competition. The importance of CMNs in the dynamics and community structure of plants may vary depending on host (Weremijewitz et al., 2016), mycorrhizal species (Hoeksema et al., 2018; Walder et al., 2012), soil nutritional status (Elumeeva et al., 2018) and climatic conditions (Bennett et al., 2018). It has been hypothesised that the trading of C and nutrients between mycorrhizae and plants is based on reciprocal rewards system, whereby those plants that can supply the most C to their fungal symbiont are supplied with the most nutrients (Lekberg et al., 2010; Hammer et al., 2011; Kiers et al., 2011; Fellbaum et al., 2014), although this has been disputed (Walder and Van der Heijden, 2015) who argue that mycorrhizal fungi use CMNs to redistribute carbohydrates amongst plants thus lessening competition (Shi et al., 2017; Van Der Heijden, 2009).

Soils comprise the largest pool of terrestrial organic C (Jobbágy and Jackson, 2000; Tarnocai et al., 2009), however, there is still much uncertainty in our understanding of the mechanisms of C storage in soils and their relative importance to C cycling (Arora et al., 2013; Todd-Brown et al., 2013). This sizable knowledge-gap is currently limiting both the reliability and robustness of the climatic change predictions, and C cycling and global circulatory models (Luo et al., 2016).

It is now generally accepted that the soil microbial biomass plays a crucial role in biogeochemical cycling as the majority of stable soil C is formed by micro-organisms oxidising belowground inputs (Schmidt et al., 2011; Luo et al., 2016). Historically competition between plants has been viewed as the primary driver of evolution and community structure, however, increasingly the relative importance of facilitative and mutualistic interactions is being appreciated (Tilman, 1988; Bruno et al., 2003; Stachowicz, 2001). This is at least in part due to the fact that in forest ecosystems most of the facilitative relationships are microscopic and therefore were not immediately obvious to those Victorians who were crucial in developing the ideas of ecological theory (Ryan, 2002). Under changing climatic conditions there is an obvious need to better understand these complex belowground symbiotic interactions to increase the accuracy of models used to predict the impact of future land-use on C cycling (Kleber et al., 2007; Schmidt et al., 2011; Davidson et al., 2014).

Radio-isotopic experimentation has allowed us to improve our understanding of C dynamics and C allocation along the plant-fungal-soil continuum. Due to the relative longevity of the photobionts involved in ectomycorrhizal symbiosis and the fact that the plants involved are generally not agricultural, this type of facilitative relationship has been relatively neglected in C flow studies (Molina et al., 1992; Simard et al., 2015). A short-term ^{14}C (half-life of 5730 years) pulse-chase was employed to quantify inter- and intra-specific C transfer occurring between two trees linked by a CMN. We used short chase times as previous studies suggest that belowground ^{14}C dilution starts to occur 48 hours post-pulse labelling event (Wu et al., 2002). An ecological understanding of the importance of ectomycorrhizal fungi to the competition dynamics of forests is needed to elucidate the black box of belowground biogeochemical cycling. In addition, this has implications for our understanding of competition and succession within forest ecosystem associated with Common Ectomycorrhizal Networks (CectoMN) could fundamentally alter our understanding of plant community dynamics (Robinson and Fitter, 1999).

Our hypotheses for this experiment were-

H₁: Transfer of C by CMNs is greater inter-specifically than intra-specifically, as a result of differences in C demand between species.

H₂: Greater mycorrhizal diversity per unit of rhizosphere soil will result in more C transfer between trees via the CMN.

2. Materials and methods

2.1. Plant and mycorrhizal establishment

Sixteen saplings of three temperate tree species were purchased from local forest nursery stock suppliers (Maelor Nursery Bronington SY13 3HZ) in November 2015. Namely, 2-year-old bare-rooted; alder (*Alnus glutinosa* (L.) Gaertn; Tree A) and silver birch (*Betula pendula* Roth; Tree B) and 1-year old cell-grown sweet chestnut (*Castanea sativa* Mill.; Tree C). The three temperate forest tree species were selected from trees already planted as part of BangorDIVERSE, an established 15-year old tree diversity experiment located at Henfaes experimental farm, Abergwyngregyn, Gwynedd, Wales, (53°14 N, 4°01 W) (Ahmed et al., 2016) on the basis of their divergent symbiotic relationships and contrasting ecological niches.

Specifically, *Betula pendula* is a wind dispersed pioneer species whereas *Castanea sativa* is an animal dispersed late successional species, also with ectomycorrhizal associations and both form ectomycorrhizal symbiotic relationships. Contrastingly, *Alnus glutinosa*, typically a riparian species with seeds dispersed by watercourses, is actinorrhizal and forms symbioses with both arbuscular and ectomycorrhizal fungi. The UK provenance zones of the three species were; zone 204 (Northumberland, England) for both *A. glutinosa* and *B. pendula* and zone 404 for *C. sativa*.

The saplings were planted into pairs of 10 L mesocosms (\varnothing 250 mm \times 200 mm) joined via a 10 cm diameter Perspex tube filled with 2 kg of horticultural grade sharp sand mixed with 20 g of bone meal and covered at both ends with a 40 μ m mesh (Normesh Limited, Lancaster, UK; Figure 1). The mesh allowed the hyphae to access and utilise the sand and bone meal but excluded the tree roots. Both mesocosms were potted using species-specific soil collected from the 0-10 cm layer of BangorDIVERSE, which had been air dried, homogenised and then sieved to pass through a 2 mm mesh. Soil is classified as a fine loamy textured Eutric Cambisol (Rheidol series) with underlying alluvial gravel (Smith et al., 2013). The trees were planted in pairs that established intra- or inter-specific combinations (A-A, A-B, A-C, B-B, B-C, and C-C) with four replicates. The tree mesocosms were then grown in a glasshouse in ambient conditions for 9 months to allow for the establishment and development of the root system and associated CMN. To apply a ^{14}C pulse-label, one tree in each pair was sealed inside 610 \times 920 mm gas-proof bags (CP lab safety, Novato, CA, USA) before being labelled with 2 MBq of $\text{NaH}^{14}\text{CO}_3$ (Amersham International, Amersham, UK) reacted in a vial containing 3 M HCl inside the bag to form $^{14}\text{CO}_2$. Each tree was exposed to the same radio-isotopic activity for 2 hours in full ambient light with a minimum photosynthetically active radiation level of 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The trees were then returned to the unheated glasshouse for the post pulse-chase period (336 hours). During the chase period the donor trees were placed with a control tree equidistant to the receiver tree (see Figure 1) to determine how much $^{14}\text{CO}_2$ was respired by the donor tree into the greenhouse and then re-fixed in photosynthesis by the control tree.

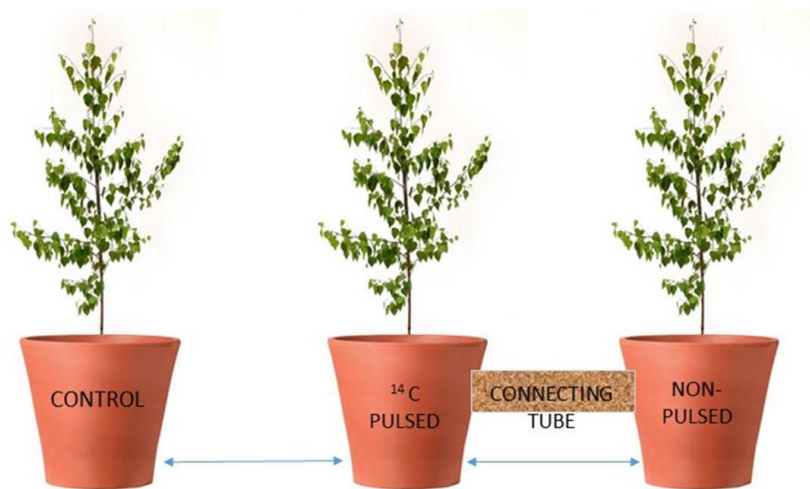


Figure 1: Experimental design with two potted trees connected by a sand and bone-meal filled Perspex tube and the control plant the same distance from the donor tree as the receiver tree. The control tree absorbed the $^{14}\text{CO}_2$ respired from the donor tree and was then subtracted from the activity found in the receiver treatment.

2.2. Sampling procedure and isotope analysis

To establish the fate of the recently fixed C, a ^{14}C pulse was used as a proxy. In the case of the ^{14}C evolved as $^{14}\text{CO}_2$ from soil respiration, a 28 mm diameter CO_2 trap that contained 3 M NaOH was suspended above the soil in both pots to allow free passage from the soil surface and replaced following 0, 24, 72, 168 and 336 hours of incubation at room temperature (21 °C). Simultaneously, dissolved ^{14}C in soil solution was sampled following 0, 24, 72, 168 and 336 hours after the pulse-label was completed, using Rhizon-MOM soil water samplers (Rhizosphere Research Products B.V., Wageningen, The Netherlands) (Jones et al., 2016). The H^{14}CO_3 content was determined by liquid scintillation spectrometry. Counts were standardised using a quench curve and regularly calibrated using standard samples and expressed in disintegrations per minute, using a Wallac 1409 scintillation counter (Wallac E G and G, Milton Keynes, UK) with automatic quench correction and Optiphase 3[®] alkali compatible scintillation fluid (Wallac E G and G). After the defined chase period (336 hours) the trees were destructively harvested and separated into foliage, branch and stem, fine and coarse roots, soil and sand from the interconnecting tube for analysis. The plant material (including the control trees), soil and sand were immediately dried at 105° C to prevent loss of isotope to microbial respiration, after which they were homogenised using a laboratory mixer ball mill (Retsch MM 200) then combusted using a Harvey Instruments Biological Oxidiser OX400

followed by trapping of the evolved $^{14}\text{CO}_2$ in Oxysolve which was then analysed as described above.

2.3. Analysis of soil chemical properties

At time point T0 (immediately post pulse-label), soil was sampled from each mesocosm using a 4 cm soil corer. The soils were then analysed to determine if the trees presence during the tree and mycorrhizal establishment period had significantly altered the soil chemical properties.

Soil samples were dried at 105 °C and ball milled before C and N analysis was measured by dry combustion using a CN Analyser (Leco Corp., St Joseph, MI, USA) and soil moisture by the gravimetric method (Reynolds, 1970). Nitrate was determined colourimetrically by photometric method ($\lambda=540$ nm) with vanadium chloride (Miranda et al., 2001) and ammonium by the salicylate-hypochlorite method of Mulvaney (1996). Soil pH was measured in 1:5 soil: water slurry according to Smith and Doran (1996). Plant available phosphorus was extracted in 0.5 M sodium bicarbonate (NaHCO_3) pH 8.5, at a ratio of 1:5 (w/v) (Olsen et al., 1954) and determined colourimetrically as molybdate-reactive P (Murphy and Riley, 1962). Soil dissolved organic C (DOC) and total dissolved N (TDN) was determined using a Shimadzu TOC-V-TN analyser (Shimadzu Corp., Kyoto, Japan) following extraction (1:5 w/v) in 0.5 M K_2SO_4 (Jones and Willett, 2006). The resultant soil chemical and properties are shown in Table 1.

2.4. Fungal DNA analysis

A subsample of soil collected at T0 was collected from the two most contrasting treatments namely the tree combination with the most transfer (C-A) and the least (A-A). The samples were freeze dried using a Lyovac GT2, benchtop freeze drier (Finn-Aqua, Huerth, Germany) and finely ground before being sieved to 500 μm and thoroughly homogenised. A representative subsample of 0.25 g of each soil sample was then weighed and added to the power bead tube for DNA extraction using the Qiagen DNeasy kit (Qiagen DNeasy, Valencia, CA, USA), closely following the manufacturers protocol. Novel fungal specific primers, ITS3 (GCATCGATGAAGAACGCAGC) and the tagged ITS4 reverse primer (CAGACTT[G/A]TA[C/T]ATGGTCCAG), were then utilised to isolate the ITS2 region of the large sub unit (LSU) of ribosomal DNA (rDNA). Subsequently, the DNA was amplified in a 25 μl Polymerase Chain Reaction (PCR) (Saiki et al., 1985), using Promega GoTaq G2 DNA polymerase (Promega, Madison USA). Each reaction contained 7.83 μl of each primer, 39.13

μl BSA, 78.26 μl dNTPs and 1.96 μl of DNA polymerase in the supplied buffer. The PCR conditions were 94 °C for 5 min (initial denaturation) followed by 30 cycles at 94 °C, 30 s (denaturation); 52 °C, 30 s (annealing); 72 °C, 30 s (extension) and a final 5 min extension at 72 °C. PCR reactions were cleaned using spin columns (NBS Biological, Huntingdon UK) and amplified DNA were quantified using NanoDrop (NanoDrop Products, Wilmington USA). DNA yield was then checked using gel electrophoresis and quality assessed using Qubex device before being exposed to an ION torrent. Primer ITS2 was linked at the 5' end to the IonTorrent A-adapter sequence (pending), the TCAG key and an IonXpress Barcode. Primer ITS4 was linked at the 5' end to Ion Torrent B adapter sequence (unpublished).

2.5. Statistical analysis

Prior to analysis all data were tested for homogeneity of variance using Levene's test. Data was structured as an independent variable group of species treatments that was comprised of *A. glutinosa*, *B. pendula* and *C. sativa*, whilst the dependant variables were plant and partition biomass, soil chemical properties, ¹⁴C activity of biomass pools, ¹⁴C labelled respiration efflux). Data were tested for normality prior to analysis using the Shapiro-Wilk Test. The EC data were log₁₀ transformed to satisfy the assumption of normality. The statistical analysis was conducted using a one-way ANOVA and Tukey's post-hoc test with SPSS® Statistics version 25.0 (IBM Corp, Chicago, 2017). Results of the analysis were considered significant at $P \leq 0.05$.

3. Results

3.1. Soil chemical properties

Growth of tree species during the 9-month establishment period did not affect soil NO₃⁻, NH₄⁺, P, pH, EC and TDN. Soil C: N ratio was found to be significantly higher under *C. sativa* compared to *A. glutinosa* and *B. pendula* ($P = 0.03$), Dissolved organic carbon (DOC) was also found to be significantly altered by tree species ($P < 0.001$) with soil under *A. glutinosa* soil containing almost 2-fold the DOC of *C. sativa* ($P < 0.001$) and significantly more than the *B. pendula* treatments ($P = 0.01$). The DOC of *C. sativa* was not found to be significantly different from the other treatments ($P = 0.86$). Soil chemical properties are shown in Table 1.

Table 1: Soil chemical properties for soil collected from the mesocosms 0 hours after the pulse completed and after a 9-month period of establishment for all three species treatments (*A. glutinosa*, *B. pendula* and *C. sativa*). Data are mean \pm SE ($n = 4$). Significant differences are highlighted in bold and the level of significance is denoted by * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Tree species	Soil chemical properties							
	NO ₃ ⁻ (mg/kg)	NH ₄ ⁺ (mg/kg)	Available P (mg/kg)	pH	EC (μ S/cm)	C:N ratio	TDN (mg/kg)	DOC (mg/kg)
<i>A. glutinosa</i>	4.81 \pm 2.74	8.54 \pm 2.21	20.4 \pm 7.3	5.54 \pm 0.06	197.0 \pm 24.1	8.30 \pm 0.13	7.43 \pm 0.78	34.5 \pm 0.7 a
<i>B. pendula</i>	2.22 \pm 0.71	7.28 \pm 1.66	13.4 \pm 1.9	5.54 \pm 0.05	247.3 \pm 35.4	8.51 \pm 0.14	8.42 \pm 2.87	24.0 \pm 2.0
<i>C. sativa</i>	7.56 \pm 3.23	5.73 \pm 2.34	14.5 \pm 4.6	5.46 \pm 0.07	251.6 \pm 36.3	9.50 \pm 0.52 a	5.03 \pm 0.39	19.7 \pm 1.6
<i>P value</i>	0.54	0.62	0.57	0.50	0.39	0.01*	0.33	0.001***

3.2 ¹⁴C transfer via the CMN

3.2.1 Total ¹⁴C activity in receiver trees Intra- vs Inter-specific

The destructive harvesting of the receiver plants tissues allowed the quantification of ¹⁴C that had been transferred through the Perspex sand-filled tube and into the receiver trees. Figure 2 shows the mean total ¹⁴C activity in all tree tissues expressed as a percentage of the total ¹⁴C activity found in the entire mesocosm as mean inter- and intra-specific data. The total mean ¹⁴C was higher in the inter-specific combinations and the results were found to be significantly different from the total ¹⁴C activity counted in the intra-specific species treatment combinations ($P = 0.03$). The mean ¹⁴C activity transferred through the CMN to the receiver plants as a percentage of total mesocosm activity was $0.39 \pm 0.15 \%$ for intra-specific treatments and $1.30 \pm 0.64 \%$.

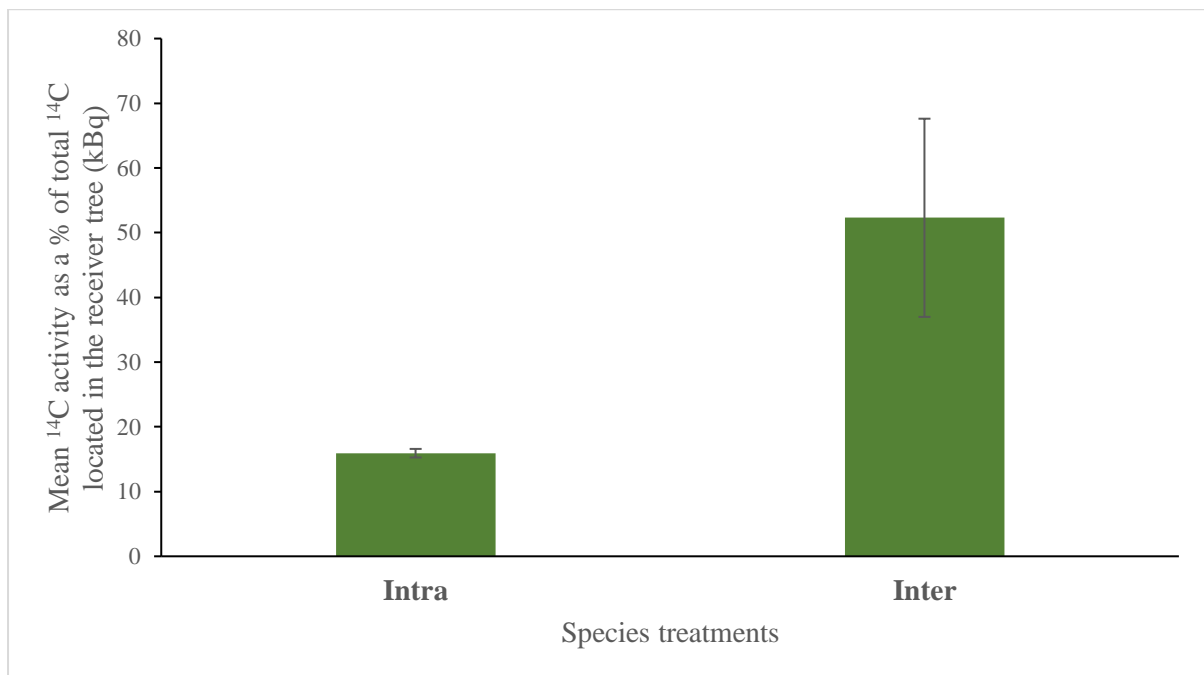


Figure 2: Mean total ¹⁴C activity located in the intra- and inter-specific species treatments of the receiver trees in kBq. Data are mean \pm SE ($n = 12$).

3.2.2. ^{14}C activity in receiver tree partitions

Table 2: The activity of the partitions (kBq) divided into intra- and interspecific treatments for comparison. Data are mean \pm SE ($n = 4$). Significant differences are highlighted in bold.

	Mesocosm partitions									
	Leaves	Branch	Coarse roots	Fine roots	Plant total	Sand	Soil CO ₂	Soil H ₂ O	Soil	Total mesocosm
Intra	0.63 \pm 0.12	0.73 \pm 0.13	0.57 \pm 0.15	0.37 \pm 0.12	2.31 \pm 0.37	2.91 \pm 0.16	0.14 \pm 0.03	0.00 \pm 0.00	13.48 \pm 0.45	15.9 \pm 0.7
Inter	0.61 \pm 0.10	0.76 \pm 0.18	16.20 \pm 10.31	6.96 \pm 3.90	24.53 \pm 14.07	4.46 \pm 0.81	1.10 \pm 0.37	0.00 \pm 0.00	26.67 \pm 5.80	52.3 \pm 15.3
<i>P-value</i>	0.92	0.90	0.14	0.11	0.13	0.73	0.02*	0.57	0.03*	0.03*

The interesting results from the analysis of the inter- and intraspecific ^{14}C activity (Table 2) of the partitions included; that the allocation to the tree tissues were similar between the treatments with no significant differences found for leaves, branches and stems, coarse roots, fine roots or total plant activity. The mean ^{14}C activity located in the interspecific soil treatments were found to be twice that of the intraspecific treatment,

(13.5 ± 0.5 kBq and 26.7 ± 5.8 kBq, respectively) and were found to be significantly different ($P = 0.03$). The soil respiration showed a similar trend with the mean interspecific treatments total soil respiration being almost eight times more than the intraspecific and the results were found to be significant ($P = 0.03$). Total mean plant ^{14}C activity was three times as much in the interspecific as the intraspecific treatments, this difference was found to be significant ($P = 0.03$). This data is presented in graphical form for ease of comparison (Figure 3).

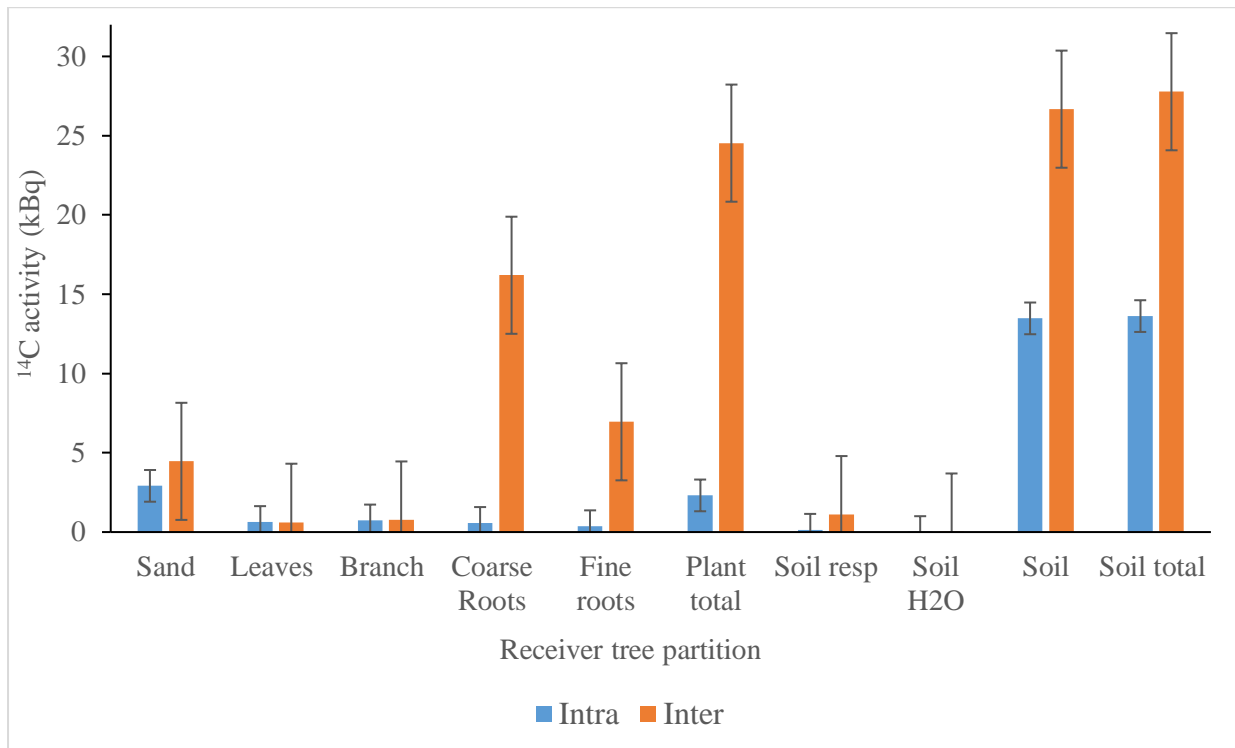


Figure 3: ^{14}C activity of intra- and inter-specific receiver tree partitions (kBq). Data is mean ($n = 12 \pm \text{SE}$).

3.5.5. Receiver plant relative ^{14}C partition allocation

The mean ^{14}C activity counted in the receiver tree had been transferred from the donor tree via CMN and this data is tabulated with the standard error in Table 3. The A-A treatments have mean ^{14}C leaf and branch activity (0.25 ± 0.11 % and 0.25 ± 0.18 %) which appears to be four times larger than most other treatments and twice as much as the next largest (Table 3). However, statistical analysis revealed no significant differences between treatments either when considered as individual treatments (6 in total), or when grouped together as two treatments (inter- and intra-specifically).

Table 3: Allocation of ^{14}C activity to the plant's tissues in the receiver trees as a percentage of the total ^{14}C activity counted in the entire mesocosm, for the six species treatment combinations (A- *A. glutinosa*, B- *B. pendula*, and C- *C. sativa*). Intraspecific and interspecific combinations are grouped together for comparison. Data are mean \pm SE ($n = 4$).

Species combination	Biomass partition			
	Leaves	Branch	Coarse root	Fine root
A-A	$0.25 \pm 0.11 \%$	$0.25 \pm 0.18 \%$	$0.14 \pm 0.09 \%$	$0.11 \pm 0.06 \%$
B-B	$0.06 \pm 0.01 \%$	$0.08 \pm 0.03 \%$	$0.08 \pm 0.03 \%$	$0.02 \pm 0.01 \%$
C-C	$0.03 \pm 0.01 \%$	$0.07 \pm 0.01 \%$	$0.04 \pm 0.01 \%$	$0.04 \pm 0.01 \%$
B-A	$0.04 \pm 0.15 \%$	$0.03 \pm 0.01 \%$	$0.97 \pm 0.93 \%$	$0.49 \pm 0.47 \%$
C-A	$0.09 \pm 0.02 \%$	$0.10 \pm 0.02 \%$	$0.24 \pm 0.18 \%$	$0.27 \pm 0.15 \%$
C-B	$0.02 \pm 0.01 \%$	$0.03 \pm 0.01 \%$	$1.22 \pm 0.39 \%$	$0.41 \pm 0.39 \%$

To try and visualise the differences between the intra and inter-specific receiver ^{14}C allocation, we expressed the receiver allocation as a percentage of total ^{14}C located in the entire receiver tree (Figure 4). This illustrates how the interspecific combinations appear to have a greater proportion of the ^{14}C activity in the belowground tissues when compared to the intraspecific combinations.

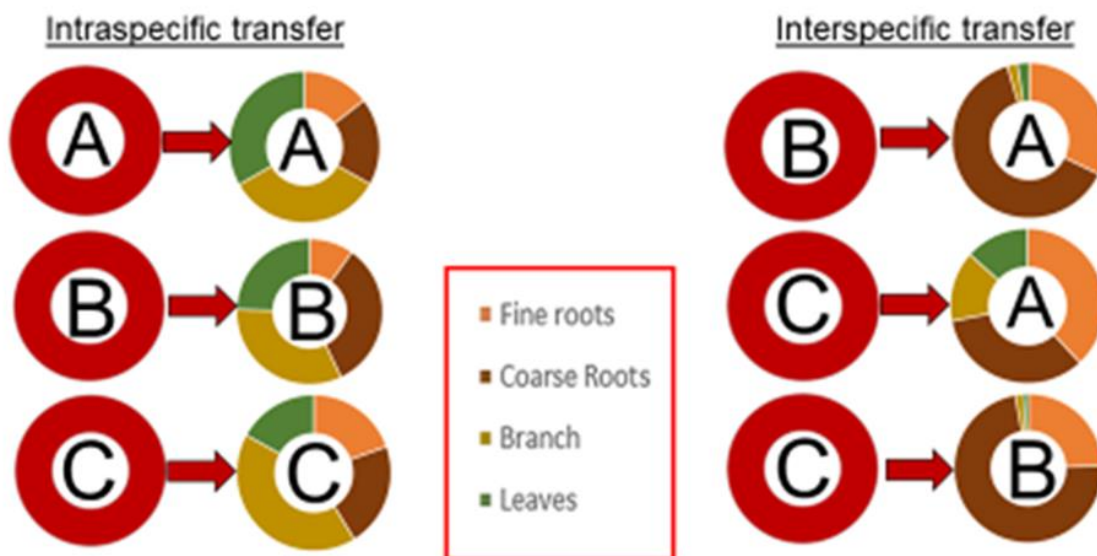


Figure 4: Allocation of ^{14}C activity to the plant's tissues in the receiver trees as a percentage of the total ^{14}C activity counted in the mesocosms, for the six species treatment combinations (A- *A. glutinosa*, B- *B. pendula*, and C- *C. sativa*). Intraspecific and interspecific combinations are grouped together for comparison. Data are means ($n = 4$).

3.6. Total transfer

The results of the total amount of ^{14}C that was transferred inter and intra-specifically through the interlinking Perspex tube showed that transfer was highest in *A. glutinosa* to *A. glutinosa* treatments (Figure 5). Overall, the treatments that included *A. glutinosa* had slightly higher transfer and those species combinations without *A. glutinosa* showed the least amount of transfer, although overall the results were not shown to be significant ($P = 0.63$).

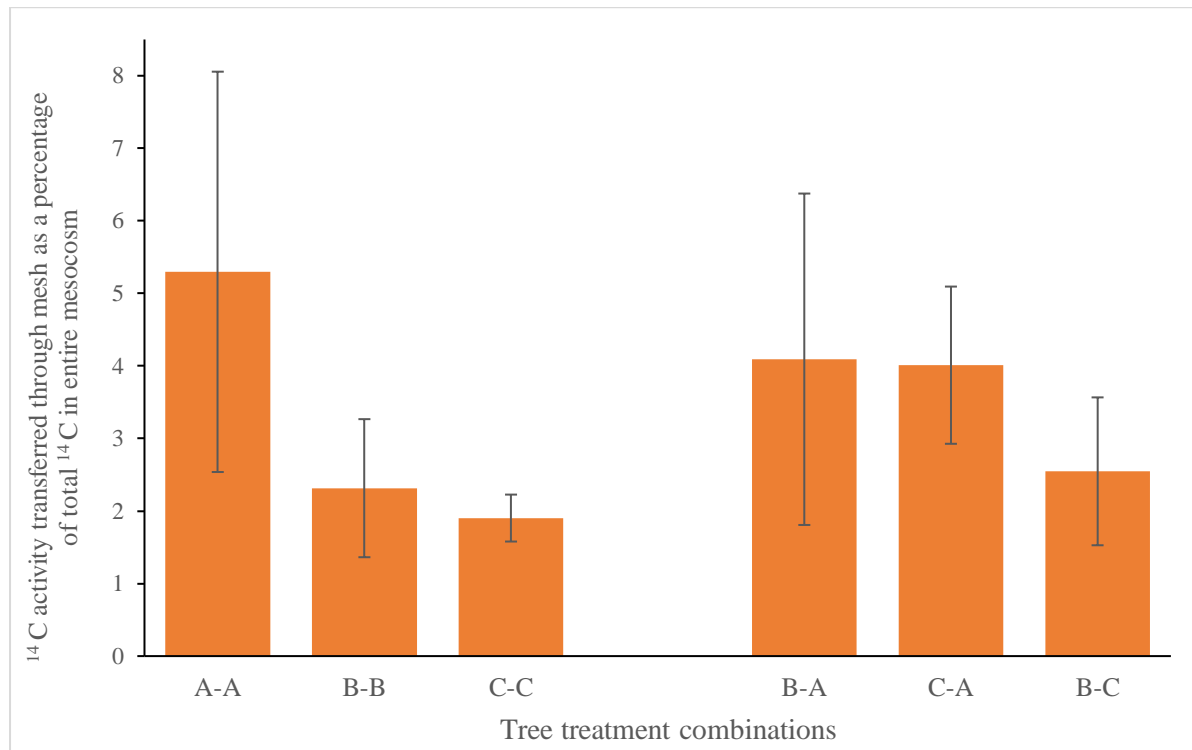


Figure 5: Total ^{14}C activity transferred through the mesh expressed as a percentage of the total ^{14}C activity in the entire mesocosm, in the six species treatment combinations (A- *A. glutinosa*, B- *B. pendula*, and C- *C. sativa*). Intra-specific and inter-specific combinations are grouped together for comparison. Data are mean \pm SE ($n = 4$).

3.7. Estimated ^{14}C transfer to micro-organisms via mycorrhizal fungi

The ^{14}C activity that was transferred through the mesh was assumed to be transferred by mycorrhizal fungal hyphae. This includes the ^{14}C activity located in the soil, the soil solution and the total soil respiration from under the receiver tree. This data is plotted in Figure 6.

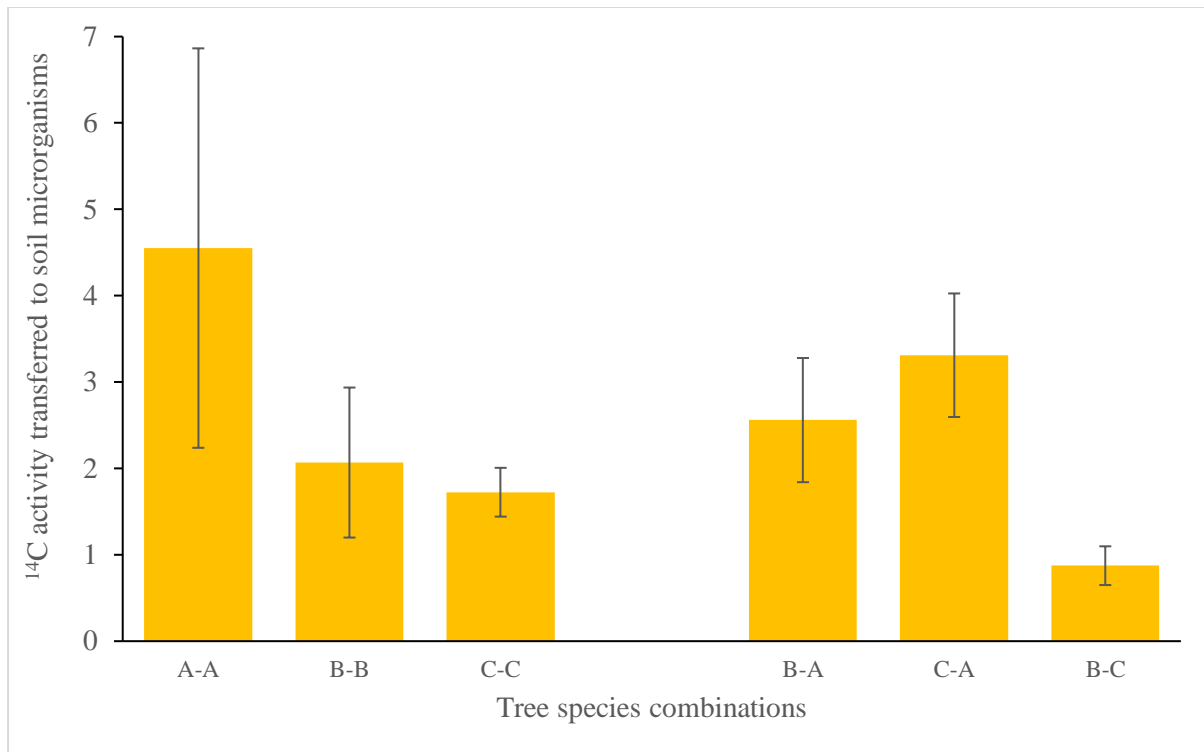


Figure 6: Total ^{14}C activity estimated to have been transferred to the soil micro-organisms in the receiver plant pot, expressed as a percentage of the total ^{14}C activity in the entire mesocosm, in the six species treatment combinations (A- *A. glutinosa*, B- *B. pendula*, and C- *C. sativa*). Intra-specific and inter-specific combinations are grouped together for comparison. Data are mean \pm SE ($n = 4$).

3.8 Fungal DNA analysis

To determine the driver of the interspecific C transfer it was decided to analyse the soil to determine which fungal symbionts were present and therefore distinguish likely conduits for biogeochemical cycling in the soil and subsequent transfer between trees. The percentage DNA is presented in Table 4.

Table 4: DNA species data tabulated as a percentage of total DNA extracted and replicated.

Fungal species	Tree species	<i>C. sativa</i>		<i>C. sativa</i>		<i>B. pendula</i>		<i>C. sativa</i>
		Ecology	Donor	Sand	Receiver	Donor	Sand	Receiver
<i>Sphaerosporella brunnea</i>	MR EM	69.03%	84.85%	78.55%		95.48%	90.95%	91.72%
<i>Inocybe curvipes</i>	MR EM	23.88%	6.69%	0.29%		0.11%	0.11%	0.23%
<i>Laccaria oblongospora</i>	MR EM	0.11%	0.16%	20.28%		0.03%	0.00%	0.63%
<i>Penicillium abidjanum</i>	SAP	0.28%	7.15%	0.27%		0.25%	1.66%	0.31%
<i>Peziza</i> sp.	MR EM	0.00%	0.00%	0.00%		0.00%	4.70%	0.08%
<i>Fusarium oxysporum</i>	PARA SAP	0.75%	0.35%	1.23%		0.87%	0.30%	1.65%
<i>Inocybe rufoalba</i>	MR EM	0.04%	4.71%	6.27%		0.00%	0.00%	0.03%
<i>Hebeloma</i> sp.	MR EM	1.23%	0.00%	1.18%		1.04%	0.08%	0.00%
<i>Trichothecium ovalisporum</i>	PATH	2.55%	0.00%	0.11%		0.05%	0.00%	1.33%
<i>Thelephora terrestris</i>	MR EM	0.63%	0.29%	1.07%		0.03%	0.00%	0.28%
<i>Capnodiales</i> sp.	SAP ENDO	1.15%	0.16%	0.07%		0.16%	0.06%	0.22%
<i>Trichothecium roseum</i>	SAP	0.00%	0.15%	3.43%		0.02%	0.00%	0.05%
<i>Aureobasidium pullulans</i>	EPI ENDO	0.00%	0.00%	0.03%		0.06%	0.01%	0.42%

We plotted the percentage frequency of the mycorrhizal species against each other (Figure 7). *Sphaerosporella brunnea* was the most abundance in all species treatments with very little difference between species treatments. No significant difference was found between the treatment combinations ($P = 0.94$).

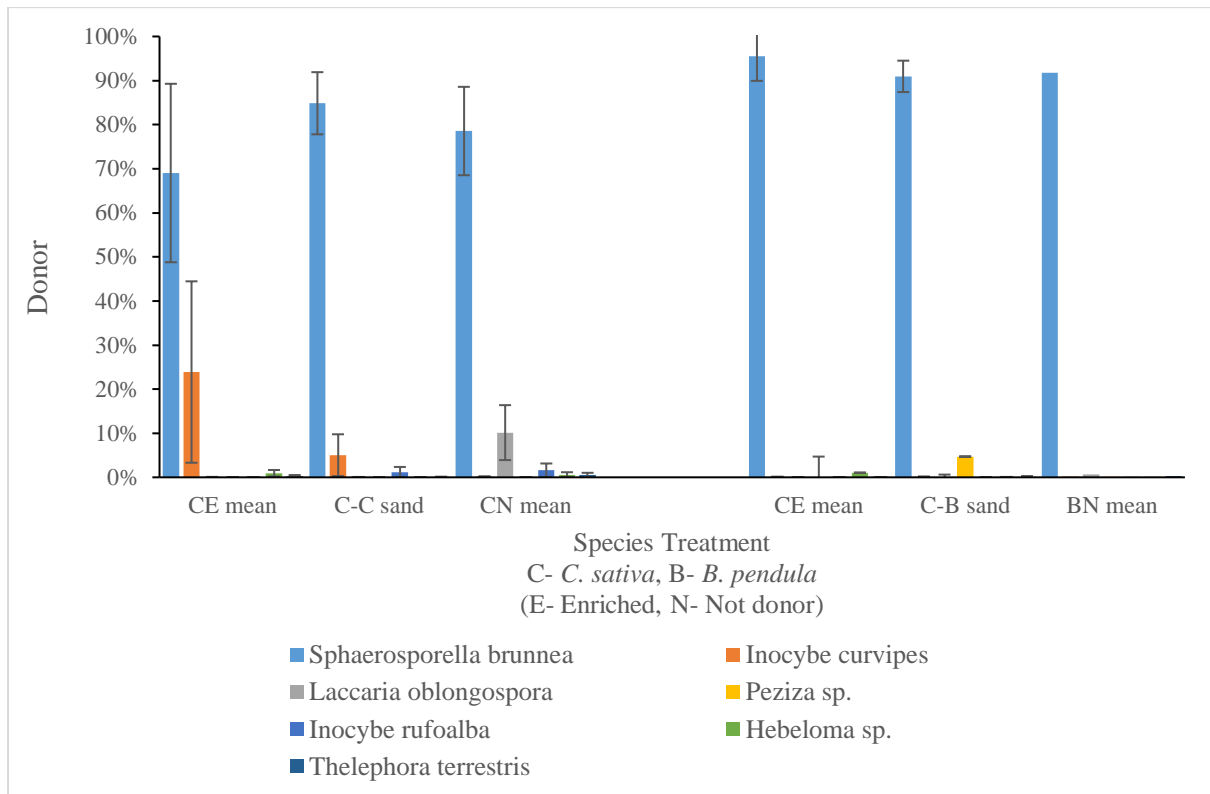


Figure 7: Results from the soil and sand DNA analysis of the two most contrasting treatments (*C. sativa* to *C. sativa* and *C. sativa* to *B. pendula*). Data are mean abundance expressed as a percentage of total of DNA extracted \pm SE ($n = 4$)

We then plotted the percentage frequency of the mycorrhizal species identified in the sand against each other (Figure 8). These data were interesting as the mycorrhizal species located in the sand must be the species responsible for the CMN formation linking the two plants and subsequent belowground ^{14}C activity transferred from the donor to the receiver trees.

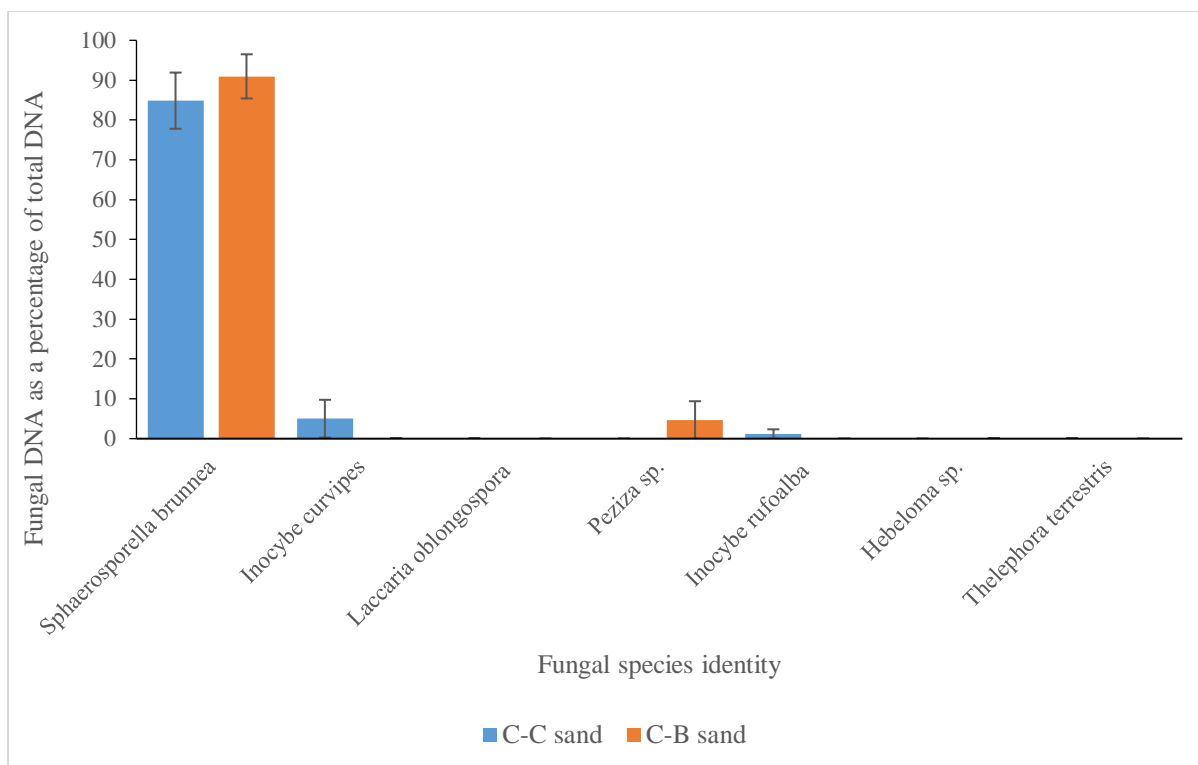


Figure 8: Comparison of the mean DNA abundance of the seven most frequently identified species, in the sand connecting the two fractions of the mesocosm, in the two treatments with the most contrasting ^{14}C transfer results expressed as a percentage of total DNA extracted \pm SE ($n = 4$)

4. Discussion

4.1. Soil chemical properties

The differences in soil conditions under the two species which we would expect after the establishment period were not observed. Therefore, the identity of the tree species made no significant difference to the soil conditions. Overall, the *A. glutinosa* pots had slightly more ammonium than the other species treatments but not nitrate, which is surprising as the *A. glutinosa* treatments are actinorhizal. This could be as a result of the nitrate fixed by the *Frankia alni* in the *A. glutinosa* root nodules being transferred to the non-actinorhizal species, further studies using ^{15}N could confirm this. This transfer of N is highly likely in both common arbuscular mycorrhizal networks (Frey and Schuepp, 1992, 1993; Johansen and Jensen, 1996; Montesinos-Navarro, 2019) and via common ectomycorrhizal networks (Arnebrant et al., 1993; Ekblad and Huss-Danell, 1995; Wipf et al., 2019).

4.2. ^{14}C activity in donor trees

The consistent mean total activity and comparable error bars of the donor trees suggests that the enrichment with $^{14}\text{CO}_2$ pulse was successful, was consistent across all species treatment combinations allowing us to compare the ^{14}C activity of the receiver plants with a degree of certainty.

The differences in the allocation of ^{14}C to the donor tree tissues illustrated the differences in relative biomass of the three species. Overall, the mean results showed that the *A. glutinosa* trees had the greatest proportion of leaves ($34.9 \pm 9.1\%$) followed by *B. pendula* ($21.5 \pm 5.5\%$), the *C. sativa* ($18.7 \pm 1.8\%$). The results found to be significant were the ^{14}C allocation to the fine root, soil respiration and soil solution. The mean ^{14}C allocation to fine-roots in the *A. glutinosa* to *A. glutinosa* species treatments was found to be significantly different from the *C. sativa* to *C. sativa* treatments. This may be as a result of the fine roots not being separated from and being homogenised with the root nodules. Later experiments will distinguish between the ^{14}C activity in the root nodules by separating them from the fine roots and analysing the ^{14}C activity independently. Interestingly, the soil solution and soil respiration had the same significant trend, which was that the *A. glutinosa* to *A. glutinosa* treatment had the highest soil respiration ($6.39 \pm 1.06\%$) and was significantly different to the treatment with the lowest soil respiration *B. pendula* to *A. glutinosa* ($2.30 \pm 1.20\%$). This could be a result of the actinorhizal relationship that *A. glutinosa* with *Frankia alni*. In addition, Burton et al., (1998) found that under non-drought conditions there is a direct correlation between increased soil respiration and fine-root N concentration and suggests that root N concentration and temperature can be used a predictor for belowground respiration. As our experiment took place in black coloured pots placed in a glasshouse, soil temperatures are likely to have been elevated and could go some way to explaining this result.

4.3. ^{14}C activity of the connecting sand tube

The ^{14}C activity results for the sand in the connecting Perspex tubes are interesting not least because they do not directly correlate to the total ^{14}C activity that was counted in the receiver trees. This suggests that some of the species treatments have transferred ^{14}C activity through the mesh via mycorrhizal hyphae but not then passed the ^{14}C activity onto the receiver tree. The *A. glutinosa* to *A. glutinosa* treatment showed the most ^{14}C activity in the sand but the least ^{14}C activity in the receiver tree. It could be that the ^{14}C activity is being utilised for hyphal growth by the fungi and not transferred or as there was only a single harvesting event it could

be that the transfer between plants of the same species is delayed and that given a longer chase-period the ^{14}C activity would have been detected in the receiver plant. Although the literature suggests that transfer can occur within 24 hours for plants of the same species in the case of volatiles believed to defend the plant against aphid attack (Babikova et al., 2013). This would be interesting to investigate in future studies.

4.4. ^{14}C activity in receiver mesocosm partitions

The soil solution data was the first data that we collected which confirmed the ^{14}C activity had passed through the sand and both the mesh Perspex tube coverings. The ^{14}C activity in the soil solution was as we would expect from the sand data, with the exception of the *B. pendula* to *A. glutinosa* treatment where the soil solution contained less ^{14}C activity than expected, suggesting that the ^{14}C activity that was transferred through the sand was retained in the soil or respired by the soil microbial community. Although no statistically significant difference in the soil ^{14}C activity was found in the soil respiration traps, the results seem to suggest that the ^{14}C activity found in the respiration traps was slightly lower in all intraspecific treatments when compared to interspecific combinations.

The interesting result from this data was the trend seen in the *B. pendula* to *A. glutinosa* treatments. Namely, the *B. pendula* to *A. glutinosa* treatments have relatively high ^{14}C activity in the soil but lower counts in soil solution and soil respiration than would be expected considering the ^{14}C activity located in the soil. This suggests that the ^{14}C activity is residing in the soil for longer, possibly as hyphal biomass and not being exuded and metabolised by the soil micro-organisms as quickly as the other treatments. It could be that the ^{14}C had been incorporated into the structural component of the fungal mycelium.

4.5. ^{14}C transfer via CMN

The total ^{14}C transfer was expected to be greater in the intra- than inter-specific treatments; this prediction was based on several previous studies which suggested that C transfer is preferential when receiver plants are full-siblings of the donor tree (Pickles et al., 2017). A theory known as the *kin selection* theory is widely supported by previous studies (Graves et al., 1997; Verbruggen et al., 2012; Platt and Bever, 2009; Dudley et al., 2013; Murphy and Dudley, 2009; Asay, 2013). The *mother tree* hypothesis goes further suggesting that recognition can go as far as trees sensing their root exudates of their offspring (Nara, 2006). The likely mechanism for this would be mediation by the mycorrhizal fungi's ability to recognise kin root exudates and for this to stimulate increased transfer (Biedrycki et al., 2010). Other studies suggest that kin

recognition could stimulate hyphal growth such as File et al., (2012), that found that the root colonization, size of mycorrhizal network, number of arbuscles were greater when *Ambrosia artemisiifolia* L. was grown with siblings compared to non-siblings but that the root lesions were reduced in number. Many of these studies focussed on the less complex symbiotic relationship with arbuscular mycorrhizae and it is possible that this trend is a result of soil or plant nutritional status and not directly related to plant fungal symbiotic interactions. Contrastingly, however, many other studies confirm the transfer of C between interspecific tree treatments (Simard, 1995; Simard et al., 1997a, Simard et al., 1997b) and a recent meta-analysis suggests that the greater the phylogenetic distance between species the greater the ectomycorrhizal facilitation (Montesinos-Navarro et al., 2019).

4.6. Total transfer

The ^{14}C activity data for the receiver trees suggested that there was more transfer in the interspecific transfer treatments. After a literature search this left us with several possible hypotheses namely;

- 1) If the mycorrhizal fungi present are species-specific, but connected to each other, then multiple tree species would mean more fungal diversity than single species mesocosms. Increased mycorrhizal diversity could then lead to more ^{14}C activity being transferred between tree species.
- 2) If the mycorrhizal fungi present are not species-specific and are soil substrate or climate specific then more ^{14}C activity could have been transferred from the donor to three receiver trees as a result these treatments having greater mycorrhizal colonisation.

However, fungal DNA data failed to prove that the treatment exhibiting the most ^{14}C transfer had either a greater fungal diversity or abundance. This led us to postulate that the differences in transfer are due to a source sink relationship, i.e. the plants with the most metabolically active below microbial symbionts will allocate the most photosynthetically fixed C belowground. Partners within the plant microbe symbiosis are able to determine the energetic demands of the partner (Kiers et al., 2011; Fellbaum et al., 2012; Fellbaum et al., 2014). It is also known that trees species can differ in the amount of photosynthetically derived C that they are prepared to invest in the CMN (Walder et al., 2012), potentially through a biological market trading system (Wyatt et al., 2014), also known as the economic game theory (Archetti et al., 2011) and in some cases the presence of the symbiotic partnership magnifies the inequity (Weremijewicz and Janos, 2013; Booth and Hoeksema, 2010). We hypothesised that transfer

would be most in the intraspecific species treatment either as plants can use CMN to transfer resources to their kin (Pickles et al., 2017) possibly mediated by volatile cues (Karban and Shiojiri, 2009; Karban et al., 2013). The results although not statistically significant suggested that the transfer was in fact more in the interspecific treatments. However, Montesinos-Navarro et al., (2019) conducted a meta-analysis of plant facilitation through mycorrhizal networks and suggested that facilitative resource sharing increases with trading plant phylogenetic distance. This could be as the nutritional demands of distantly related plants and their nutrient resource strategies may differ (Cornwell et al., 2014).

4.7. Fungal DNA analysis

Analysis of the fungal DNA in the sand and soil revealed that the communities were dominated by three mycorrhizal species namely *Sphaerospora brunnea*, *Inocybe curvipes* and *Laccaria oblongospora* with the mean % of total DNA being 85.1 ± 4.0 %, 5.2 ± 3.9 % and 3.5 ± 3.4 % respectively for the three fungi species, making these three species 93.9 % of the total DNA on average. The fact that the *Laccaria* sp. could be important as Chen et al., (2000) found that in a *Eucalyptus* species which exhibits dual mycorrhizal symbiosis (both arbuscular and ectomycorrhizal), that the presence of *Laccaria* sp. significantly reduces colonisation by arbuscular mycorrhizal symbionts. The mechanism for this is not yet fully understood (Lodge, 2000) but several theories have been postulated including; that as *Laccaria* is an ectomycorrhizal fungi with a mantle, that this may act as a physical barrier to arbuscular mycorrhizal colonisation (Chilvers et al., 1987; Chilvers and Gust, 1982), or the fungi may be exuding chemical deterrents to the colonisation of the root by arbuscular mycorrhizal fungi (Lodge and Wentworth, 1990), or it could just be as a result of greater competition for plant derived carbohydrates (Lodge, 2000). Chen et al., (2000) did find that the presence of *Laccaria* sp. modified the root architecture, specifically by reducing the growth of new root tips and thereby reducing the availability of suitable spots for arbuscular fungal colonisation.

The barcodes used for identifying species are located within ribosomal RNA genes (George et al., 2019). Specifically, for fungi, the internal transcribed spacer (ITS) section is utilised to identify taxonomic groupings (Schoch et al., 2012). This novel methodology has led to advances in our understanding of fungal diversity (Kumar et al., 2019), but it also has been criticised for potential bias based on the ITS region chosen and the fungal database used to identify species (Tedersoo et al., 2016; Xue et al., 2019). ITS barcoding is considered particularly limited when concerned with unknown samples as analysis can only identify to

family level, at best (Cavender-Bares et al., 2009). Use of ITS barcodes has also been found to be particularly unreliable in identifying the Glomeromycetes (Stockinger et al., 2010). For example, in bulk soil this method has been found to underestimate Glomeromycetes (Berruti et al., 2017), which would in this case be arbuscular mycorrhizal associated with *A. glutinosa*. Future studies could use the 18S region which is better suited to identifying the presence of Glomeromycetes

5. Conclusions

H₁: Transfer of C by CMNs is greater inter-specifically than intra-specifically.

H₂: Greater mycorrhizal diversity per unit of rhizosphere soil will result in more C transfer between trees via the CMN.

H₁: The transfer appeared to be more in the inter-specific species treatments than the intra-specific although this was not borne out statistically. This could be at least in part as a result of multiple biological interactions with potential variations within tree species examples of this are differences in; root architecture leading to differences in belowground sink strength and aboveground biomass differences and C allocation differences. These are then confounded by more potential differences in root colonisation by mycorrhizal fungi. In short, the sample size was probably too small for a complex biological chain such as this and as a result very few differences were found to be significant.

H₂: DNA analysis of the soil under the two most contrasting treatments (in terms of the amount of photosynthetically derived C that was transferred via CMN to the receiver tree), failed to find differences in species diversity. This hypothesis was therefore rejected.

The transfer between plants of resources, in this case C, does not appear to be driven by either the diversity or abundance of mycorrhizal fungi in the interconnected sand tube. We postulate therefore that the fungal mediated resource transfer is most likely determined by the supply and demand of the partners participating in the symbiotic relationship. It could be that each species, due to differences in nutrient demand during different developmental stages, has a period of supplying the other with photosynthetically derived, only for this to be reciprocated at a later stage once the other species enters a period of increased growth rate.

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7. References

- Ahmed, I.U., Smith, A.R., Jones, D.L. and Godbold, D.L., 2016. Tree species identity influences the vertical distribution of labile and recalcitrant carbon in a temperate deciduous forest soil. *Forest Ecology and Management*, 359, pp.352-360.
- Archetti, M., Scheuring, I., Hoffman, M., Frederickson, M.E., Pierce, N.E. and Yu, D.W., 2011. Economic game theory for mutualism and cooperation. *Ecology Letters*, 14(12), pp.1300-1312.
- Arnebrant, K., Ek, H., Finlay, R.D. and Söderström, B., 1993. Nitrogen translocation between *Alnus glutinosa* (L.) Gaertn. seedlings inoculated with *Frankia* sp. and *Pinus contorta* Doug. ex Loud seedlings connected by a common ectomycorrhizal mycelium. *New Phytologist*, 124(2), pp.231-242.
- Arora, V.K., Boer, G.J., Friedlingstein, P., Eby, M., Jones, C.D., Christian, J.R., Bonan, G., Bopp, L., Brovkin, V., Cadule, P. and Hajima, T., 2013. Carbon–concentration and carbon–climate feedbacks in CMIP5 Earth system models. *Journal of Climate*, 26(15), pp.5289-5314.
- Asay, A.K., 2013. *Mycorrhizal facilitation of kin recognition in interior Douglas-fir (Pseudotsuga menziesii var. glauca)* (Doctoral dissertation, University of British Columbia).
- Averill, C., Turner, B.L. and Finzi, A.C., 2014. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature*, 505(7484), p.543.
- Babikova, Z., Johnson, D., Bruce, T., Pickett, J. and Gilbert, L., 2013. How rapid is aphid-induced signal transfer between plants via common mycelial networks? *Communicative and Integrative Biology*, 6(6), pp.835-43.
- Biedrzycki, M.L., Jilany, T.A., Dudley, S.A. and Bais, H.P., 2010. Root exudates mediate kin recognition in plants. *Communicative and Integrative Biology*, 3(1), pp.28-35.

- Beiler, K.J., Durall, D.M., Simard, S.W., Maxwell, S.A. and Kretzer, A.M., 2010. Architecture of the wood-wide web: *Rhizopogon* spp. genets link multiple Douglas-fir cohorts. *New Phytologist*, 185(2), pp.543-553.
- Berruti, A., Desirò, A., Visentin, S., Zecca, O. and Bonfante, P., 2017. ITS fungal barcoding primers versus 18S AMF-specific primers reveal similar AMF-based diversity patterns in roots and soils of three mountain vineyards. *Environmental Microbiology Reports*, 9(5), pp.658-667.
- Bennett, J.A. and Klironomos, J., 2018. Climate, but not trait, effects on plant–soil feedback depend on mycorrhizal type in temperate forests. *Ecosphere*, 9(3).
- Booth, M.G. and Hoeksema, J.D., 2010. Mycorrhizal networks counteract competitive effects of canopy trees on seedling survival. *Ecology*, 91(8), pp.2294-2302.
- Brownlee, C., Duddridge, J.A., Malibari, A. and Read, D.J., 1983. The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. In *Tree Root Systems and Their Mycorrhizas* (pp. 433-443). Springer, Dordrecht.
- Bruno, J.F., Stachowicz, J.J. and Bertness, M.D., 2003. Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution*, 18(3), pp.119-125.
- Bücking, H., Mensah, J.A. and Fellbaum, C.R., 2016. Common mycorrhizal networks and their effect on the bargaining power of the fungal partner in the arbuscular mycorrhizal symbiosis. *Communicative and Integrative Biology*, 9(1), p.e1107684.
- Burton, A.J., Pregitzer, K.S., Zogg, G.P. and Zak, D.R., 1998. Drought reduces root respiration in sugar maple forests. *Ecological Applications*, 8(3), pp.771-778.
- Cavender-Bares, J., Kozak, K.H., Fine, P.V. and Kembel, S.W., 2009. The merging of community ecology and phylogenetic biology. *Ecology Letters*, 12(7), pp.693-715.
- Chen, Y.L., Brundrett, M.C. and Dell, B., 2000. Effects of ectomycorrhizas and vesicular–arbuscular mycorrhizas, alone or in competition, on root colonization and growth of *Eucalyptus globulus* and *E. urophylla*. *The New Phytologist*, 146(3), pp.545-555.
- Chilvers GA, Gust LW. 1982. Comparison between the growth rates of mycorrhizas, uninfected roots and a mycorrhizal fungus of *Eucalyptus st-johnii* R. T. Bak. *New Phytologist* 91. pp.453 – 466.

- Chilvers, G.A., Lapeyrie, F.F. and Horan, D.P., 1987. Ectomycorrhizal vs endomycorrhizal fungi within the same root system. *New Phytologist*, 107(2), pp.441-448.
- Cornwell, W.K., Westoby, M., Falster, D.S., FitzJohn, R.G., O'Meara, B.C., Pennell, M.W., McGlinn, D.J., Eastman, J.M., Moles, A.T., Reich, P.B. and Tank, D.C., 2014. Functional distinctiveness of major plant lineages. *Journal of Ecology*, 102(2), pp.345-356.
- Davidson, E.A., Savage, K.E. and Finzi, A.C., 2014. A big-microsite framework for soil carbon modeling. *Global Change Biology*, 20(12), pp.3610-3620.
- Dennis, P.G., Miller, A.J. and Hirsch, P.R., 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS Microbiology Ecology*, 72(3), pp.313-327.
- Dudley, S.A., Murphy, G.P. and File, A.L., 2013. Kin recognition and competition in plants. *Functional Ecology* 27, pp.898-906.
- Ekblad, A. and Huss-Daniel, K., 1995. Nitrogen fixation by *Alnus incana* and nitrogen transfer from *A. incana* to *Pinus sylvestris* influenced by macronutrients and ectomycorrhiza. *New Phytologist*, 131(4), pp.453-459.
- Elumeeva, T.G., Onipchenko, V.G., Cornelissen, J.H., Semenova, G.V., Perevedentseva, L.G., Freschet, G.T., van Logtestijn, R.S. and Soudzilovskaia, N.A., 2018. Is intensity of plant root mycorrhizal colonization a good proxy for plant growth rate, dominance and decomposition in nutrient poor conditions? *Journal of Vegetation Science*, 29(4), pp.715-725.
- Fellbaum, C.R., Gachomo, E.W., Beesetty, Y., Choudhari, S., Strahan, G.D., Pfeffer, P.E., Kiers, E.T. and Bücking, H., 2012. Carbon availability triggers fungal nitrogen uptake and transport in arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences*, 109(7), pp.2666-2671.
- Fellbaum, C.R., Mensah, J.A., Cloos, A.J., Strahan, G.E., Pfeffer, P.E., Kiers, E.T. and Bücking, H., 2014. Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants. *New Phytologist*, 203(2), pp.646-656.
- File, A.L., Klironomos, J., Maherali, H. and Dudley, S.A., 2012. Plant kin recognition enhances abundance of symbiotic microbial partner. *PLoS One*, 7(9), p. e45648.

- Finlay, R.D., 2008. Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. *Journal of Experimental Botany*, 59(5), pp.1115-1126.
- Francis, R., and Read, D. J., 1984. Direct transfer of carbon between plants connected by vesicular–arbuscular mycorrhizal mycelium. *Nature* 307, pp.53 – 56.
- Frey, B. and Schuepp, H. 1992. Transfer of symbiotically fixed nitrogen from berseem (*Trifolium alexandrinum* L.) to maize via vesicular-arbuscular mycorrhizal hyphae. *New Phytologist*. 122, pp.447–454.
- Frey, B. and Schuepp, H. 1993. A role of vesicular arbuscular (VA) mycorrhizal fungi in facilitating interplant nitrogen transfer. *Soil Biology and Biochemistry*. 25, pp.651–658.
- George, P.B., Creer, S., Griffiths, R.I., Emmett, B.A., Robinson, D.A. and Jones, D.L., 2019. Primer and database choice affect fungal functional but not biological diversity findings in a national soil survey. *Frontiers in Environmental Science*, 7, p.173.
- Graves, J.D., Watkins, N.K., Fitter, A.H., Robinson, D. and Scrimgeour, C., 1997. Intraspecific transfer of carbon between plants linked by a common mycorrhizal network. *Plant and Soil*, 192(2), pp.153-159.
- Grime, J.P., Mackey, J.M.L., Hillier, S.H. and Read, D.J., 1987. Floristic diversity in a model system using experimental microcosms. *Nature*, 328(6129), p.420.
- Hammer, E.C., Pallon, J., Wallander, H. and Olsson, P.A., 2011. Tit for tat? A mycorrhizal fungus accumulates phosphorus under low plant carbon availability. *FEMS Microbiology Ecology*, 76(2), pp.236-244.
- Hirrel, M.C. and Gerdemann, J.W., 1979. Enhanced carbon transfer between onions infected with a vesicular-arbuscular mycorrhizal fungus. *New Phytologist*, 83(3), pp.731-738.
- Hoeksema, J.D., Bever, J.D., Chakraborty, S., Chaudhary, V.B., Gardes, M., Gehring, C.A., Hart, M.M., Housworth, E.A., Kaonongbua, W., Klironomos, J.N. and Lajeunesse, M.J., 2018. Evolutionary history of plant hosts and fungal symbionts predicts the strength of mycorrhizal mutualism. *Communications Biology*, 1(1), p.116.
- Hulvey, K. B., Hobbs, R. J., Standish, R. J., Lindenmayer, D. B., Lach, L., and Perring, M. P., 2013. Benefits of tree mixes in carbon plantings. *Nature Climate Change*, 3(10), pp.869-874.

- Jobbágy, E.G. and Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications*, 10(2), pp.423-436.
- Johnson, D. and Gilbert, L., 2015. Interplant signalling through hyphal networks. *New Phytologist*, 205(4), pp.1448-1453.
- Johansen, A. and Jensen, E. S. 1996. Transfer of N and P from intact or decomposing roots of pea to barley interconnected by an arbuscular mycorrhizal fungus. *Soil Biology and Biochemistry*, 28, pp.73–81.
- Jones, D.L. and Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology and Biochemistry*, 38(5), pp.991-999.
- Jones, D.L., Nguyen, C. and Finlay, R.D., 2009. Carbon flow in the rhizosphere: carbon trading at the soil–root interface. *Plant and Soil*, 321(1-2), pp.5-33.
- Jones, T.G., Evans, C.D., Jones, D.L., Hill, P.W. and Freeman, C., 2016. Transformations in DOC along a source to sea continuum; impacts of photo-degradation, biological processes and mixing. *Aquatic Sciences*, 78(3), pp.433-446.
- Karban, R. and Shiojiri, K., 2009. Self-recognition affects plant communication and defense. *Ecology Letters*, 12(6), pp.502-506.
- Karban, R., Shiojiri, K., Ishizaki, S., Wetzal, W.C. and Evans, R.Y., 2013. Kin recognition affects plant communication and defence. *Proceedings of the Royal Society B: Biological Sciences*, 280(1756), p.20123062.
- Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J.A., Franken, O., Verbruggen, E., Fellbaum, C.R., Kowalchuk, G.A., Hart, M.M., Bago, A. and Palmer, T.M., 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*, 333(6044), pp.880-882.
- Kleber, M., Sollins, P. and Sutton, R., 2007. A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. *Biogeochemistry*, 85(1), pp.9-24.
- Klein, T., Siegwolf, R. T., and Körner, C. 2016. Belowground carbon trade among tall trees in a temperate forest. *Science*, 352(6283), pp.342-344.

- Kumar, V., Soni, R., Jain, L., Dash, B. and Goel, R., 2019. Endophytic Fungi: Recent Advances in Identification and Explorations. In *Advances in Endophytic Fungal Research* (pp. 267-281). Springer, Cham.
- Lekberg, Y., Hammer, E.C. and Olsson, P.A., 2010. Plants as resource islands and storage units—adopting the mycocentric view of arbuscular mycorrhizal networks. *FEMS Microbiology Ecology*, 74(2), pp.336-345.
- Lodge, D.J., 2000. Ecto-or arbuscular mycorrhizas—which are best? *The New Phytologist*, 146(3), pp.353-354.
- Lodge DJ, Wentworth TR. 1990. Negative associations among VA-mycorrhizal fungi and some ectomycorrhizal fungi inhabiting the same root system. *Oikos*. 57, pp.347 – 356.
- Lukac, M. and Godbold, D.L., 2011. *Soil ecology in northern forests: a belowground view of a changing world*. Cambridge University Press.
- Luo, Y., Ahlström, A., Allison, S.D., Batjes, N.H., Brovkin, V., Carvalhais, N., Chappell, A., Ciais, P., Davidson, E.A., Finzi, A. and Georgiou, K., 2016. Toward more realistic projections of soil carbon dynamics by Earth system models. *Global Biogeochemical Cycles*, 30(1), pp.40-56.
- Miranda, K.M., Espey, M.G. and Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*, 5(1), pp.62-71.
- Molina, R., Massicotte, H. and Trappe, J.M., 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. *Mycorrhizal functioning: an integrative plant-fungal process*, 357, p.423.
- Montesinos-Navarro, A., Valiente-Banuet, A. and Verdú, M., 2019. Plant facilitation through mycorrhizal symbiosis is stronger between distantly related plant species. *New Phytologist*.
- Montesinos-Navarro, A., Verdú, M., Querejeta, J.I., Sortibrán, L. and Valiente-Banuet, A., 2016. Soil fungi promote nitrogen transfer among plants involved in long-lasting facilitative interactions. *Perspectives in Plant Ecology, Evolution and Systematics*, 18, pp.45-51.
- Mulvaney, R.L., 1996. Nitrogen—inorganic forms. *Methods of soil analysis: Part 3: Chemical methods*, 5, pp.1123-1184.

- Murphy, G.P. and Dudley, S.A., 2009. Kin recognition: competition and cooperation in *Impatiens* (Balsaminaceae). *American Journal of Botany*, 96(11), pp.1990-1996.
- Murphy, J. and Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27, pp.31-36.
- Nara, K., 2006. Ectomycorrhizal networks and seedling establishment during early primary succession. *New Phytologist*, 169(1), pp.169-178.
- Neumann, G. and Römheld, V., 2007. The release of root exudates as affected by the plant physiological status. *The Rhizosphere: Biochemistry and organic substances at the soil-plant interface*, 2, pp.23-72.
- Olsen, S.R., 1954. *Estimation of available phosphorus in soils by extraction with sodium bicarbonate* (No. 939). US Department of Agriculture.
- Pausch, J. and Kuzyakov, Y., 2018. Carbon input by roots into the soil: quantification of rhizodeposition from root to ecosystem scale. *Global change biology*, 24(1), pp.1-12.
- Pfeffer, P.E., Douds, D.D., Bücking, H., Schwartz, D.P. and Shachar-Hill, Y., 2004. The fungus does not transfer carbon to or between roots in an arbuscular mycorrhizal symbiosis. *New Phytologist*, 163(3), pp.617-627.
- Pickles, B.J., Wilhelm, R., Asay, A.K., Hahn, A.S., Simard, S.W. and Mohn, W.W., 2017. Transfer of ^{13}C between paired Douglas-fir seedlings reveals plant kinship effects and uptake of exudates by ectomycorrhizas. *New Phytologist*, 214(1), pp.400-411.
- Platt, T.G. and Bever, J.D., 2009. Kin competition and the evolution of cooperation. *Trends in ecology and evolution*, 24(7), pp.370-377.
- Reynolds, S.G., 1970. The gravimetric method of soil moisture determination Part IA study of equipment, and methodological problems. *Journal of Hydrology*, 11(3), pp.258-273.
- Robinson, D. and Fitter, A., 1999. The magnitude and control of carbon transfer between plants linked by a common mycorrhizal network. *Journal of Experimental Botany*, 50(330), pp.9-13.
- Ryan, F., 2002. *Darwin's blind spot: evolution beyond natural selection*. Houghton, Mifflin Harcourt.
- Saiki, R.K., Scharf, S., Faloona, F., Mullis, K., Hoorn, G.T. and Arnheim, N., 1985. Polymerase chain reaction. *Science*, 230, pp.1350-1354.

- Selosse, M.A., Richard, F., He, X. and Simard, S.W., 2006. Mycorrhizal networks: des liaisons dangereuses?. *Trends in Ecology and Evolution*, 21(11), pp.621-628.
- Schmidt, M.W., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A. and Nannipieri, P., 2011. Persistence of soil organic matter as an ecosystem property. *Nature*, 478(7367), p.49.
- Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Consortium, F.B., Bolchacova, E., Voigt, K. and Crous, P.W., 2012. From the cover: nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences of the United States of America*, 109(16), p.6241.
- Shi, N.N., Gao, C., Zheng, Y. and Guo, L.D., 2017. Effects of ectomycorrhizal fungal identity and diversity on subtropical tree competition. *Journal of Plant Ecology*, 10(1), pp.47-55.
- Simard S.W., 1995. *Interspecific carbon transfer in ectomycorrhizal tree species mixtures*. Ph.D. Dissertation. Oregon State University, Corvallis. 210 p.
- Simard, S., Asay, A., Beiler, K., Bingham, M., Deslippe, J., He, X., Philip, L., Song, Y. and Teste, F., 2015. Resource transfer between plants through ectomycorrhizal fungal networks. In *Mycorrhizal networks* (pp. 133-176). Springer, Dordrecht.
- Simard, S.W., Durall, D.M. and Jones, M.D., 1997a. Carbon allocation and carbon transfer between t *Betula papyrifera* and t *Pseudotsuga menziesii* seedlings using a ¹³C pulse-labeling method. *Plant and Soil*, 191(1), pp.41-55.
- Simard, S. W., Perry, D. A., Jones, M. D., Myrold, D. D., Durall, D. M., and Molina, R., 1997b. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature*, 388(6642), 579-582.
- Smith, J.L. and Doran, J.W., 1997. Measurement and use of pH and electrical conductivity for soil quality analysis. *Methods for assessing soil quality*, 49, pp.169-185.
- Smith, A.R., Lukac, M., Hood, R., Healey, J.R., Miglietta, F. and Godbold, D.L., 2013. Elevated CO₂ enrichment induces a differential biomass response in a mixed species temperate forest plantation. *New Phytologist*, 198(1), pp.156-168.
- Smith, S.E. and Read, D.J., 2010. *Mycorrhizal symbiosis*. Academic press. London.

Stachowicz, J.J., 2001. Mutualism, facilitation, and the structure of ecological communities: positive interactions play a critical, but underappreciated, role in ecological communities by reducing physical or biotic stresses in existing habitats and by creating new habitats on which many species depend. *AIBS Bulletin*, 51(3), pp.235-246.

Tarnocai, C., Canadell, J.G., Schuur, E.A.G., Kuhry, P., Mazhitova, G. and Zimov, S., 2009. Soil organic carbon pools in the northern circumpolar permafrost region. *Global biogeochemical cycles*, 23(2).

Tedersoo, L., Bahram, M., Pölme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A. and Smith, M.E., 2014. Global diversity and geography of soil fungi. *Science*, 346(6213), p.1256688.

Tilman, D., 1988. *Plant strategies and the dynamics and structure of plant communities*. (No. 26). Princeton University Press. Princetown.

Todd-Brown, K.E.O., Randerson, J.T., Post, W.M., Hoffman, F.M., Tarnocai, C., Schuur, E.A.G. and Allison, S.D., 2013. Causes of variation in soil carbon simulations from CMIP5 Earth system models and comparison with observations. *Biogeosciences*, 10(3), pp.1717-1736.

Van Der Heijden, M.G., 2016. Underground networking. *Science*, 352(6283), pp.290-291.

Van Der Heijden, M.G. and Horton, T.R., 2009. Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology*, 97(6), pp.1139-1150.

Verbruggen, E., El Mouden, C., Jansa, J., Akkermans, G., Bücking, H., West, S.A. and Kiers, E.T., 2012. Spatial structure and interspecific cooperation: theory and an empirical test using the mycorrhizal mutualism. *The American Naturalist*, 179(5), pp.E133-E146.

Vogt, K.A., Grier, C.C., Meier, C.E. and Edmonds, R.L., 1982. Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis* ecosystems in Western Washington. *Ecology*, 63(2), pp.370-380.

Wallander, H., Nilsson, L.O., Hagerberg, D. and Bååth, E., 2001. Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. *New Phytologist*, 151(3), pp.753-760.

- Walder, F., Niemann, H., Natarajan, M., Lehmann, M.F., Boller, T. and Wiemken, A., 2012. Mycorrhizal networks: common goods of plants shared under unequal terms of trade. *Plant physiology*, 159(2), pp.789-797.
- Walder, F. and van der Heijden, M.G., 2015. Regulation of resource exchange in the arbuscular mycorrhizal symbiosis. *Nature Plants*, 1(11), p.15159.
- Weremijewicz, J. and Janos, D.P., 2013. Common mycorrhizal networks amplify size inequality in *Andropogon gerardii* monocultures. *New Phytologist*, 198(1), pp.203-213.
- Weremijewicz, J., Sternberg, L.D.S.L.O. and Janos, D.P., 2016. Common mycorrhizal networks amplify competition by preferential mineral nutrient allocation to large host plants. *New Phytologist*, 212(2), pp.461-471.
- Wipf, D., Krajinski, F., van Tuinen, D., Recorbet, G. and Courty, P.E., 2019. Trading on the arbuscular mycorrhiza market: from arbuscules to common mycorrhizal networks. *New Phytologist*. (in Press).
- Wu, B., Nara, K. and Hogetsu, T., 2002. Spatiotemporal transfer of carbon-14-labelled photosynthate from ectomycorrhizal *Pinus densiflora* seedlings to extraradical mycelia. *Mycorrhiza*, 12(2), pp.83-88.
- Wyatt, G.A., Kiers, E.T., Gardner, A. and West, S.A., 2014. A biological market analysis of the plant-mycorrhizal symbiosis. *Evolution*, 68(9), pp.2603-2618.
- Xue, C., Hao, Y., Pu, X., Penton, C.R., Wang, Q., Zhao, M., Zhang, B., Ran, W., Huang, Q., Shen, Q. and Tiedje, J.M., 2019. Effect of LSU and ITS genetic markers and reference databases on analyses of fungal communities. *Biology and fertility of soils*, 55(1), pp.79-88.

8. Appendices/ Supplementary

8.1. ^{14}C activity in donor trees

8.1.1 Total ^{14}C activity in donor trees

The activity of the donor trees was used as an indication that the pulse labelling event was successful across all species treatment combinations (Figure 8). ^{14}C activity was seen to be consistent across species treatment combinations with no significant differences found between species treatment combinations ($P = 0.62$) and standard errors were seen to be very similar for all treatments.

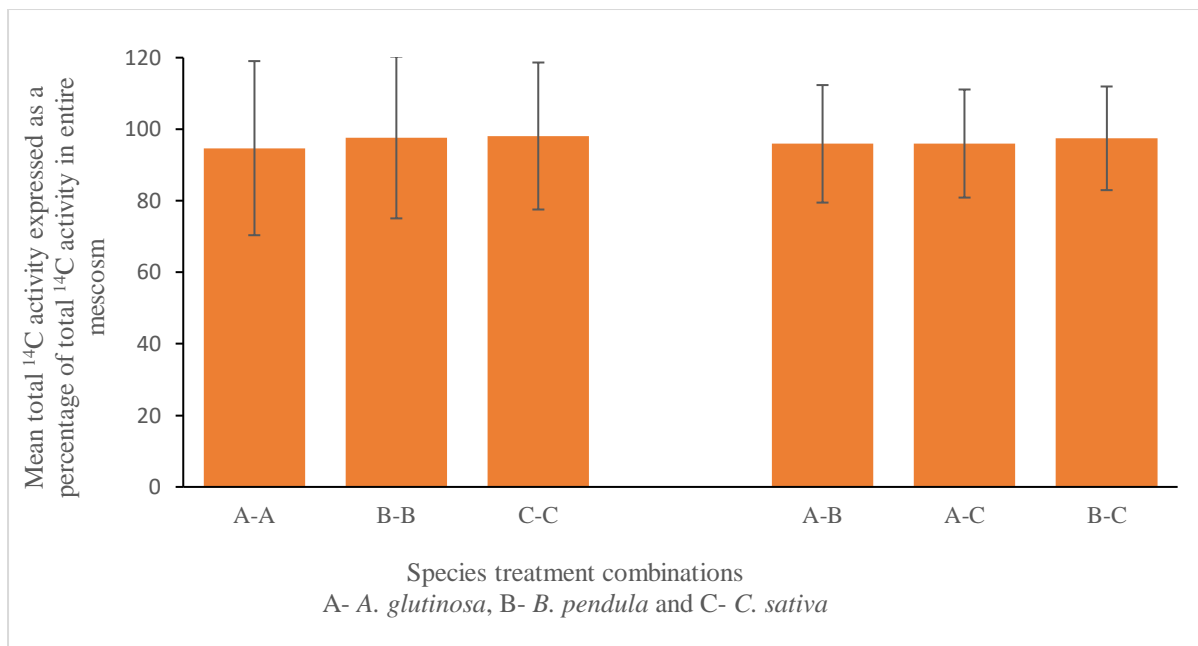


Figure S1: ^{14}C activity measured in donor plant partitions expressed as a percentage of total ^{14}C activity counted in entire mesocosm in the six species treatment combinations (A- *A. glutinosa*, B- *B. pendula*, and C- *C. sativa*). Data are mean \pm SE ($n = 4$).

8.1.2 Partition allocation in donor plants

To establish if there were differences in belowground C allocation between species the ^{14}C partition allocation of the donor plants was investigated and the results are shown in Table 4.

Table S1: Mean ^{14}C activity expressed as a percentage of the ^{14}C activity in the entire mesocosm \pm SE of the six species treatment combinations (A- *A. glutinosa*, B- *B. pendula* and C- *C. sativa*) determined by destructive harvesting 336 hours after the $^{14}\text{CO}_2$ pulse ($n= 4$). Statistically significant results are given in bold and denoted by superscript letters and asterisks (*, $P < 0.1$).

Treatment	Leaves	Branch	Donor plant partition				
			Coarse root	Fine root	Soil respiration	Soil solution	Soil
A-A	34.98 \pm 9.31	12.57 \pm 2.16	7.16 \pm 1.88	2.60 \pm 0.50	6.39 \pm 1.06	<0.01 \pm 0.00	31.00 \pm 9.42
B-B	21.15 \pm 5.35	21.39 \pm 4.34	5.35 \pm 1.59	4.88 \pm 1.67	5.44 \pm 0.67	<0.01 \pm 0.00	39.48 \pm 9.00
C-C	18.67 \pm 1.76	16.73 \pm 4.36	9.98 \pm 2.05	8.28 \pm 1.33	5.16 \pm 0.91	<0.01 \pm 0.00	39.28 \pm 10.13
B-A	26.60 \pm 3.44	23.92 \pm 4.07	9.55 \pm 0.49	5.14 \pm 1.03	2.30 \pm 1.20	<0.01 \pm 0.00	28.40 \pm 6.20
C-A	21.94 \pm 3.54	13.11 \pm 2.04	7.50 \pm 1.83	5.71 \pm 1.13	3.91 \pm 0.52	<0.01 \pm 0.00	43.82 \pm 6.05
C-B	28.76 \pm 4.59	27.03 \pm 4.67	7.37 \pm 0.63	2.90 \pm 0.96	3.61 \pm 0.63	<0.01 \pm 0.00	27.79 \pm 3.01
<i>P value</i>	0.30	0.07	0.50	0.03*	0.03*	0.03*	0.59

The allocation of ^{14}C activity results show that the highest percentage of ^{14}C was retained in the leaves in the donor *A. glutinosa*, although this was not found to be significant ($P = 0.30$). In the branch and stem partition the donor *B. pendula* treatments retained more ^{14}C as a percentage of overall ^{14}C activity than the other species, although this was not found to be significant ($P = 0.50$). Coarse roots appeared not to show allocation trends by species. In contrast, the fine root results showed more activity in the *C. sativa* to *C. sativa* species treatment combinations ($P = 0.03$). A post-hoc Tukey test revealed that the mean ^{14}C activity located in the fine-roots of the *A. glutinosa* to *A. glutinosa* species treatment combination was significantly different to the *C. sativa* to *C. sativa* ($P = 0.03$). No other combinations were found to be significantly different. Soil solution and soil respiration were both also found to have results that were significantly different. A post-hoc Tukey test showed in both the soil respiration and soil solution the significant differences in mean ^{14}C activity were between the *A. glutinosa* to *A. glutinosa* species treatments and the *A. glutinosa* to *B. pendula* combinations

($P = 0.03$). The soil ^{14}C activity showed no trends and showed no significant differences between treatments.

8.2. Activity of the connecting sand tube

The results of the ^{14}C analysis of the sand enabled us to assess the transfer of ^{14}C from the donor tree to the mycorrhizal hyphae regardless of if the ^{14}C was then passed on to the receiver tree. The sand in the *A. glutinosa* to *A. glutinosa* treatment was seen to have the largest amount ^{14}C activity when expressed as a percentage of the total ^{14}C activity counted in the entire mesocosm (Figure 10). The species treatments were not found to be significantly different from each other ($P = 0.43$).

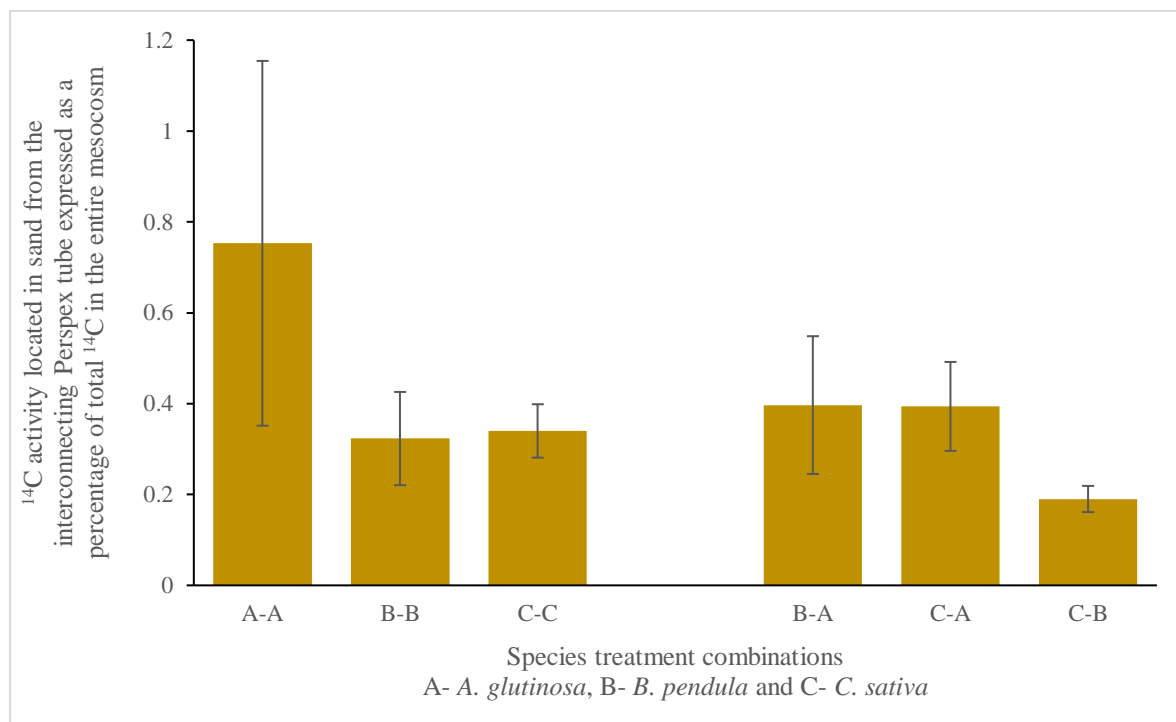


Figure S2: ^{14}C activity counted in the sand from the Perspex interconnecting tube in the six species treatment combinations (A- *A. glutinosa*, B- *B. pendula*, and C- *C. sativa*), post destructive harvesting 336 hours after the $^{14}\text{CO}_2$ pulse. Intraspecific and interspecific combinations are grouped together for comparison. Data are mean \pm SE ($n = 4$).

8.3. ^{14}C activity in receiver mesocosm partitions

8.3.1 Soil solution ^{14}C activity

The soil water ^{14}C activity collected by Rhizon suction sampling devices from the receiver fraction showed that the intraspecific combinations seemed to have slightly higher ^{14}C activity counts (Figure 11), however, the results of the intraspecific combination were not found to be significantly different to the interspecific combinations ($P = 0.41$).

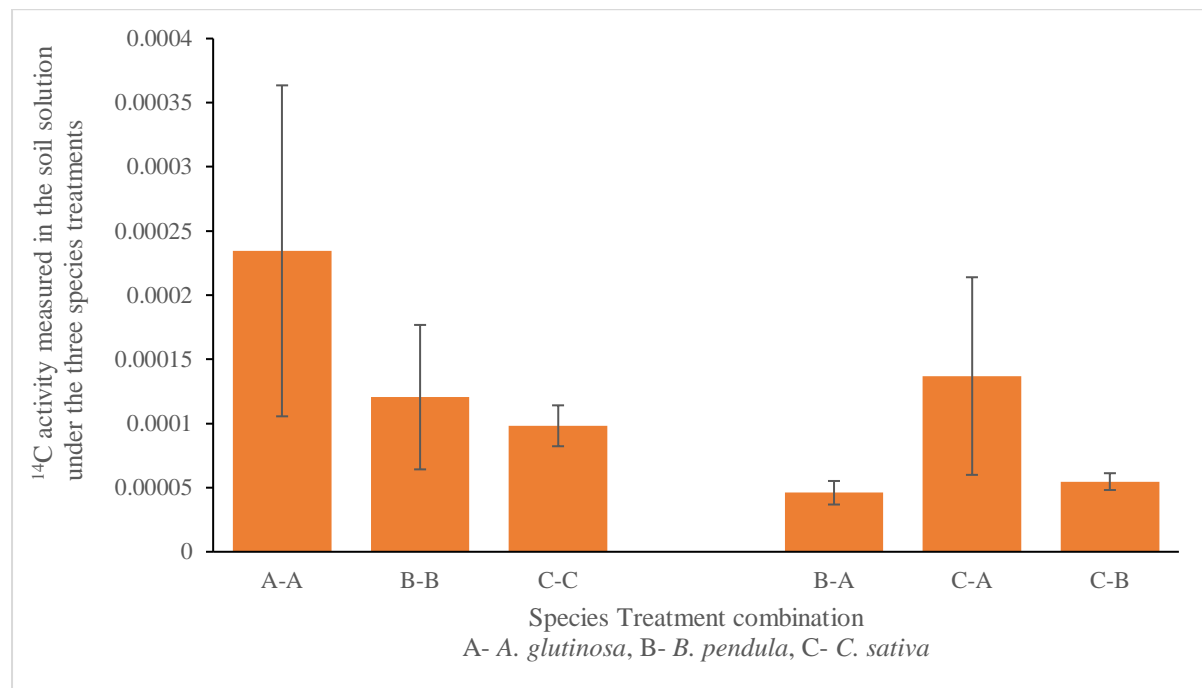


Figure S3: ^{14}C activity measured in soil solution extracted from the soil using a Rhizon suction sampler under the six species treatment combinations (A- *A. glutinosa*, B- *B. pendula*, and C- *C. sativa*). Data are mean \pm SE ($n = 4$).

8.3.2. Soil ^{14}C activity

The ^{14}C activity in the soil under the receiver tree (Figure 12) showed largely similar results to that of the soil solution ^{14}C activity (Figure 11), with the exception of the combination B-A which appeared to have retained the ^{14}C activity in the soil relative to the ^{14}C counted in the soil respiration traps. The species treatments were not found to be significantly different to each other ($P = 0.23$).

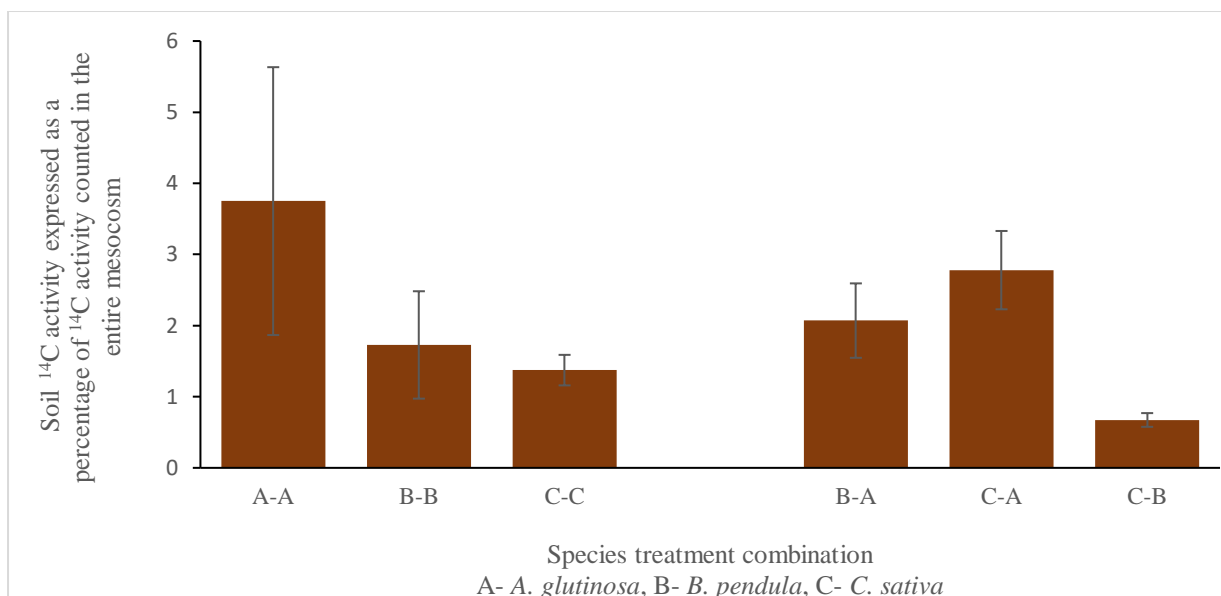


Figure S4: ¹⁴C activity measured in soil under the six species treatment combinations and expressed as a percentage of total ¹⁴C activity in the entire mesocosm (A- *A. glutinosa*, B- *B. pendula*, and C- *C. sativa*). Data are mean ± SE ($n = 4$)

8.3.3. Soil respiration

The accumulative soil respiration results (Figure 13) revealed some interesting results as the trend approximately followed those seen in both the soil water ¹⁴C activity and soil ¹⁴C activity. However, the soil respiration was lower than expected in the intraspecific species combinations based on the soil and soil water ¹⁴C activity, although the intra and interspecific soil respiration were not found to be significantly different to each other ($P = 0.09$).

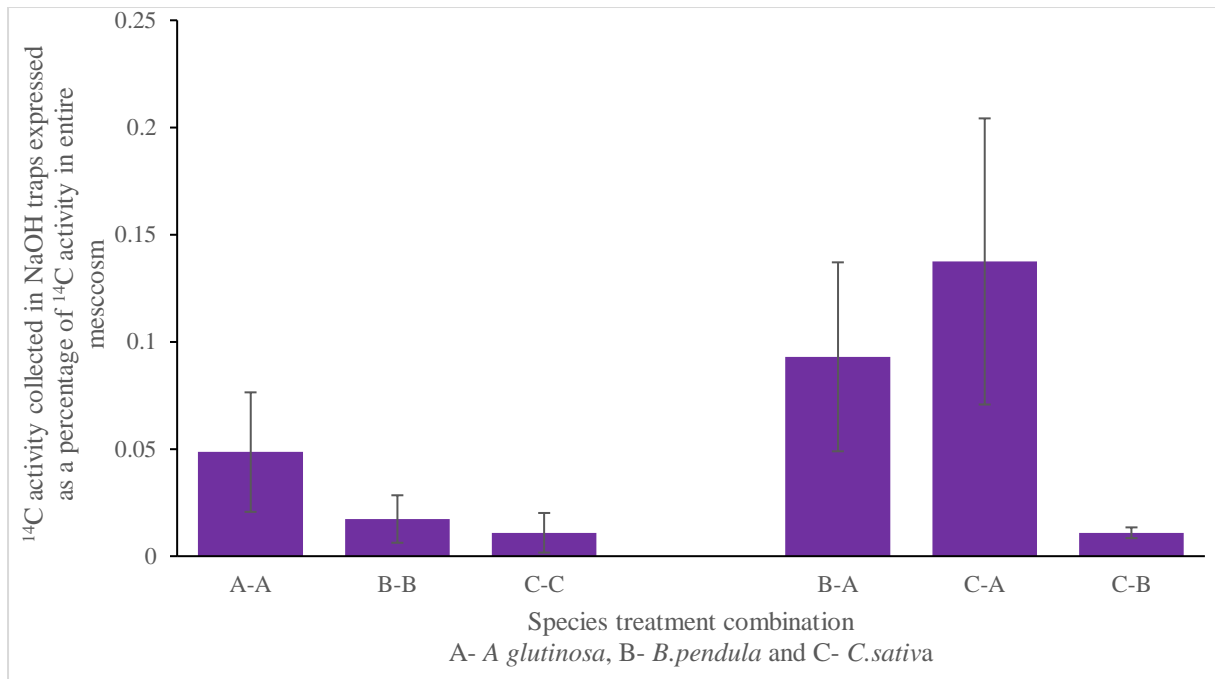


Figure S5: Accumulated soil respiration ¹⁴C activity collected in sodium hydroxide soil respiration traps under the receiver tree in the six species treatment combinations (A- *A. glutinosa*, B- *B. pendula*, and C- *C. sativa*). Intraspecific and interspecific combinations are grouped together for comparison. Data are mean ± SE ($n = 4$).

8.3.4. ¹⁴C activity in the receiver trees leaves

To try to further understand the differences in C allocation in the receiver trees, we investigated the ¹⁴C in each of the plant tissues. The ¹⁴C activity located in the receiver tree leaves is shown in Figure 2. The ¹⁴C activity was higher in the *A. glutinosa* to *A. glutinosa* treatments and overall the mean leaf ¹⁴C activity were found to be significantly different from each other ($P = 0.02$). A post-hoc test revealed that the *A. glutinosa* to *A. glutinosa* treatments had a mean leaf ¹⁴C activity that was significantly different to the; *C. sativa* to *C. sativa* treatments ($P = 0.04$), the *A. glutinosa* to *B. pendula* treatments ($P = 0.04$) and the *B. pendula* to *C. sativa* treatments ($P = 0.04$).

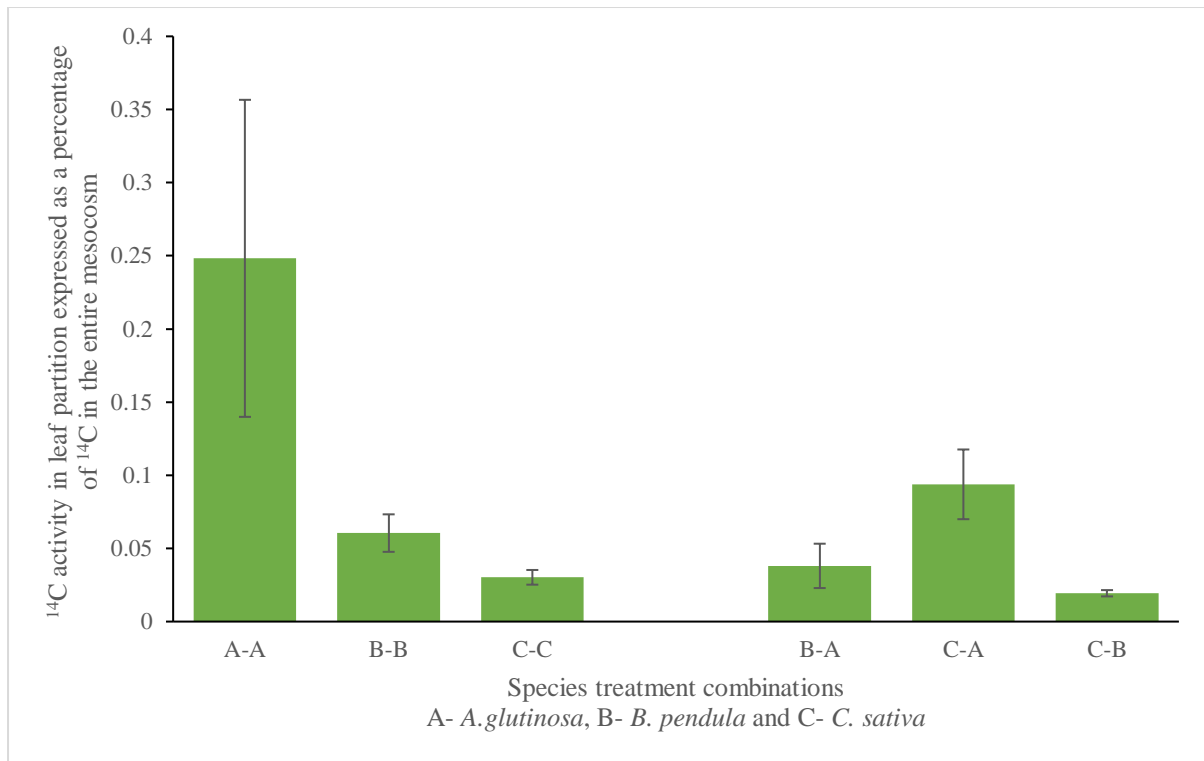


Figure S6: ^{14}C activity counted in the leaf partition of the receiver tree and expressed as a percentage of the total ^{14}C activity in the entire mesocosm, in the six species treatment combinations (A- *A. glutinosa*, B- *B. pendula*, and C- *C. sativa*). Intraspecific and interspecific combinations are grouped together for comparison. Data are mean \pm SE ($n = 4$).

8.3.5. ^{14}C activity in receiver tree branches

The results of the receiver ^{14}C allocation to the branch and stem shows largely the same trend as was seen in the leaf tissue (shown in Figure 14), with a larger percentage of the entire mesocosm ^{14}C activity located in the branch and stem in the *A. glutinosa* to *A. glutinosa* species treatment combination, although again this was not a significant result ($P = 0.39$).

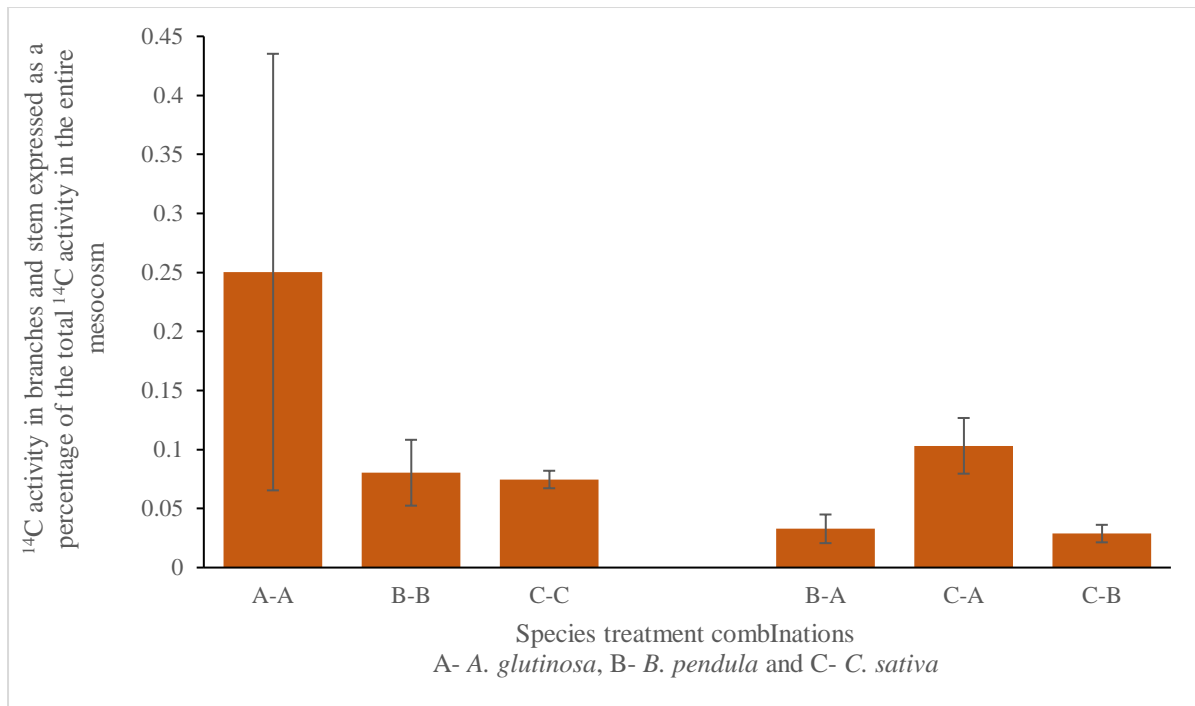


Figure S7: ¹⁴C activity counted in the branch and stem partition of the receiver tree and expressed as a percentage of the total ¹⁴C activity in the entire mesocosm, in the six species treatment combinations (A- *A. glutinosa*, B- *B. pendula*, and C- *C. sativa*). Intraspecific and interspecific combinations are grouped together for comparison. Data are mean ± SE ($n = 4$).

8.3.6. ¹⁴C activity in receiver tree roots

The results of the analysis of the ¹⁴C activity located in the coarse and fine roots are shown in Figure 16. Here the intraspecific treatment combinations appear to have proportionately more ¹⁴C activity in them compared to the interspecific combinations. Overall, the total mean ¹⁴C activity found in the roots results were not found to be significant ($P = 0.66$) neither were they significant for the coarse roots alone ($P = 0.62$) or the fine roots ($P = 0.70$).

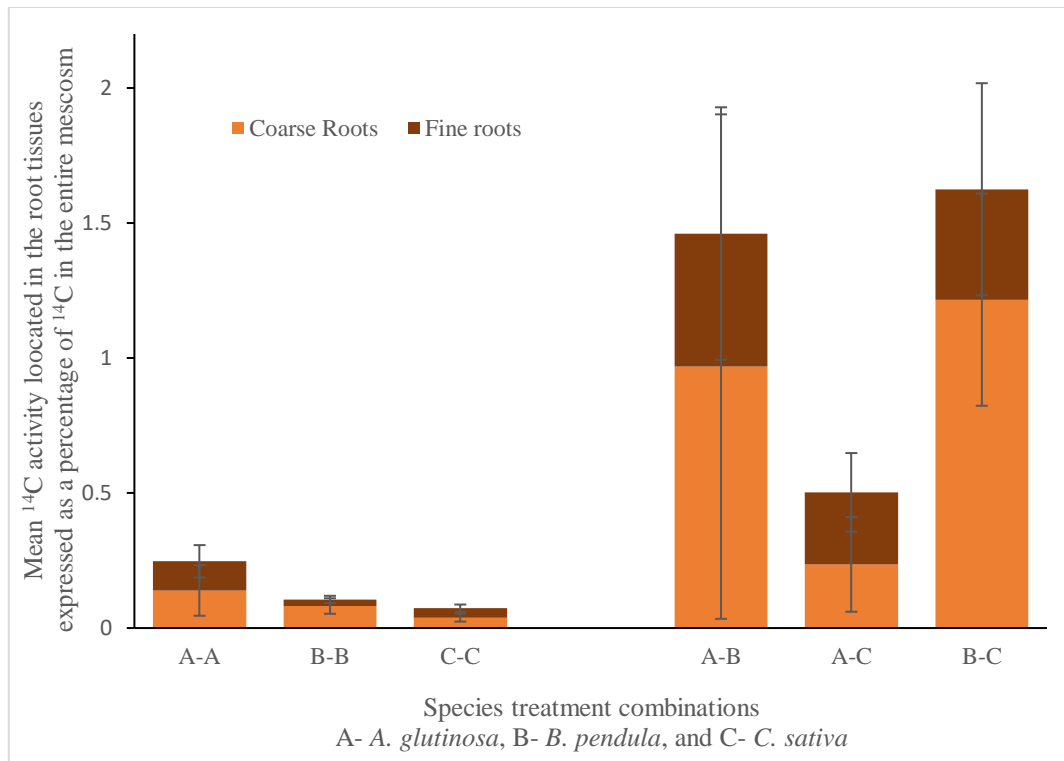


Figure S8: ^{14}C activity counted in the fine and coarse roots of the receiver tree and expressed as a percentage of the total ^{14}C activity in the entire mesocosm, in the six species treatment combinations (A- *A. glutinosa*, B- *B. pendula*, and C- *C. sativa*). Intraspecific and interspecific combinations are grouped together for comparison. Data are mean \pm SE ($n = 4$).

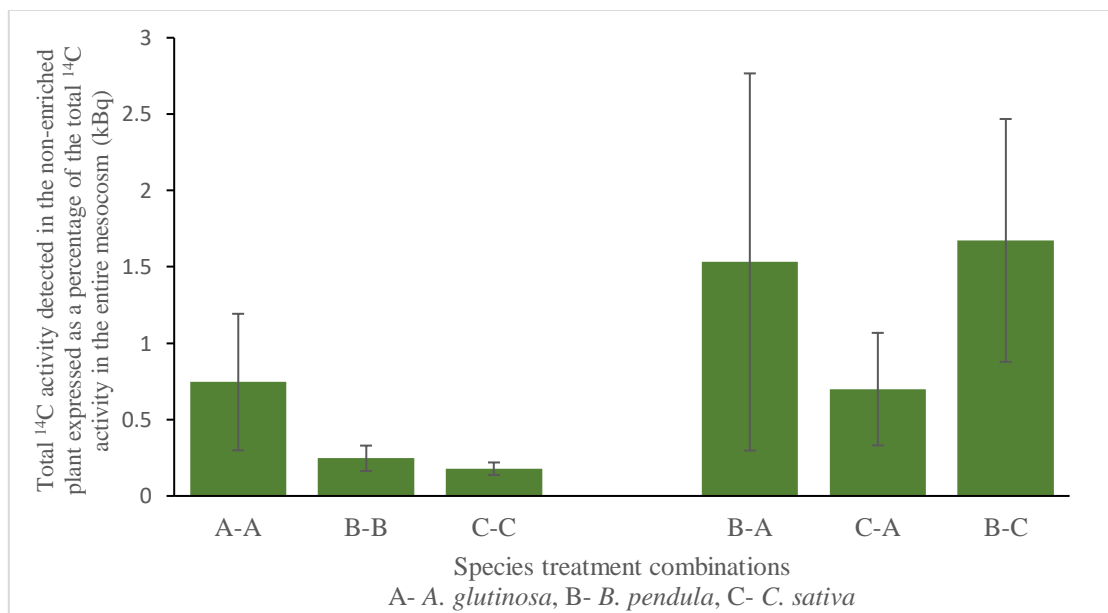


Figure S9: Total ^{14}C activity located in the receiver plant expressed as a percentage of the total ^{14}C activity in the entire mesocosm, in the six species treatment combinations (A- *A. glutinosa*, B- *B. pendula*, and C- *C. sativa*). Intraspecific and interspecific combinations are grouped together for comparison. Data are mean \pm SE ($n = 4$).

Chapter – 5

Preferential allocation of carbon to the root nodules of common alder (*Alnus glutinosa*) via common mycorrhizal networks.

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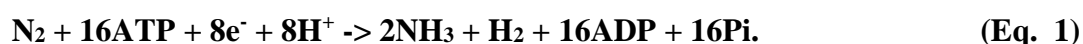
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TDP conducted all empirical work with guidance on design, execution and data analysis from ARS and DLJ. TDP wrote the manuscript with all authors contributing to the final version.

1. Introduction

Nitrogen (N) is an essential building block for all life as it is required to produce nucleic acids, amino acids and proteins (Ogle and Brown, 1997). Despite the Earth's atmosphere being 78% di-nitrogen, this gaseous form of N is unavailable to plants and is often a major limitation to plant productivity (Geddes et al., 2015). Plant-bacterial symbiosis has evolved to overcome this limitation through a process known as N-fixation that is widely accepted to be the second most important biological process in the world, after photosynthesis (Gruber and Galloway, 2008). During the process of N-fixation, the plant host supplies N-fixing bacteria with carbon (C) that is exchanged for N in the form of ammonium following the reduction of di-nitrogen by bacteria that produce the enzyme nitrogenase as shown in Equation 1 (Vessey et al., 2005; Herridge et al., 2008; Postgate, 2012).



As can be seen in the equation, the requirement for 16 ATP molecules shows that the process is extremely metabolically costly (Tjepkema and Winship, 1980).

Plant: bacterial symbiotic N-fixation has evolved in many divergent plant phyla (Mus et al., 2016) and occurs when N-fixing bacteria enter a plant and inhabit root nodules. Plants have

concurrently evolved multiple strategies to facilitate and foster these relationships and become hospitable hosts to the relevant bacterium (Holmer et al., 2017). These diazotrophic bacteria fall broadly into two categories: (i) Rhizobia, a group of paraphyletic bacteria; and (ii) the diverse actinobacterial genus *Frankia* (Holmer et al., 2017).

The most commonly identified N-fixing bacteria are *Rhizobia*, these bacteria represent 15 genera in 8 families of α -, β - and γ -Proteobacteria (Remigi et al., 2016). Globally this form of symbiosis is the most economically important for agricultural production (Desbrosses and Stougaard, 2011) and consequently the most extensively studied (Tariq et al., 2017). The second most frequent N-fixing bacteria is the filamentous sporangia forming *Frankia*, which forms root nodules known as actinorhizas in woody plants known as actinorhizals (Ghedira et al., 2018). *Frankia* was first identified in 1886 by Jørgen Brunchorst but was not successfully isolated until 1978 (Callaham, 1978). Consequently, these actinomycetes are poorly understood in comparison to *Rhizobia*. Indeed, *Frankia* was originally classified as a fungus until Becking (1970) identified *Frankia* as an obligate prokaryotic actinomycete, and created a new family, Frankiaceae, within the family Actinomycetales (Becking, 1970; Benson and Sylvester, 1993). Actinorhizals are a diverse range of 150 species of non-leguminous woody dicotyledonous plants from 17 genera, 8 families and 3 orders (Fagales, Rosales, Cucurbitales), which has led to postulation that they are likely the result of several evolutionary events (Pawlowski and Demchenko, 2012). Many of these specialist plants are pioneers that excel in colonising poor nutrient status soils (Ogle and Brown, 1997; Van Nguyen and Pawlowski, 2017) or land area devoid of soil (del Moral and Wood 1993, Walker et al., 2003).

Since the development of the Haber-Bosch process in the first-half of the twentieth century the production of man-made fertilisers has grown exponentially and currently two-thirds of the world's human population are reliant on this process to fertilise their food-crops (Vicente and Dean, 2017). Although this process has been heralded as one of the most important technological advances of the 20th century, over application of industrially fixed N in agriculture has resulted in myriad of environmental and human-health hazards (Tariq et al., 2017). Among these externalities are eutrophication, enhanced N-deposition, greenhouse gas production, particularly nitrous oxide (N₂O), and in part has facilitated the human population explosion (Vicente and Dean, 2017). Grassland species mixtures which include N-fixing plants have the potential to increase agricultural production by improving resource efficiency, by reducing the need for industrially produced N inputs (Peyraud et al., 2009). It is worth considering that every 1 kg of mineral N requires 55 MJ of energy to produce, transport and

spread, whereas nitrogen-fixation requires only sunlight (Peyraud et al., 2009). A better understanding of N-fixation combined with modern genetic engineering techniques could lead to artificially extending the range of N-fixing plant hosts to reduce reliance on fertiliser use, with its associated fossil-fuel consumption (Mus et al., 2016; Beatty and Good, 2011).

N-fixation is ecologically important in temperate forest ecosystems as non-N-fixing plants can benefit from the presence of N-fixing plants by the releases of N_2 by the root nodules, transfer of N containing molecules via common mycorrhizal networks (CMN), or through the decomposition of above- and below-ground N rich plant biomass (Roggy et al., 2004; Daudin and Sierra, 2008). CMN are fungal networks which connect two or more plants together underground and can be used to transport water, carbon, nitrogen, as well as other nutrients, and signalling chemicals (Simard et al., 2012). It is known that actinorhizal trees can transfer N fixed to the surrounding soil and therefore indirectly to neighbouring non-N-fixing plants (Ekbald and Huss-Danell, 1995). In a study of the indirect translocation of N via CMN Arnebrant et al. (1993) used $^{15}N_2$ gas as a proxy for N_2 to quantify the transfer of atmospheric N fixed by *Alnus glutinosa* to neighbouring *Pinus contorta* trees and found significant transfer occurred. The net benefit of including N-fixing species of tree in temperate forests communities could be equivalent to the annual addition of 150 kg N ha^{-1} (Cleveland et al., 1999). However, temperate tree species that have developed actinorhizal symbiosis are uncommon and consequently have not been widely studied. In this study we investigate the role of *Frankia alni* on the C source-sink dynamics of common mycorrhizal networks, on allocation belowground and subsequent transfer to root nodules, using ^{14}C as a proxy.

Here we investigate the transfer of C between two trees: (i) a ^{14}C pulse-labelled “donor” tree; and (ii) a CMN conjoined “receiver” tree; to assess the importance of actinorhizal N-fixation to belowground C allocation and elucidate the importance of plant: bacterial symbiosis in combination with the ubiquitous plant: fungal, mycorrhizal symbiotic relationships.

Our hypothesis for this experiment was:

H_{null} Belowground allocation of C via inter- and intra-specific CMNs will be greatest in “receiver” trees with actinorhizal associations, due to an increased C sink strength generated by the metabolic processes of the root nodule.

2. Materials and methods

2.1. Plant, root nodule and CMN establishment

1-year old cell-grown sweet chestnut (*Castanea sativa* Mill; Tree C) and bare rooted saplings of two temperate tree species namely; common alder (*Alnus glutinosa* (L.) Gaertn; Tree A) and silver birch (*Betula pendula* Roth; Tree B) were purchased from local forest nursery stock suppliers (Maelor Forest Nurseries Ltd, Bronington, SY13 3HZ) in November 2015. The tree selections were based on their contrasting belowground symbioses and functional traits, which was anticipated to maximise the positive effect on net productivity. Explicitly, *A. glutinosa* is actinorhizal, intermediate in shade tolerance, tolerates waterlogged soils and has seeds generally dispersed by watercourses. In contrast, *B. pendula* is a relatively short-lived, light demanding, early successional pioneer species with wind dispersed seeds and *C. sativa* is a late successional, shade tolerant, long-lived species with seed dispersed by animals. The saplings were planted into two connected 10 L pots (Ø 250 mm × 200 mm) which were coupled via a 10 cm diameter Perspex tube. Soil to backfill the pots was collected from the 0-10 cm layer of plots from the BangorDiverse experiment (Ahmed et al., 2016) where, since 2004, the Fluventic Dystrochrept soil had developed under the aforementioned species grown in monoculture. Prior to backfilling, the soil was coarse sieved to remove large stones, air dried, homogenised and sieved to pass 2 mm. The connecting tubes were filled with a homogenised mixture of 2 kg horticultural grade sharp sand and 20 g of bone meal (to encourage hyphal colonisation), which was covered at both ends with a 40 µm mesh (Normesh Limited, Lancaster, UK; Figure 1). The mesh pore size excluded trees roots but allowed penetration of the connecting tube by hyphae.

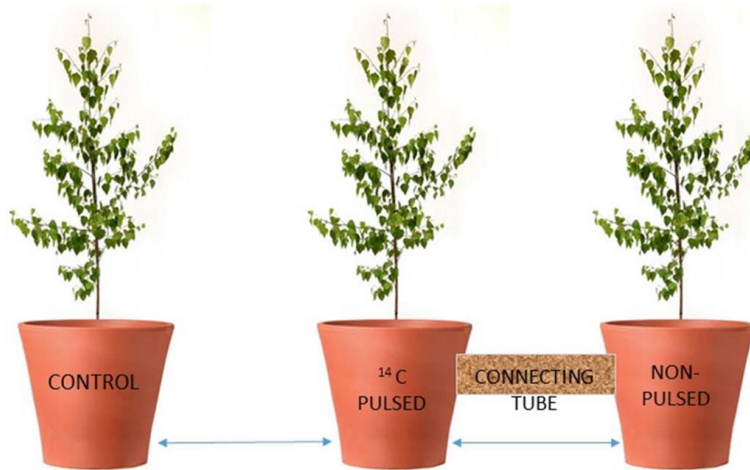


Figure 1: Diagram showing experimental design with two potted trees connected with the sand filled tube and the control plant.

Trees were planted in intra- or inter-specific combinations, creating three treatments (i.e., A-A, B-A, C-A; $n = 4$) with *A. glutinosa* consistently used as the ^{14}C receiver tree, with *A. glutinosa*, *B. pendula* and *C. sativa* all being ^{14}C donor trees. In all cases, the donor tree was the tree which was enriched in ^{14}C with a $^{14}\text{CO}_2$ pulse, whereas the receiver trees had no direct enrichment and therefore it was assumed that all ^{14}C measured in the receiver had been translocated belowground via a CMN or reabsorbed $^{14}\text{CO}_2$ that had previously been respired. To account for the possibility of re-photo assimilated $^{14}\text{CO}_2$ being counted in the receiver tree, a control tree was used and kept at the same distance from the donor as was the receiver (Figure 1). The conjoined pots were then grown in ambient conditions inside a glasshouse for 9 months to allow for the development of roots, mycorrhizal hyphae and root-nodule development, before being ^{14}C pulse-labelled using $\text{NaH}^{14}\text{CO}_3$ (Amersham International, Amersham, UK) which was reacted to $^{14}\text{CO}_2$ with an excess of 3 M HCl. To achieve ^{14}C enrichment, trees were allowed to photo assimilate the 2 MBq of $^{14}\text{CO}_2$ for 2 hours in full sunlight with a minimum photosynthetically active radiation intensity of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$.

2.2. Sampling procedure and isotope analysis

To trace the ^{14}C through the plant-microbe-soil continuum we measured the ^{14}C content of soil and plant biomass pools using a range of analytical methodologies. To estimate the plant and microbial respiration we measured mineralisation by suspending 28 mm diameter CO_2 traps containing 3 M NaOH above the soil allowing gaseous free passage from the soil surface. These were regularly replaced before saturation of the NaOH occurred and counted by liquid

scintillation spectrometry using a Wallac 1409 scintillation counter (Wallac EG and G, Milton Keynes, UK) with automatic quench correction and Optiphase Hisafe 3[®] (Perkin Elmer, UK) compatible scintillation fluid. Dissolved ¹⁴C in soil pore water was sampled simultaneously using mini-rhizon suction sampling devices (Rhizosphere Research Products B.V., Wageningen, The Netherlands) (Jones et al., 2016) and liquid scintillation counting as above. Disintegrations per minute were counted, standardised against a quench curve, and regularly calibrated using standards. Once the defined pulse chase period had elapsed (336 hours) the trees were harvested destructively and separated into the following partitions: foliage, branch and stem, fine and coarse roots, root nodules soil and sand from the interconnecting tube for ¹⁴C quantification (Figure 2). To prevent further plant and microbial losses of ¹⁴CO₂ after harvest, the plant partition, soil and sand were immediately dried at 105 °C for 24 hours. Subsequently, the partitions were weighed, homogenised and ground using a Retsch MM200 laboratory ball mill (Retsch GmbH, Hann, Germany) before being combusted using a Harvey Instruments Biological Oxidiser OX400 (R.J. Harvey Instrument Corp., Hillsdale, NJ, USA) and mineralised into Oxysolve C-400 (Zinsser Analytic GmbH, Frankfurt, Germany) and counted by liquid scintillation counting as described above.

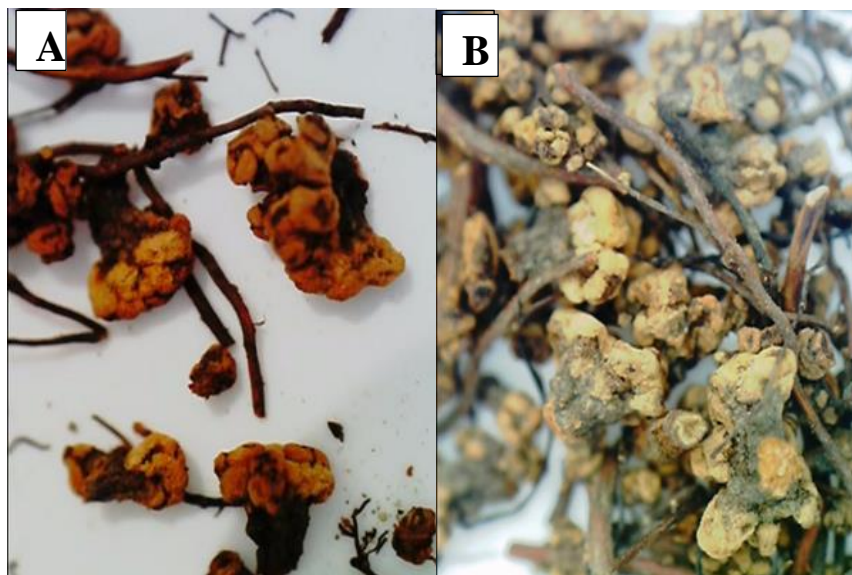


Plate 1: The root nodules were painstakingly separated from the wet roots (panel A) and then dried (panel B) in an oven 105 °C for 24 hours, whereby the original orange colouration was lost.

2.3. Soil chemical properties analysis

Immediately post pulse-label, soil was sampled from each mesocosm using a 4 cm soil corer then the chemical properties were analysed to determine if the trees presence during the tree and mycorrhizal establishment period had significantly altered the soil. The soils were weighed, then dried at 105 °C and ball milled before gravimetric determination of moisture content (Rowell, 1994) and C and N analysis, measured by dry combustion (Elementar analyzer). Nitrate was determined colourimetrically by the vanadium chloride method of Miranda et al. (2001), ammonium by the salicylate-hypochlorite method of Mulvaney (1996) both extracted in K₂SO₄, and plant-available phosphate by the molybdate-reactive method of Murphy and Riley (1962) following a 0.5 M sodium bicarbonate (NaHCO₃) pH 8.5 (Olsen et al., 1954) extraction. Soil pH and electrical conductivity (EC) were measured in a 1:5 (w/v) slurry of soil and distilled water (Smith and Doran, 1996). Soil dissolved organic carbon (DOC) and total dissolved N (TDN) was determined using a Shimadzu TOC-V-TN analyser (Shimadzu Corp., Kyoto, Japan) following extraction (1:5 w/v) in 0.5 M K₂SO₄ (Jones and Willett, 2006). The resultant soil chemical properties are shown in Table 1.

2.4. Statistical analysis

Prior to analysis all data were tested for homogeneity of variance using Levene's test. The independent variable was the species treatment (*A. glutinosa*, *B. pendula* and *C. sativa*). The dependant data (total plant biomass and partition biomass, soil chemical properties and ¹⁴C activity of biomass pools, ¹⁴C labelled respiration efflux) were tested for normality prior to analysis using the Shapiro-Wilk Test and no transformations were required. The statistical analysis was conducted using a one-way ANOVA and Tukey's posthoc test with SPSS® Statistics version 25.0 (IBM Corp, Chicago, 2017). Results were considered significant at $P \leq 0.05$.

3. Results

3.1. Soil chemical properties

Table 1 shows the results of the soil chemical analysis. As the soil samples were collected from the mesocosms below the three tree species at time point zero (directly post pulse label) they were interpreted based on the species identity rather than the treatment combination.

Table 1: Soil chemical properties for soil collected from the mesocosms 0 hours after the pulse was complete for all three species treatments (*A. glutinosa*, *B. pendula* and *C. sativa*). Data are mean \pm SE ($n = 4$ for *B. pendula* and *C. sativa*, $n = 16$ for *A. glutinosa*). Significant differences are highlighted in bold and the level of significance is denoted by $^*P < 0.05$.

Tree species	NO ₃ ⁻ (mg/kg)	NH ₄ ⁺ (mg/kg)	Available-P (mg/kg)	pH	EC (μ S/cm)	C: N	TDN (mg/kg)	DOC (mg/kg)
<i>A. glutinosa</i>	2.06 \pm 1.02	6.61 \pm 1.38	17.44 \pm 4.77	5.49 \pm 0.07	190.75 \pm 22.36	8.30 \pm 0.13 ab	7.11 \pm 0.91	34.09 \pm 4.10
<i>B. pendula</i>	0.84 \pm 0.40	6.64 \pm 2.01	12.56 \pm 1.29	5.63 \pm 0.11	141.25 \pm 32.61	7.78 \pm 0.28 b	6.08 \pm 0.67	31.62 \pm 6.88
<i>C. sativa</i>	1.94 \pm 1.37	3.79 \pm 1.28	13.55 \pm 2.99	5.56 \pm 0.14	220.75 \pm 78.78	8.95 \pm 0.40 a	5.54 \pm 0.84	20.50 \pm 0.85
<i>P-value</i>	0.83	0.59	0.62	0.79	0.82	0.03*	0.28	0.63

The soil chemical properties did not show major differences between species, meaning that the effect of the trees presence during the period of tree and mycorrhizal establishment of the soil was negligible and as result there were no significant differences in soil properties, except for the soil C: N ratio. The C: N mean data indicated that the C: N ratio was significantly lower (by ca. 15) for *B. pendula* than *C. sativa*, however, there were no statistically significant differences between *A. glutinosa* and *B. pendula* or *A. glutinosa* and *C. sativa*. The total amount of C and N in the soils collected from below the three tree species is presented in Table 2 to investigate which of the two variables was driving the differences between species. As can be seen in Table 2, the variable that differs is the % of C. The C content of soil under *C. sativa* trees is ca.15 % higher when compared with the C content of the soil under *A. glutinosa* and *B. pendula*.

Table 2: Total C (%) and N (%) of the three species (irrespective of the species treatment combination). Data are mean \pm SE ($n = 4$ for *B. pendula* and *C. sativa* and $n = 16$ for *A. glutinosa*).

Species identity	% C	% N
<i>A. glutinosa</i>	2.52 \pm 0.07	0.30 \pm 0.01
<i>B. pendula</i>	2.44 \pm 0.02	0.31 \pm 0.02
<i>C. sativa</i>	2.82 \pm 0.03	0.31 \pm 0.02

3.2. Plant biomass

To reduce nutrient availability in the mesocosm soil and to therefore increase the chances of the *A. glutinosa* trees forming root nodules, the trees were grown in 10 litre pots for nine months before pulse-labelling. On visual inspection at the point of destructive harvesting the alder plants exhibited root nodules in all treatments and replicates.

After 336 hours of the ^{14}C pulse being applied, destructive harvest of the plant biomass showed that the total mean ($n = 16$) dry weight of the three species irrespective of the species treatment combinations was 73.3 ± 4.8 g. At the final harvesting time point (336 hours after pulse completed), the mean ($n = 4$) plant biomass of *A. glutinosa* was 106.8 ± 7.5 g, for *B. pendula* it was 47.4 ± 4.6 g, and for *C. sativa* it was 65.5 ± 2.4 g. The total biomass of the three species

was found to be different from each other ($P < 0.001$; Table 3). Mean total biomass of *A. glutinosa* was almost twice that of *B. pendula* and half as much as *C. sativa* and the difference between the conjoined *A. glutinosa* and *B. pendula*, and *A. glutinosa* and *C. sativa* were found to be significant, ($P < 0.001$) and ($P = 0.09$), respectively. However, the biomass of *B. pendula* and *C. sativa* were not significantly different to each other ($P = 0.50$). The relative dry weights of the plant partitions were also quantified and a post-hoc Tukey test showed that there were significant differences between the biomass partitions of the three species. All biomass partitions of *A. glutinosa* were larger than those of both *B. pendula* and *C. sativa* ($P < 0.001$). *A. glutinosa* was shown to have twice the mean leaf biomass of the *B. pendula* treatments and half as much as the *C. sativa* ($P < 0.001$), treatments. Total leaf biomass of *B. pendula* and *C. sativa* were not found to be significantly different to each other ($P = 0.48$), whereas branch and stem total mean biomass were significantly different to each other ($P < 0.001$) for all treatment comparisons, with the exception of *B. pendula* and *C. sativa*, which were not significantly different from each other ($P = 0.80$).

Analysis of coarse and fine root biomass data revealed that *A. glutinosa* had a larger mean dry root biomass that was significantly different from that of *B. pendula* and *C. sativa* ($P = 0.03$ and $P = 0.01$) for fine and coarse root, respectively. The root biomass of *B. pendula* and *C. sativa* were not significantly different from each other ($P = 0.53$). As root nodules are only present in *A. glutinosa*, no comparison could be undertaken. Overall, at the time of pulse-labelling, the mean total biomass of the *A. glutinosa* (14.2 ± 0.9 g) was significantly greater than *B. pendula* (5.8 ± 1.2 g; $P = 0.001$) and *C. sativa* (8.4 ± 0.7 g; $P = 0.01$).

Table 3: Mean biomass (g dwt) \pm SE of five biomass partitions and total biomass of *A. glutinosa*, *B. pendula* and *C. sativa* determined by destructive harvesting at 336 hours after a $^{14}\text{CO}_2$ pulse was applied. ($n = 4$ for *B. pendula* and *C. sativa*; $n = 16$ for *A. glutinosa*). Significant differences are highlighted in bold and the level of significance is denoted by asterisks (*, $P < 0.1$; **, $P < 0.05$; ***, $P < 0.001$).

Tree species	Tree partition					
	Leaf	Branch	Coarse root	Fine root	Root nodule	Total biomass
<i>A. glutinosa</i>	27.92 \pm 1.88	40.58 \pm 1.97	22.52 \pm 2.06	14.48 \pm 1.44	1.31 \pm 0.14	106.8 \pm 7.49
<i>B. pendula</i>	13.61 \pm 1.67	17.18 \pm 1.14	9.99 \pm 1.17	6.61 \pm 0.58	N/A	47.39 \pm 4.56
<i>C. sativa</i>	19.46 \pm 1.01	20.31 \pm 0.23	15.41 \pm 0.43	10.34 \pm 0.74	N/A	65.52 \pm 2.42
<i>P-value</i>	<0.001**	<0.001***	0.01**	0.02**	<0.001***	<0.001***

3.3. Partitioning of ^{14}C activity within mesocosms C pools

In order to determine the relative concentration of ^{14}C activity between the mesocosm C pools of the different treatments, the ^{14}C activity per gram of dried biomass was calculated (Table 4). The total biomass of *A. glutinosa* was twice that of the *B. pendula* and almost twice that of the mean total *C. sativa* biomass. Table 3 clearly shows that all the mesocosm's C pools were almost twice as large in the *A. glutinosa* trees and all were found to have significant differences except for the soil ^{14}C activity per gram, which was not dependent on the tree species ($P = 0.26$). A post-hoc Tukey test showed that ^{14}C activity per gram of leaf was significantly different between the *A. glutinosa* and *B. pendula* treatments ($P < 0.001$), between *B. pendula* and *C. sativa* treatments ($P = 0.03$), but not between the *A. glutinosa* and *C. sativa* treatments ($P = 0.67$). The branch data also showed significant differences between the *A. glutinosa* and *B. pendula* treatments ($P < 0.001$), between *B. pendula* and *C. sativa* treatments ($P = 0.03$), but not between the *A. glutinosa* and *C. sativa* treatments ($P = 0.41$). The same trend was apparent from both the coarse and fine root data, with *A. glutinosa* showing significant differences from *B. pendula* ($P < 0.001$ and $P < 0.001$ in coarse and fine root respectively). *B. pendula* showing significant differences from *C. sativa* ($P = 0.01$ and $P = 0.04$, in coarse and fine roots

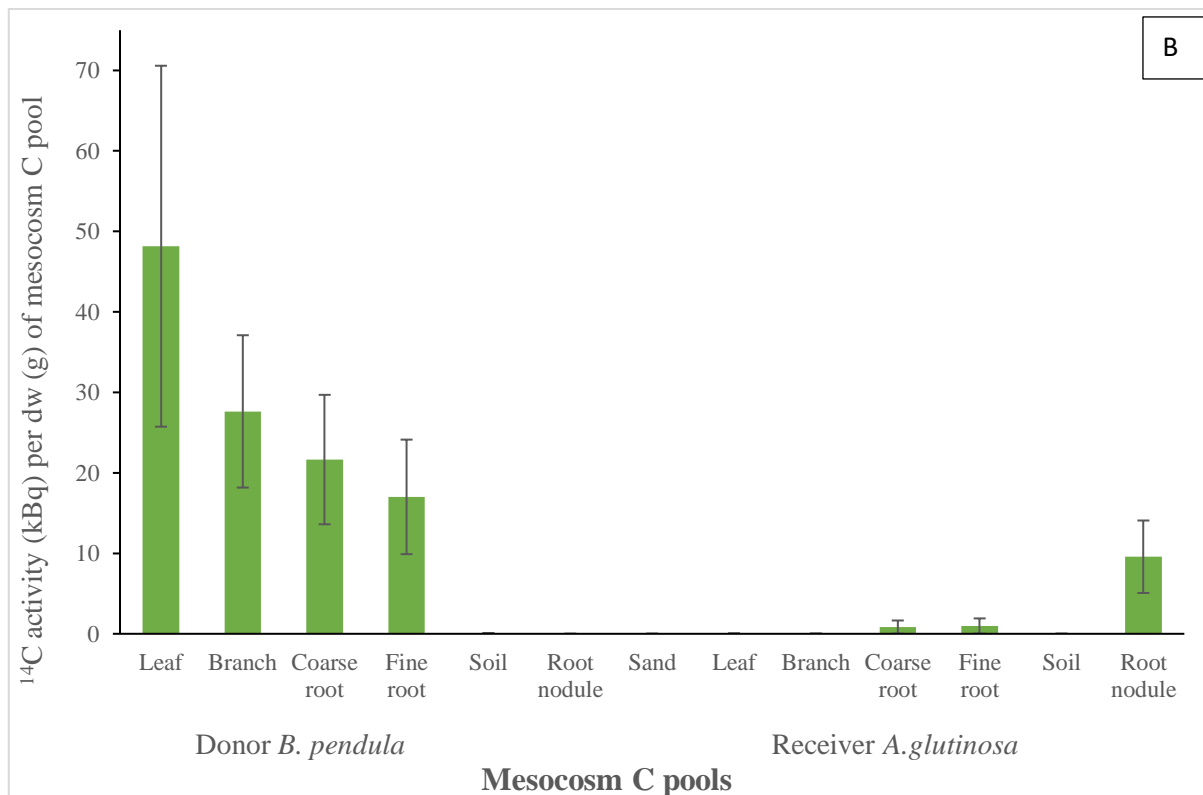
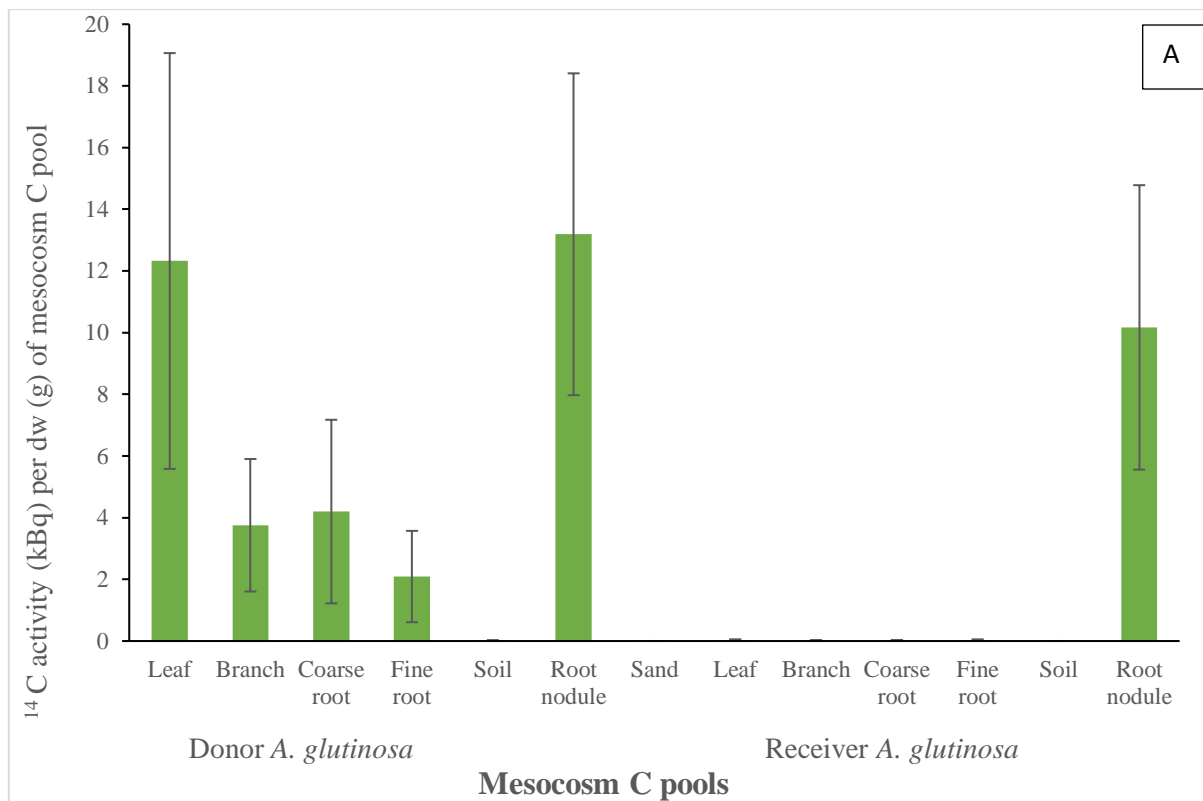
respectively). In contrast, in both root partitions, *B. pendula* was found not to be significantly different to *C. sativa* in coarse ($P = 0.54$) and fine roots ($P = 0.23$).

Mean ^{14}C activity was examined when the receiver species was *A. glutinosa* to establish if the identity of the donor tree significantly altered the ^{14}C activity detected in the receiver fraction. Statistical analysis of these data suggests that there were no significant differences between the ^{14}C activity detected in the receiver plant and the species identity of the donor plant.

Table 4: ^{14}C activity of each C pool within the mesocosm determined after destructive harvesting expressed as total ^{14}C activity (kBq) per gram of mean plant biomass for each biomass partition (g dwt) \pm SE ($n = 4$ for A-A, B-A, C-A). Significant differences are highlighted in bold and the level of significance is denoted by asterisks (*, $P < 0.1$; **, $P < 0.05$; ***, $P < 0.001$). The sand C pool was determined from sand collected in the conjoined tube.

^{14}C activity (kBq) per gram of mesocosm C pools							<i>P</i> -value
Species combination	A-A	A-A	B-A	B-A	C-A	C-A	
Tree species	<i>A. glutinosa</i>	<i>A. glutinosa</i>	<i>B. pendula</i>	<i>A. glutinosa</i>	<i>C. sativa</i>	<i>A. glutinosa</i>	
^{14}C status	Donor	Receiver	Donor	Receiver	Donor	Receiver	
Leaf	12.32 \pm 6.74	0.04 \pm 0.01	48.16 \pm 22.42	0.02 \pm 0.01	11.98 \pm 2.44	0.03 \pm 0.01	0.001**
Branch	3.75 \pm 2.15	0.02 \pm 0.00	27.63 \pm 9.46	0.01 \pm 0.00	6.47 \pm 1.19	0.03 \pm 0.01	0.000***
Fine root	2.09 \pm 1.48	0.03 \pm 0.02	17.01 \pm 7.12	0.97 \pm 0.94	6.28 \pm 1.57	0.26 \pm 0.18	0.000***
Coarse root	4.20 \pm 2.98	0.02 \pm 0.01	21.65 \pm 8.04	0.84 \pm 0.81	5.34 \pm 1.46	0.13 \pm 0.10	0.000***
Root nodule	13.19 \pm 5.22	10.17 \pm 4.61	19.63 \pm 7.56	9.57 \pm 4.5	5.70 \pm 1.45	8.11 \pm 3.02	N/A
Soil $\times 10^{-3}$	16.28 \pm 4.32	1.41 \pm 0.13	4.75 \pm 0.86	3.42 \pm 0.73	49.45 \pm 16.24	3.37 \pm 1.45	0.261
Sand $\times 10^{-3}$	1.35 \pm 0.05	1.35 \pm 0.05	3.17 \pm 1.12	3.17 \pm 1.12	1.8 \pm 0.23	1.80 \pm 0.23	0.000***

Transfer of pulsed-labelled ^{14}C from the donor tree species to the conjoined *A. glutinosa* tree are shown in Figure 1a-c. N/A denotes not applicable.



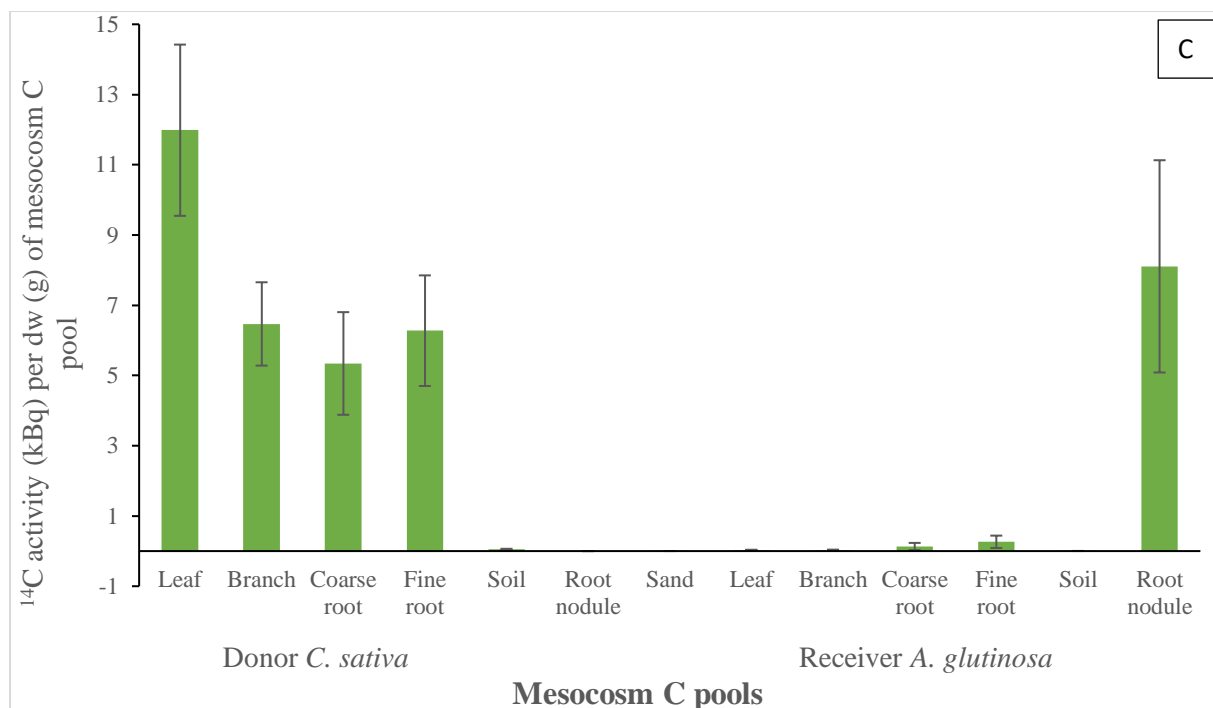


Figure 2: Panel A shows the mean dried weight (gram) of the partitions in both the donor and receiver fractions of the *A. glutinosa* to *A. glutinosa* species treatment combinations and SE ($n = 4$); Panel B shows the mean dried weight (grams) of the partitions in both the donor and receiver fractions of the *B. pendula* to *A. glutinosa* species treatment combinations and SE ($n = 4$); Panel C shows the mean dried weight (grams) of the partitions in both the donor and receiver fractions of the *C. sativa* to *A. glutinosa* species treatment combinations and SE ($n = 4$)

3.4. Effect of treatment (identity of donor plant) on ¹⁴C activity in root nodule

The ¹⁴C activity per gram of biomass data allowed the differences in size of tree partitions to be accounted for and therefore more direct comparison to be made. The mean root nodule activity per gram ($n = 4$) for the donor trees was 13.2 ± 5.2 kBq/g, and for the receiver trees was 10.2 ± 4.6 kBq/g when the identity of the donor was *A. glutinosa*, 9.6 ± 4.5 kBq/g when the donor trees identity was *B. pendula* and 8.1 ± 3.0 kBq/g when the donor trees identity was *C. sativa*. A comparison of the ¹⁴C activity per gram of the receiver tree root nodules is shown in Figure 2b and a comparison between ¹⁴C activity per gram of the donor root nodules is shown in Figure 2c. There were no significant differences in the ¹⁴C activity regardless of the species identity of the donor plant ($P = 0.72$). In the case of the data plotted in Figure 4, no significant difference was observed between the ¹⁴C activity of the donor *A. glutinosa* tree root nodules and the ¹⁴C activity measured in the receiver trees. For the relative ¹⁴C activity of the donor and receiver trees as a percentage of total ¹⁴C recovered in all plant partitions see Section 3.5.

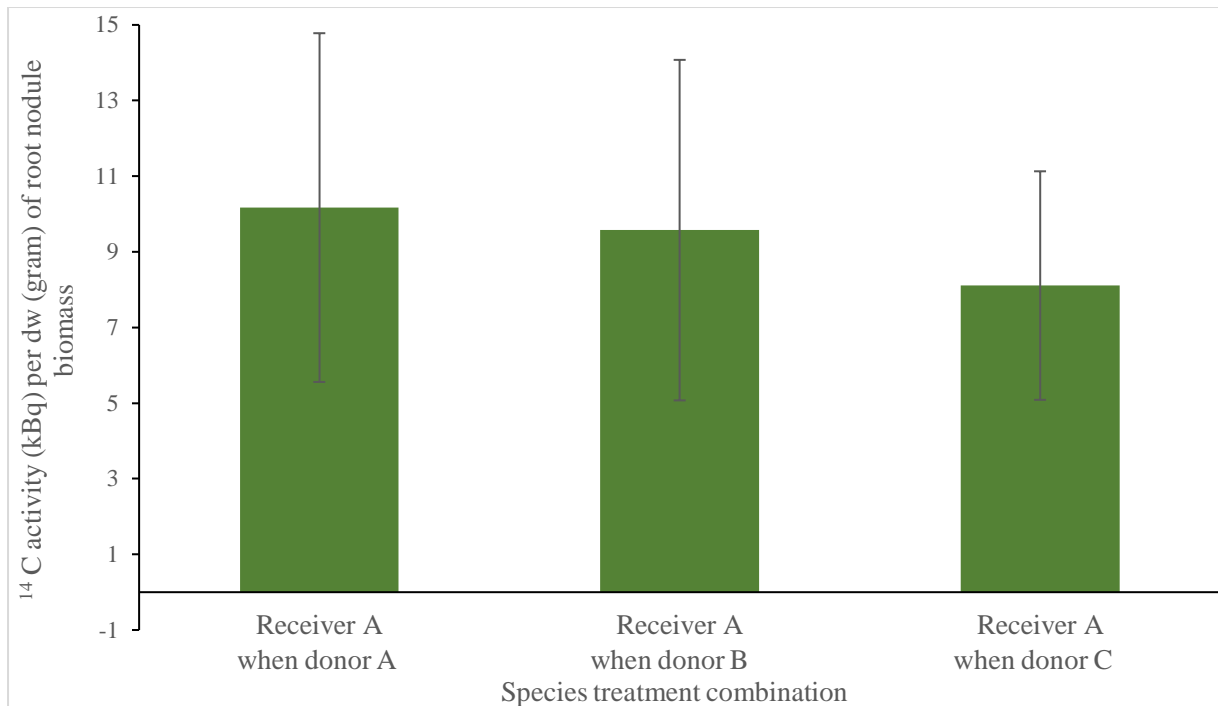


Figure 3: Root nodule ¹⁴C activity (kBq) of the three treatments where in all cases the receiver plant is the actinorhizal *A. glutinosa* but the donor tree is of three tree species both intra- and inter-specifically with the identity of the pulse tree also being *A. glutinosa*, and interspecifically *B. pendula* and *C. sativa*. Data is mean ($n = 4$) with SE.

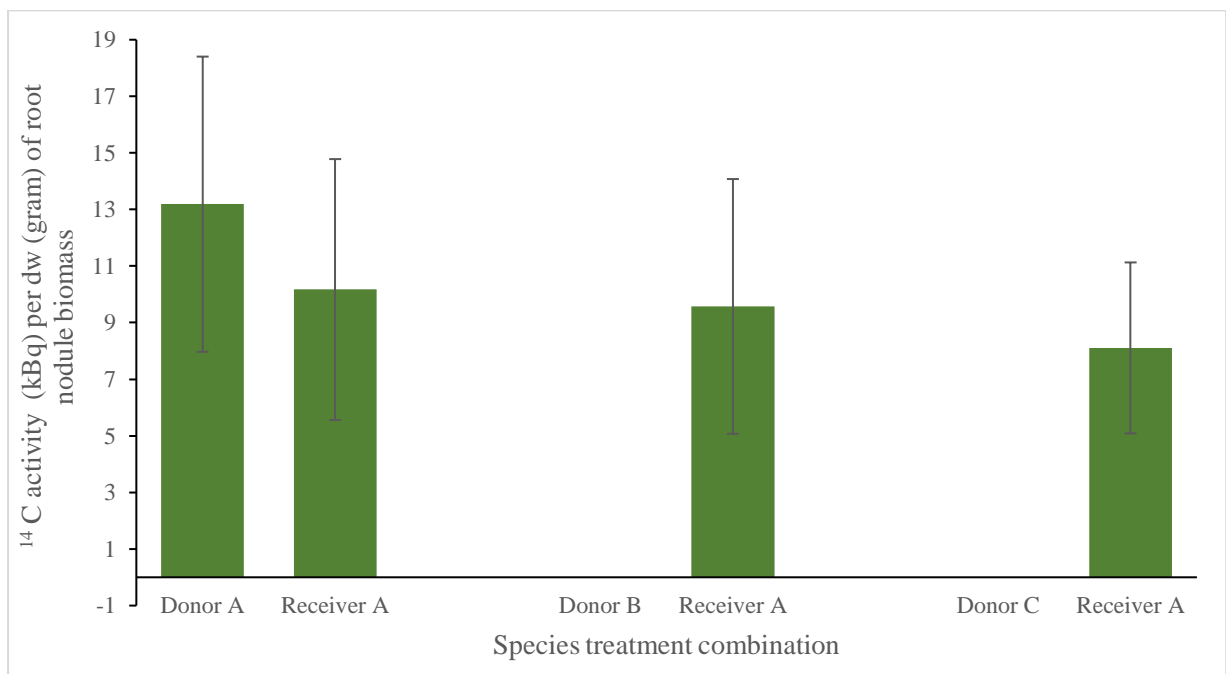


Figure 4: Root nodule ¹⁴C activity (kBq) quantified from the nodules separated from the roots of the pulsed (donor) *A. glutinosa*, and the activity of the root nodules for each of the receiver *A. glutinosa* in each species combination. Data is mean \pm SE ($n = 4$).

3.5. Root nodule activity as % of total ^{14}C in receiver plants

To normalise the ^{14}C activity of the three species by biomass, the ^{14}C activity detected in the root nodules of the receiver tree as a percentage of the total ^{14}C activity in the receiver tree biomass was calculated. As can be seen in Table 5, in both the *A. glutinosa* to *A. glutinosa* treatments and the *C. sativa* to *A. glutinosa* treatments over 50 % of the total ^{14}C activity in the whole tree was detected in the root nodules (65.8 ± 21.9 % and 56.8 ± 18.0 %, respectively). The *B. pendula* to *A. glutinosa* treatments had slightly less ^{14}C activity as a percentage of the ^{14}C detected in the whole tree on average with just slightly under 50 % of the total (49.8 ± 24 %). A statistical comparison of the ^{14}C activity detected in the root nodule as a percentage of the total ^{14}C activity detected in the entire receiver trees biomass partitions revealed no significant difference between the treatments (Table 5).

Table 5: ^{14}C activity of each biomass partition determined after destructive harvesting expressed as a percentage of the total ^{14}C activity detected in the receiver tree \pm SE ($n = 4$).

Donor tree identity	Biomass partitions of receiver tree (<i>A. glutinosa</i>)				
	Leaf	Branch	Coarse root	Fine root	Root nodule
<i>A. glutinosa</i>	11.09 ± 4.82	7.31 ± 4.08	6.63 ± 5.29	9.18 ± 8.43	65.79 ± 21.94
<i>B. pendula</i>	7.66 ± 5.40	8.56 ± 7.03	22.99 ± 13.73	11.09 ± 6.60	49.76 ± 23.99
<i>C. sativa</i>	5.99 ± 1.06	8.25 ± 3.21	14.20 ± 10.12	14.73 ± 8.03	56.84 ± 17.99
<i>P-value</i>	0.69	0.98	0.55	0.88	0.87

4. Discussion

Using ^{14}C as a proxy for recently photosynthetically fixed C, this study indicated that C fixed by a conjoined tree was transferred between trees via a CMN and that the C was preferentially allocated to the root nodules of *A. glutinosa*. Figures 2a-c showed that ^{14}C was transferred to the root nodule of *A. glutinosa* (recipient) whatever conjoined species (donor) was ^{14}C pulse labelled. This suggests that the identity of the donor tree species is not important in determining the quantity of ^{14}C activity that is transferred to the root nodules of the receiver *A. glutinosa* tree. This result was unexpected but can be explained by the considerable energetic requirement of nitrogen fixation, as the nitrogen fixing cells require a supply of energy (ATP) and a reductant, commonly $\text{Na}_2\text{S}_2\text{O}_4$ (Huss-Danell and Hahlin, 1988). This huge energy requirement alters the balance between the C source (shoots, leaf) and the C sinks (growing roots,

mycorrhizal fungi, extramatrical mycorrhizal fungi and endophytic nitrogen-fixing bacteria), which could be the mechanism mediating the transfer of C between trees via a CMNs (Simard et al., 1997; Simard and Durall, 2004; Selosse et al., 2006). It would appear that the C demand of the root nodule in the receiver tree is sufficient enough to translocate C from the roots of the donor tree to root nodules in the receiver. Presumably, the same is simultaneously occurring in reverse i.e. the C fixed by the receiver tree is being conveyed across the CMN to the donor root nodules, although this unfortunately cannot be substantiated from the data collected in this study.

Additionally, the transfer of C between the *A. glutinosa* and *A. glutinosa* species combination treatment showed that the receiver plant-microbe soil system allocated the most ^{14}C activity to the root nodules, with very little present in either the fine or coarse root. In contrast, when the *B. pendula* to *A. glutinosa* species combination treatment was examined it appeared that *B. pendula* retained more of the ^{14}C in plant tissues relative to the other donor species (Figure 3).

4.2. Plant biomass

Differences in the biomass of the three species were expected due to the differential growth rates of the tree species. Of the two early successional species *B. pendula* typically yields a net primary production (NPP; gross photosynthesis minus plant respiration) of $8.5 \text{ t C ha}^{-1} \text{ year}^{-1}$ (Varik et al., 2015) or $5.3 \text{ m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$ to $11.4 \text{ m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$ (Karlsson et al., 1997), whereas *A. glutinosa* has a typical NPP of 4 to $14 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$. (Claessens et al., 2010). In contrast, the late-successional and generally fast-growing species *C. sativa* has a typical mean NPP of $11 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$, when grown in the UK (Everard and Christie, 1995). Depending on the growing conditions of the three species, *A. glutinosa* has the potential to have the largest annual yield and has a distinct advantage being actinorhizal species in nutritionally deficient soil conditions (Isopi et al., 1994). However, Orfanoudaki et al., (2010) found that even when *A. glutinosa* trees were supplied with an excess of nitrogen and phosphorus, (phosphorus at 240 mg kg^{-1} and nitrogen 83 mg kg^{-1}) that those inoculated with mycorrhizal fungi and nitrogen fixing bacteria still outperformed those without. When the plant tissues were analysed for nitrogen and phosphorus content, it was found that they were the same in both cases, suggesting that the growth promotion properties of *Frankia* and mycorrhizal fungi are not confined to purely nutritional benefits. It has been suggested that other benefits of the symbiosis include growth assistance through modification to the root system (Hooker and Atkinson, 1996), more specifically through increased root branching (Berta et al., 1990).

It seems logical that the greater biomass of *A. glutinosa* could be accounted for by the N status of the soil. However, the only soil chemical property that was different between the species treatments was the C: N ratios of *B. pendula* and *C. sativa* whereas, the C: N ratio of soil under *A. glutinosa* was similar to both *B. pendula* and *C. sativa*. This was quite surprising as *A. glutinosa* is actinorhizal and we would therefore expect the levels of nitrate to be slightly higher under this species than the other two. A more detailed investigation of what was driving the differences between the C: N ratio of the *C. sativa* soil and the other two species shows the mean C % under *C. sativa* was higher than the other two species. Whereas in contrast, the N % was almost identical under the three species (Table 2). The differences could be explained by the trees species having slightly different rates of growth and nutritional requirements during the periods of growth and therefore as they use resources from the soil at different rates the stoichiometry of the remaining nutrients would be altered by the identity of the tree species potted into the mesocosm or most likely to be a result of organic N and C inputs (i.e. root turnover, litter inputs, hyphal turnover, microbial [fungi/bacterial turnover]). The biomass inputs will eventually change the soil C: N ratio. An explanation for the lack of differences in N % under the three species could be that because the mycorrhizal hyphae are spanning both pots through the sand that the fungi may be redistributing the N fixed by the *Frankia* in the root nodules to the non-actinorhizal species in the case of the interspecific species combination. It well-known that the presence of nitrogen-fixing nodules not only improves the N status of the host plant but can also benefit plants in the vicinity through litter inputs either above or belowground, root exudation and leaching from leaves (He et al., 2009). This has been experimentally substantiated as when leguminous plants are planted in mixtures with non-leguminous plants, a significant increase in growth and yield in the non-leguminous plants is often observed (Herridge et al., 2008; Li et al., 2007; Malezieux et al., 2009). In a study of nitrogen fixing *Canavalia ensiformis* (jackbean), Sierra and Defontaines (2009) showed that N transferred to *Musa acuminata* (banana) 6% had originated from exudates, 32 % from root turnover, and 62 % from soil N. However, common genes are needed for the colonisation of roots by both nitrogen-fixing bacteria and mycorrhizal fungi (Ané et al., 2004; Sprent and James, 2007) and therefore most nitrogen fixing tree species display dual symbiosis (Teste et al., 2019). The effect of dual symbiosis could further increase the transfer to non-leguminous plants not only by direct release from nitrogen-fixing plants (Ledgard, 2001; Paynel et al., 2001; Shen and Chu, 2004; Sierra and Desfontaines, 2009; Sierra and Nygren, 2006), but by the following potential mechanisms; mycorrhizal N uptake and transfer (Koltai and Kapulnik, 2010; Smith and Read, 2010); through CMNs (Koltai and Kapulnik, 2010; Smith and Read,

2010; Moyer-Henry et al., 2006). Arbuscular mycorrhizae in CMN are known to mediate this (Johansen and Jensen, 1996; Moyer-Henry et al., 2006); as well as ectomycorrhizal networks where N-transfer through ecto-CMN has been detected between *Alnus* spp. (*A. glutinosa* and *A. incana*) and non-actinorhizal *Pinus contorta* (Lodgepole pine) and *P. sylvestris* (Scots pine) (Arnebrant et al., 1993; Ekblad and Huss-Danell, 1995). Single-directional N transfer by CMN has been reported to range between 0 to 80 % (Newman et al., 1994; He et al., 2009). In N limited conditions in single-species stands, the advantage conferred to *A. glutinosa* through N-fixation has been shown to result in a faster growth rate in the seedling phase than other temperate tree species (Kupper et al., 2018).

4.3. ^{14}C activity of mesocosms C pool

^{14}C activity detected in the soil below the three receiver species was not significantly altered by the species identity that was potted in it suggesting that there was not a measurable difference in the ^{14}C exuded by the roots of the three species, or that any difference in exudation was quickly eliminated due to consumption of exudates by the soil microbiota and eventually respired to the atmosphere. Ryan et al. (2001) added realistic concentrations of soluble C to the rhizosphere to quantify rates of mineralisation and confirmed what others had found, that microbial consumption of root exudates was extremely quick (Jones et al., 2004; Kuzyakov and Cheng, 2001; Nguyen et al., 1999) and that substrate half-lives are between 0.5 and 2 h for the most common exudates such as sugars, amino and organic acids (Ryan et al., 2001). The partition data itself revealed significant differences in ^{14}C activity as a % of total ^{14}C activity in the mesocosm; this is at least in part due to the significant differences between species in the relative sizes of the biomass partitions reported in sections 3.2 and 4.2. The *A. glutinosa* treatments had significantly greater mean rate of growth and resultantly had larger biomass partitions. As the size of the ^{14}C activity was the same for each species treatment a significant difference in biomass will result in a significant difference in % ^{14}C activity in each biomass compartment.

Statistical analysis to establish if the mean ^{14}C activity of the root nodule was determined by the species identity of the donor tree. This could distinguish if the differences in recently fixed C allocation alters the quality or quantity of root exudates, which would then impact the supply of ^{14}C to the mycorrhizal fungi and subsequently to the receiver tree linked to the common mycorrhizal network. It was found that the ^{14}C activity located in the receiver tree was not determined by the species identity of the donor tree. This suggested that the transfer of C to the

root nodules is mediated by the fungal network and not by the tree, as has been previously suggested in several theories such as the Mother tree theory (Simard, 2017) and Kin hypothesis (Pickles et al., 2017). Our results show that although the growth, relative biomass partitions and ensuing ^{14}C activity in the partitions is significantly different, that these differences are mediated by the fungal component of the plant-microbe symbiosis.

4.4. Effect of treatment (identity of donor plant) on ^{14}C activity in root nodule

The results from the *A. glutinosa* to *A. glutinosa* treatment (Figure 4) revealed that the majority of the ^{14}C activity located in the receiver fraction was in the root nodules. This is slightly unexpected as we would expect the ^{14}C to have travelled through the mycorrhizal fungal hyphae through the sand filled Perspex tube where it colonises the receiver tree fine roots before being translocated into the root nodules. The data shows, however, very small ^{14}C activity in both the fine and coarse roots suggesting the possibility that the mycorrhizal fungi are colonising the root in the proximity of the root nodules or possibly even colonising the root nodules themselves. This has been suggested in studies but only confirmed in leguminous *Rhizobium* containing root nodules rather the *Frankia* root nodules that we have on the *A. glutinosa* roots. Scheublin et al. (2004) found that one type of arbuscular mycorrhizal fungi, known as Glo3 was found in the roots of legumes (39 %) and in higher concentrations in the root nodules (63 %), while the control non-leguminous plants had lower results (13 %). This suggestion that some mycorrhizal fungi can link directly into the root nodules may go some way to explaining the very high ^{14}C activity located in the root nodules but not the fine or coarse roots. Scheublin et al. (2004) go on to suggest that the arbuscular mycorrhizal community was significantly different in roots nodules than roots in the leguminous plants, again suggesting more abundant or diverse colonisation of the root nodules when compared to the roots of the same plant. Orfanoudakis et al. (2010) suggested that there are interactions between arbuscular mycorrhizal fungi, *Frankia* and *A. glutinosa* that can alter belowground root architecture. Specifically, the presence of tripartite symbiosis increased the number of lateral roots, root hairs and the amount of branching whilst decreasing root hair density (Orfanoudakis et al., 2010). In this study unfortunately the root architecture was not analysed to confirm this hypothesis.

4.5. Root nodule activity as % of total ^{14}C in receiver plants

The root nodule activity as a percentage of total ^{14}C in receiver plants shows us that they appear to be some differences in the allocation to partitions in the receiver *A. glutinosa* tree depending

on the species of the donor tree. Although the results were not found to be significantly different from each other, they did show some interesting differences. The *A. glutinosa* receiver trees had more ^{14}C activity in the leaves than the other species treatments (11.09 ± 4.82 % for *A. glutinosa* donor), compared with 7.66 ± 5.40 % in treatments when *B. pendula* was the donor tree and 5.99 ± 1.06 % for *C. sativa* treatments). This could be as the *A. glutinosa* had a larger leaf biomass and therefore may have been more efficient at reabsorbing ^{14}C that had been respired from the donor tree from the glasshouse atmosphere, or the ^{14}C that had been transferred via the CMN into the roots and then reallocated by the tree to the leaves. During an earlier experiment we used control trees to quantify the amount of ^{14}C reuptake from the ^{14}C respired by the donor tree. It was found that although respired ^{14}C reuptake did occur it was an extremely small percentage of the ^{14}C in the entire mesocosm and therefore it seems more likely that the leaves reallocation rather than reabsorption was the cause of the higher ^{14}C activity in the leaves.

The percentage of ^{14}C activity in the branches of the receiver trees showed no differences with the *A. glutinosa*, *B. pendula* and *C. sativa* donor treatments all showing very similar results (7.31 ± 4.08 %, 8.56 ± 7.03 % and 8.25 ± 3.21 %, respectively; $P = 0.98$). Coarse and fine roots seemed to show greater differences, although not significantly different. Treatments with *B. pendula* as the donor had more ^{14}C activity in the coarse roots than the other two treatments (22.99 ± 13.73 % compared with 6.63 ± 5.29 % for *A. glutinosa* and 14.20 ± 10.12 % for *C. sativa*). The literature provided no mechanistic explanation for these differences due to the novel nature of the experimental design. Fine roots showed less differences between the species treatments although the highest percentage was found in the treatments with *C. sativa* as the donor tree (14.20 ± 10.12 % compared with 9.18 ± 8.43 % and 11.09 ± 6.60 % for the *A. glutinosa* and *B. pendula* treatments).

The percentage of total ^{14}C in the root nodules appeared to show slight differences depending of the donor tree species identity, although these differences were not found to be significantly different. The most ^{14}C activity in the root nodule was located in the root nodules of the *A. glutinosa* donor treatments (65.79 ± 21.94 % compared with 49.76 ± 23.99 % for *B. pendula* and 56.84 ± 17.99 % for *C. sativa* donor tree treatments).

In a world with increasing atmospheric carbon dioxide concentration, plant primary production will likely be limited by the availability of other essential nutrients, including nitrogen (Norby et al., 2010). It is believed that root nodulation as a functional trait in higher plants evolved

approximately between 56- 50 million years ago, at a time when atmospheric carbon dioxide concentrations, temperature and humidity were much higher than we observe today (Sprent and James, 2007; Bowen et al., 2004; Sprent, 2008). It has been theorised that because establishing N-fixing symbiosis with bacteria has substantial C cost to the plant, that the excess of atmospheric carbon dioxide that was available to plants at this time could have been a driver in the evolution of this fundamental plant-microbe symbiosis (Sprent and James, 2007). We suggest that in future, the primary producers will be limited by nutrients such as N and, that once again, this will give actinorhizal and leguminous plants a competitive advantage over species without N-fixing symbiotic associations.

The dynamics of tripartite mutualistic symbiosis are not yet fully understood, due to difficulties in quantifying nutrient flows and the highly complex nature of these belowground interactions. Transfer of C via CMNs is probably mediated by both the trees and the mycorrhizal fungi, but in our study, we showed that the C demand of N-fixation processes resulted in preferential transfer of ^{14}C to the root nodules of the receiver plant. This could be as a result of a source-sink relationship (Fellbaum, 2014). For example, in experiments where a shaded tree was connected to another via CMN, C was preferentially translocated from the photosynthetically active tree (the source) to the more demanding shaded tree (sink). It could also be explained by the belowground trading market (Wipf et al., 2019), which postulates that the accessibility of nitrogen and phosphorus drives the rates of mycorrhizal colonisation and whether the assistance this provides outweighs the cost to the plant (Johnson et al., 2015). This seems unlikely as Orfanoudaki et al. (2010) discovered that tripartite symbiosis developed successfully and still delivered the plant host growth benefits even when N and P were unlikely to be limiting. The other possibility is that the plants are individuals acting as with firms and co-ops (Noë and Kiers, 2018) operating based on the principles of mutualistic reciprocal rewards (Kiers et al., 2011). In this model, neither partner has overall control of the symbiosis and are therefore both willing participants and the equilibrium is maintained through both rewarding the other (Noë and Hammerstein, 1995; Schwartz and Hoeksema, 1998; Hammerstein, 2003). Further experimental work using N and C isotopes simultaneously could begin to elucidate the dynamics of belowground trading in these tripartite symbioses.

5. Conclusion

Our hypothesis for this experiment was:

H_{null} Belowground allocation of C via inter- and intra-specific CMNs will be greatest in “receiver” trees with actinorhizal associations, due to an increased C sink strength generated by the metabolic processes of microbial symbionts.

It was found that belowground allocation was not greater in the species treatments with actinorhizal symbiosis, however the soil respiration was greater in the species treatments with actinorhizal symbiosis and the C storage was greatest in the *C. sativa*. This suggests that the energetic demand of the nitrogen fixation is utilising the photosynthates as an energy supply and that this is driving the increase in soil respiration. Whereas for the *C. sativa* treatments the belowground biomass compartments are less demanding and implying that unutilised exudates in the soil has led to the increased ^{14}C activity measured in the soil under these trees. The hypothesis was therefore rejected.

The most fascinating result from this research was that the energetic demand for carbohydrates of their root nodules nitrogen-fixation is enough of a sink to expedite the ^{14}C transfer from the donor tree across the CMN and into the root nodule of the receiver plant. It was not possible to determine if the ^{14}C was moving directly from the mycorrhizal hyphae into the root nodule or if it as first passing through the receiver trees fine roots before residing in the nodule biomass.

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7. References

- Ahmed, I.U., Smith, A.R., Jones, D.L. and Godbold, D.L., 2016. Tree species identity influences the vertical distribution of labile and recalcitrant carbon in a temperate deciduous forest soil. *Forest Ecology and Management*, 359, pp.352-360.
- Ané, J.M., Kiss, G.B., Riely, B.K., Penmetsa, R.V., Oldroyd, G.E., Ayax, C., Lévy, J., Debellé, F., Baek, J.M., Kalo, P. and Rosenberg, C., 2004. *Medicago truncatula* DMI1 required for bacterial and fungal symbioses in legumes. *Science*, 303(5662), pp.1364-1367.
- Arnebrant, K., Ek, H., Finlay, R.D. and Söderström, B., 1993. Nitrogen translocation between *Alnus glutinosa* (L.) Gaertn. seedlings inoculated with *Frankia* sp. and *Pinus contorta* Doug, ex Loud seedlings connected by a common ectomycorrhizal mycelium. *New Phytologist*, 124(2), pp.231-242.
- Beatty, P.H. and Good, A.G., 2011. Future prospects for cereals that fix nitrogen. *Science*, 333(6041), pp.416-417.
- Becking, J.H., 1970. *Frankiaceae* fam. nov.(*Actinomycetales*) with one new combination and six new species of the genus *Frankia* Brunchorst 1886, 174. *International Journal of Systematic and Evolutionary Microbiology*, 20(2), pp.201-220.
- Benson, D.R. and Silvester, W.B., 1993. Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants. *Microbiological Reviews*, 57(2), pp.293-319.
- Berta, G., Fusconi, A., Trotta, A. and Scannerini, S., 1990. Morphogenetic modifications induced by the mycorrhizal fungus *Glomus* strain E3 in the root system of *Allium porrum* L. *New Phytologist*, 114(2), pp.207-215.
- Bowen, G.J., Beerling, D.J., Koch, P.L., Zachos, J.C. and Quattlebaum, T., 2004. A humid climate state during the Palaeocene/Eocene thermal maximum. *Nature*, 432(7016), p.495.
- Callaham, D., Deltredici, P. and Torrey, J.G., 1978. Isolation and cultivation in vitro of the actinomycete causing root nodulation in *Comptonia*. *Science*, 199(4331), pp.899-902
- Claessens, H., Oosterbaan, A., Savill, P. and Rondeux, J., 2010. A review of the characteristics of black alder (*Alnus glutinosa* (L.) Gaertn.) and their implications for silvicultural practices. *Forestry*, 83(2), pp.163-175.

- Cleveland, C.C., Townsend, A.R., Schimel, D.S., Fisher, H., Howarth, R.W., Hedin, L.O., Perakis, S.S., Latty, E.F., Von Fischer, J.C., Elseroad, A. and Wasson, M.F., 1999. Global patterns of terrestrial biological nitrogen (N₂) fixation in natural ecosystems. *Global biogeochemical cycles*, 13(2), pp.623-645.
- Daudin, D. and Sierra, J., 2008. Spatial and temporal variation of below-ground N transfer from a leguminous tree to an associated grass in an agroforestry system. *Agriculture, ecosystems and environment*, 126(3-4), pp.275-280.
- del Moral, R. and Wood, D.M., 1993. Early primary succession on the volcano Mount St. Helens. *Journal of Vegetation Science*, 4(2), pp.223-234.
- Desbrosses, G.J. and Stougaard, J., 2011. Root nodulation: a paradigm for how plant-microbe symbiosis influences host developmental pathways. *Cell Host and Microbe*, 10(4), pp.348-358.
- Dilkes, N.B., Jones, D.L. and Farrar, J., 2004. Temporal dynamics of carbon partitioning and rhizodeposition in wheat. *Plant Physiology*, 134(2), pp.706-715.
- Ekblad, A. and Huss-Danell, K., 1995. Nitrogen fixation by *Alnus incana* and nitrogen transfer from *A. incana* to *Pinus sylvestris* influenced by macronutrients and ectomycorrhiza. *New Phytologist*, 131(4), pp.453-459.
- Everard, J. and Christie, J.M., 1995. Sweet chestnut: silviculture, timber quality and yield in the forest of Dean. *Forestry: An International Journal of Forest Research*, 68(2), pp.133-144.
- Fellbaum, C.R., Mensah, J.A., Cloos, A.J., Strahan, G.E., Pfeffer, P.E., Kiers, E.T. and Bücking, H., 2014. Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants. *New Phytologist*, 203(2), pp.646-656.
- Geddes, B.A., Ryu, M.H., Mus, F., Costas, A.G., Peters, J.W., Voigt, C.A. and Poole, P., 2015. Use of plant colonizing bacteria as chassis for transfer of N₂-fixation to cereals. *Current opinion in biotechnology*, 32, pp.216-222.
- Ghedira, K., Harigua-Souiai, E., Hamda, C., Fournier, P., Pujic, P., Guesmi, S., Guizani, I., Miotello, G., Armengaud, J., Normand, P. and Sghaier, H., 2018. The PEG-responding desiccume of the alder microsymbiont *Frankia alni*. *Scientific Reports*, 8(1), p.759.
- Gruber, N. and Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen cycle. *Nature*, 451(7176), p.293

Hammerstein, P. ed., 2003. Genetic and cultural evolution of cooperation. MIT press Cambridge, MA.

He, X., Xu, M., Qiu, G.Y. and Zhou, J., 2009. Use of ^{15}N stable isotope to quantify nitrogen transfer between mycorrhizal plants. *Journal of Plant Ecology*, 2(3), pp.107-118.

Herridge, D.F., Peoples, M.B. and Boddey, R.M., 2008. Global inputs of biological nitrogen fixation in agricultural systems. *Plant and soil*, 311(1-2), pp.1-18.

Holmer, R., Rutten, L., Kohlen, W., van Velzen, R. and Geurts, R., 2017. Commonalities in Symbiotic Plant-Microbe Signalling. In *Advances in Botanical Research* (Vol. 82, pp. 187-221). Academic Press.

Hooker, J.E. and Atkinson, D., 1996. Arbuscular mycorrhizal fungi-induced alteration to tree-root architecture and longevity. *Zeitschrift für Pflanzenernährung und Bodenkunde*, 159(3), pp.229-234.

Huss-Danell, K. and Hahlin, A.S., 1988. Nitrogenase activity decay and energy supply in Frankia after addition of ammonium to the host plant *Alnus incana*. *Physiologia Plantarum*, 74(4), pp.745-751.

Isopi, R., Lumini, E., Frattegiani, M., Puppi, G., Bosco, M., Favilli, F. and Buresti, E., 1994. Inoculation of *Alnus cordata* with selected microsymbionts: Effects of *Frankia* and *Glomus* spp. on seedling growth and development. Symbiosis (Philadelphia, Pa.)(USA).

Johansen, A. and Jensen, E.S., 1996. Transfer of N and P from intact or decomposing roots of pea to barley interconnected by an arbuscular mycorrhizal fungus. *Soil Biology and Biochemistry*, 28(1), pp.73-81.

Johnson, N.C., Wilson, G.W., Wilson, J.A., Miller, R.M. and Bowker, M.A., 2015. Mycorrhizal phenotypes and the Law of the Minimum. *New Phytologist*, 205(4), pp.1473-1484.

Jones, D.L., Hodge, A. and Kuzyakov, Y., 2004. Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist*, 163(3), pp.459-480.

Jones, D.L. and Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology and Biochemistry*, 38(5), pp.991-999.

- Jones, T.G., Evans, C.D., Jones, D.L., Hill, P.W. and Freeman, C., 2016. Transformations in DOC along a source to sea continuum; impacts of photo-degradation, biological processes and mixing. *Aquatic Sciences*, 78(3), pp.433-446.
- Karlsson, A., Albrektson, A. and Sonesson, J., 1997. Site index and productivity of artificially regenerated *Betula pendula* and *Betula pubescens* stands on former farmland in southern and central Sweden. *Scandinavian journal of Forest research*, 12(3), pp.256-263.
- Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J.A., Franken, O., Verbruggen, E., Fellbaum, C.R., Kowalchuk, G.A., Hart, M.M., Bago, A. and Palmer, T.M., 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*, 333(6044), pp.880-882.
- Koltai, H. and Kapulnik, Y. eds., 2010. *Arbuscular mycorrhizas: physiology and function*. Springer Science and Business Media, Dordrecht.
- Kupper, P., Ivanova, H., Söber, A., Rohula-Okunev, G. and Sellin, A., 2018. Night and daytime water relations in five fast-growing tree species: Effects of environmental and endogenous variables. *Ecohydrology*, 11(6), p.e1927.
- Kuzyakov, Y. and Cheng, W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. *Soil Biology and biochemistry*, 33(14), pp.1915-1925.
- Ledgard, S.F., 2001. Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/grass pastures. *Plant and Soil*, 228(1), pp.43-59.
- Li, L., Li, S.M., Sun, J.H., Zhou, L.L., Bao, X.G., Zhang, H.G. and Zhang, F.S., 2007. Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. *Proceedings of the National Academy of Sciences*, 104(27), pp.11192-11196.
- Malézieux, E., Crozat, Y., Dupraz, C., Laurans, M., Makowski, D., Ozier-Lafontaine, H., Rapidel, B., De Tourdonnet, S. and Valantin-Morison, M., 2009. Mixing plant species in cropping systems: concepts, tools and models: a review. In *Sustainable agriculture* (pp. 329-353). Springer, Dordrecht.
- Miranda, K.M., Espey, M.G. and Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric oxide*, 5(1), pp.62-71.
- Moyer-Henry, K.A., Burton, J.W., Israel, D.W. and Rufty, T.W., 2006. Nitrogen transfer between plants: a 15 N natural abundance study with crop and weed species. *Plant and Soil*, 282(1-2), pp.7-20.

- Mulvaney, R.L., 1996. Nitrogen-inorganic forms. In 'Methods of soil analysis. Part 3. Chemical methods'. 3rd edn.(Eds DL Sparks, AL Page, PA Helmke, RH Loeppert, PN Soltanpour, MA Tabatabai, CT Johnston, ME Sumner) pp. 1123–1184. Soil Science Society of America: Madison, WI.
- Murphy, J. and Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica chimica acta*, 27, pp.31-36.
- Mus, F., Crook, M.B., Garcia, K., Costas, A.G., Geddes, B.A., Kouri, E.D., Paramasivan, P., Ryu, M.H., Oldroyd, G.E., Poole, P.S. and Udvardi, M.K., 2016. Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. *Applied and environmental microbiology*, 82(13), pp.3698-3710.
- Newman, E.I., Devoy, C.L.N., Easen, N.J. and Fowles, K.J., 1994. Plant species that can be linked by VA mycorrhizal fungi. *New Phytologist*, 126(4), pp.691-693.
- Nguyen, C., Todorovic, C., Robin, C., Christophe, A. and Guckert, A., 1999. Continuous monitoring of rhizosphere respiration after labelling of plant shoots with $^{14}\text{CO}_2$. *Plant and Soil*, 212(2), pp.189-199.
- Noë, R. and Hammerstein, P., 1995. Biological markets. *Trends in Ecology and Evolution*, 10(8), pp.336-339.
- Noë, R. and Kiers, E.T., 2018. Mycorrhizal markets, firms, and co-ops. *Trends in ecology and evolution*, 33(10), pp.777-789.
- Norby, R.J., Warren, J.M., Iversen, C.M., Medlyn, B.E. and McMurtrie, R.E., 2010. CO₂ enhancement of forest productivity constrained by limited nitrogen availability. *Proceedings of the National Academy of Sciences*, 107(45), pp.19368-19373.
- Ogle, H. and Brown, J., 1997. Plant-microbe symbioses. *Plant pathogens and plant diseases*. Rockvale Publications, pp.21-37.
- Olsen, S.R., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture; Washington.
- Orfanoudakis, M., Wheeler, C.T. and Hooker, J.E., 2010. Both the arbuscular mycorrhizal fungus *Gigaspora rosea* and *Frankia* increase root system branching and reduce root hair frequency in *Alnus glutinosa*. *Mycorrhiza*, 20(2), pp.117-126.

- Pawlowski, K. and Demchenko, K.N., 2012. The diversity of actinorhizal symbiosis. *Protoplasma*, 249(4), pp.967-979.
- Paynel, F., Murray, P.J. and Cliquet, J.B., 2001. Root exudates: a pathway for short-term N transfer from clover and ryegrass. *Plant and soil*, 229(2), pp.235-243.
- Peyraud, J.L., Le Gall, A. and Lüscher, A., 2009. Potential food production from forage legume-based-systems in Europe: an overview. *Irish Journal of Agricultural and Food Research*, pp.115-135.
- Pickles, B.J., Wilhelm, R., Asay, A.K., Hahn, A.S., Simard, S.W. and Mohn, W.W., 2017. Transfer of ^{13}C between paired Douglas-fir seedlings reveals plant kinship effects and uptake of exudates by ectomycorrhizas. *New Phytologist*, 214(1), pp.400-411.
- Postgate, J. ed., 2012. *The chemistry and biochemistry of nitrogen fixation*. Springer Science and Business Media. New York.
- Remigi, P., Zhu, J., Young, J.P.W. and Masson-Boivin, C., 2016. Symbiosis within symbiosis: evolving nitrogen-fixing legume symbionts. *Trends in microbiology*, 24(1), pp.63-75.
- Roggy, J.C., Moiroud, A., Lensi, R. and Domenach, A.M., 2004. Estimating N transfers between N_2 -fixing actinorhizal species and the non- N_2 -fixing *Prunus avium* under partially controlled conditions. *Biology and fertility of soils*, 39(5), pp.312-319.
- Rowell, D.L., 1994. Soil science: methods and applications. Department of Soil Science, University of Reading.
- Ryan, P.R., Delhaize, E. and Jones, D.L., 2001. Function and mechanism of organic anion exudation from plant roots. *Annual review of plant biology*, 52(1), pp.527-560.
- Scheublin, T.R., Ridgway, K.P., Young, J.P.W. and Van Der Heijden, M.G., 2004. Non-legumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. *Applied and Environmental Microbiology*, 70(10), pp.6240-6246.
- Schwartz, M.W. and Hoeksema, J.D., 1998. Specialization and resource trade: biological markets as a model of mutualisms. *Ecology*, 79(3), pp.1029-1038.
- Selosse, M.A., Richard, F., He, X. and Simard, S.W., 2006. Mycorrhizal networks: des liaisons dangereuses?. *Trends in Ecology and Evolution*, 21(11), pp.621-628.

- Shen, Q. and Chu, G., 2004. Bi-directional nitrogen transfer in an intercropping system of peanut with rice cultivated in aerobic soil. *Biology and fertility of soils*, 40(2), pp.81-87.
- Sierra, J. and Desfontaines, L., 2009. Role of root exudates and root turnover in the below-ground N transfer from *Canavalia ensiformis* (jackbean) to the associated *Musa acuminata* (banana). *Crop and Pasture Science*, 60(3), pp.289-294.
- Sierra, J. and Nygren, P., 2006. Transfer of N fixed by a legume tree to the associated grass in a tropical silvopastoral system. *Soil Biology and Biochemistry*, 38(7), pp.1893-1903.
- Simard, S.W., 2017. The mother tree. The word for world is still forest. Berlin, Germany: K. Verlag and the Haus der Kulturen der Welt.
- Simard, S.W., Beiler, K.J., Bingham, M.A., Deslippe, J.R., Philip, L.J. and Teste, F.P., 2012. Mycorrhizal networks: mechanisms, ecology and modelling. *Fungal Biology Reviews*, 26(1), pp.39-60.
- Simard, S.W. and Durall, D.M., 2004. Mycorrhizal networks: a review of their extent, function, and importance. *Canadian Journal of Botany*, 82(8), pp.1140-1165.
- Simard, S.W., Perry, D.A., Jones, M.D., Myrold, D.D., Durall, D.M. and Molina, R., 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature*, 388(6642), p.579.
- Smith, J.L. and Doran, J.W., 1996. Measurement and use of pH and electrical conductivity for soil quality analysis. In *Methods for assessing soil quality* (Vol. 49). Soil Science Society of America Madison, WI
- Smith, A.R., Lukac, M., Hood, R., Healey, J.R., Miglietta, F. and Godbold, D.L., 2013. Elevated CO₂ enrichment induces a differential biomass response in a mixed species temperate forest plantation. *New Phytologist*, 198(1), pp.156-168.
- Smith, S.E. and Read, D.J., 2010. *Mycorrhizal symbiosis*. Academic press. London.
- Sprent, J.I., 2008. Evolution and diversity of legume symbiosis. In Nitrogen-fixing leguminous symbioses (pp. 1-21). Springer, Dordrecht.
- Sprent, J.I. and James, E.K., 2007. Legume evolution: where do nodules and mycorrhizas fit in? *Plant Physiology*, 144(2), pp.575-581.
- Tariq, M., Hameed, S., Khan, H.U., Munir, M.I. and Nushin, F., 2017. Role of microsymbionts in plant microbe symbiosis. *Journal of Applied Microbiology and Biochemistry*, 1(2), p.6.

- Teste, F.P., Jones, M.D. and Dickie, I.A., 2019. Dual-mycorrhizal plants: their ecology and relevance. *New Phytologist*. (Preprint).
- Tjepkema, J.D. and Winship, L.J., 1980. Energy requirement for nitrogen fixation in actinorhizal and legume root nodules. *Science*, 209(4453), pp.279-281.
- Van Nguyen, T. and Pawlowski, K., 2017. Frankia and Actinorhizal Plants: Symbiotic Nitrogen Fixation. In *Rhizotrophs: Plant Growth Promotion to Bioremediation* (pp. 237-261). Springer, Singapore.
- Varik, M., Kukumägi, M., Aosaar, J., Becker, H., Ostonen, I., Lõhmus, K. and Uri, V., 2015. Carbon budgets in fertile silver birch (*Betula pendula* Roth) chronosequence stands. *Ecological Engineering*, 77, pp.284-296.
- Vessey, J.K., Pawlowski, K. and Bergman, B., 2005. Root-based N₂-fixing symbioses: legumes, actinorhizal plants, *Parasponia* sp. and cycads. In *Root physiology: from gene to function* (pp. 51-78). Springer, Dordrecht.
- Vicente, E.J. and Dean, D.R., 2017. Keeping the nitrogen-fixation dream alive. *Proceedings of the National Academy of Sciences*, 114(12), pp.3009-3011.
- Walker, L.R., Clarkson, B.D., Silvester, W.B. and Clarkson, B.R., 2003. Colonization dynamics and facilitative impacts of a nitrogen-fixing shrub in primary succession. *Journal of Vegetation Science*, 14(2), pp.277-290.
- Wipf, D., Krajinski, F., van Tuinen, D., Recorbet, G. and Courty, P.E., 2019. Trading on the arbuscular mycorrhiza market: from arbuscules to common mycorrhizal networks. *New Phytologist*. (Preprint).

Chapter 6

Investigating carbon allocation in two temperate tree species to understory vegetation via mycorrhizae.

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1. Introduction

Mycorrhizal symbiosis is the commonest global plant-fungal relationship that involves a diverse range of fungi from both divergent taxonomic groups and functional guilds colonising over 90 % of known plant species (Nguyen et al., 2016; Prasad et al., 2017). These ecologically fundamental associations are comprised of two or more partners trading nitrogen (N), phosphorus and/or a variety of trace elements for photosynthates (Bever, 2015). Research has often focussed on the improved nutritional status of the host. Plants that possess these relationships imbibe water and nutrients, however, fewer studies have attempted to quantify the carbon (C) fluxes from host plant to mycorrhizal fungi and the mechanisms that support allocation of C belowground (Slavíková et al., 2017). It is well-known that, as heterotrophs, ectomycorrhizal fungi are nearly always reliant on C derived from the photosynthesis of their plant-host (Lindahl and Tunlid, 2015), although unlike arbuscular mycorrhizae in some cases ectomycorrhizal fungi have been found to derive some C from decomposition of non-living organic matter (Talbot et al., 2008). However, this flux has not been reliably quantified, with current estimates for arbuscular symbiosis ranging from 4 to 20 % of gross photosynthetic production (Grimoldi et al., 2006; Jakobsen and Rosendahl 1990; Wright et al., 1998a) and ectomycorrhizal symbiosis is reported to vary from 3 to 36 % of gross primary production

(Bryla and Eissenstat, 2005; Řezáčová et al., 2017). Variation in the estimates of C allocation to mycorrhizal partners is thought to be influenced by the species identity of the fungal partner, nutrient availability and the photosynthetic photon flux density that drives photosynthesis and ultimately the plant-fungi source-sink relationship (Lendenmann et al., 2011; Konvalinková and Jansa, 2016).

When two or more trees of either the same or different species are involved in a relationship with the same fungi, this is known as a common mycorrhizal network (CMN) or colloquially the Wood Wide Web (Simard et al., 1997; Helgason et al., 1998). CMNs have been shown to occur in all global biomes (Francis and Read, 1994; Simard and Durall, 2004; Simard et al., 2012; Molina and Horton, 2015), in both natural and agricultural ecosystems (Gosling et al., 2006). Agricultural soils tend to be dominated by arbuscular CMNs whereas in forest ecosystems ectomycorrhizal species tend to prevail. Arbuscular mycorrhizal fungi are the oldest form of mycorrhizal association within the order Glomales (Zygomycetes), whereas ectomycorrhizal associations are more recently evolved symbioses that consist of a small number of ascomycetes and some *basiomycetes* mycobionts (Brundrett and Tedersoo, 2018). Arbuscular and ecto-mycorrhizae exhibit different physiological characteristics and as a result can benefit the host and ecosystem in different ways (Lodge, 2000). For example, ecto-mycorrhizae are known to protect trees from pathogens by forming a complex branched structure between the cortical and epidermal root cells known as the Hartig net and sheath like mantle around the short lateral root tips. This physically isolates the plants roots from the pathogen whilst simultaneously increasing nutrient uptake (Smith and Read, 2010). It has been found that the rate of ectomycorrhizal colonisation directly promotes plant disease resistance (Machón et al., 2009), whereas arbuscular mycorrhizal fungi do not have this ability (Johnson et al., 2018); untangling the relative importance of each of the two types of relationship to the tree host is extremely difficult (Jones et al., 1998).

Tripartite mycorrhizal symbiotic relationships, commonly known as “bridge trees” are of particular ecological importance as they are an interface between the arbuscular and ectomycorrhizal hyphal networks found in temperate forest soils (Lodge, 2000). Examples of tree species that form both arbuscular and ectomycorrhizal associations are uncommon, but do occur in some cases in temperate forest ecosystems e.g. aspen (*Populus tremula*) (Brundrett et al., 1998) and common alder (*Alnus glutinosa*) (Brundrett et al., 1990) and in Australia e.g. *Acacia* spp., *Casuarina* (Brundrett et al., 1998) and jarrah (*Eucalyptus marginata*) (Brundrett et al., 1994). There is some evidence that the dominant type of symbiotic association changes

during the lifetime of a tree, with arbuscular relationships being more important at the beginning of a tree's life and then later giving way to the ecto-mycorrhizae has been observed in both field experiments (Bellei et al., 1992; Dominik, 1956; Gardner and Malajczuk, 1988; Lodge and Wentworth, 1990; Adjoud-Sadadou and Halli-Hargas, 2017) and glasshouse experiments (Lapyeyrie and Chilvers, 1985; Chen et al., 1998). Multiple mechanistic explanations have been suggested for this replacement of arbuscular with ectomycorrhizal fungal associations in tripartite plant species over time (Lodge, 2000). These include: (i) that initially the ectomycorrhizal fungi are slower to colonise plant roots than arbuscular fungi (Chilvers et al., 1987), but once the ectomycorrhizae have established that the mantle creates a physical barrier preventing arbuscular mycorrhizal colonisation (Chilvers et al., 1987); (ii) that chemicals are released by either the fungi or plant host which allows the dominance of ectomycorrhizal fungi (Lodge, 2000), or (iii) that the ectomycorrhizae can outcompete the arbuscular for plant-derived carbohydrates (Lodge and Wentworth, 1990). Chen et al. (2000) were able to demonstrate that multiple drivers were at play simultaneously, the sheaths were shown to quickly colonise the roots blocking colonisation by competitors (see also Chilvers et al., 1987; Chilvers and Gust, 1982), the presence of ectomycorrhizal fungi was also shown to reduce the growth of fine roots and reduce the ability of new arbuscular colonisation (Chen et al., 2000). Some ectomycorrhizal species were found to reduce arbuscular mycorrhizal success more than others, particularly *Laccaria* spp. suggesting a chemical interaction. Although these studies clearly indicate that mycorrhizal fungi have an effect on succession either by interference and/or competition, they also suggest that rates of arbuscular mycorrhizal fungi decrease over time even without the presence of ectomycorrhizae, suggesting changes in the plants physiology altering the colonisation receptivity (Lodge, 2000). In the case of *A. glutinosa*, the tree's roots are quadripartite, that is, that they not only symbiotic relationships with arbuscular and ecto-mycorrhizae, but also form a symbiotic relationship with the N-fixing bacteria *Frankia alni*.

Complex interactions between multiple symbionts within the rhizosphere are understood to be important: (i) as mediators of plant health by protecting the plant host from antagonists (Berendsen, 2012; Laliberté et al., 2015; Vannette and Rasmann, 2012); (ii) in modifying soil structure (Rillig, 2004); (iii) for nutrient acquisition (Bhandari and Garg, 2017); (iv) biogeochemical cycling (Leake and Read, 2017); (v) population dynamics of forest ecosystem plant communities (Wilson et al., 2009; Bennett et al., 2017); (vi) seedling survival (Wężowicz et al., 2017); and (vii) in mediating successional development (Allen et al., 2018). The type of

symbiont present can alter plant successional dynamics as ectomycorrhizal fungi can improve rates of seedling survival more than the presence of arbuscular fungi (Van Der Heijden and Horton, 2009).

Transfer of resources through CMNs is a well-established phenomenon. However, primarily due to differentiation between the type of mycorrhizal associations of species used in agriculture (predominantly arbuscular mycorrhizae) and those associations found in temperate forests (predominantly ectomycorrhizae), arbuscular mycorrhizae have not been widely researched in forest ecosystems. An understanding of belowground C dynamics that details which fungal symbionts mediate biogeochemical cycling are increasingly salient in a world of changing environmental conditions (Pickles et al., 2016; Richter and Billings, 2015). C isotope labelling is a powerful technique that can be used to improve our understanding of the complex role the belowground microbial community has in the C cycle (Körner et al., 2005; Klein et al., 2016). Isotopic pulse techniques can utilise both radio-isotopes such as ^{14}C (half-life of 5730 years) and ^{11}C (half-life of 20.4 min) or stable isotopes like ^{13}C , which have increased in popularity since stable isotope ratio mass spectrometry has increased in availability (Epron et al., 2012). An advantage in using the radioisotope ^{14}C in field experiments over the stable isotope ^{13}C is that relatively small amounts can be detected as the ^{14}C isotope does not exist in any significant amount naturally (Epron et al., 2012).

In this field-based experiment we use ^{14}C as a proxy for recently fixed C to monitor the transfer of C from trees to other plant species in the field. Specifically, a ^{14}C pulse chase was conducted to establish the fate of C fixed by two temperate tree species to establish the relative importance of mycorrhizal type to belowground C allocation and subsequent transfer of C to mycorrhizal hyphal networks. The two temperate tree species studied were *Alnus glutinosa* (commonly known as black alder, common alder or European alder), a species with multiple symbiotic relationships and *Castanea sativa* (known commonly as sweet chestnut), a species with only ectomycorrhizal associations.

Our hypothesis for this experiment was:

H₁ Transfer of C to ground vegetation will be greatest via the CMN of *A. glutinosa*, that has a symbiosis with both ecto- and arbuscular mycorrhizae, rather than *C. sativa*, an obligate ectomycorrhizal species.

2. Materials and methods

2.1. Study site

The two-tree species studied were *Castanea sativa* Mill. and *Alnus glutinosa* (L.) Gaertn and were chosen from species that comprised BangorDIVERSE, a tree diversity experiment located at the Henfaes experimental farm, Abergwyngregyn, Gwynedd, Wales, (53°14 N, 4°01 W). The experiment was established in March 2004, on two previously agricultural fields, with a total area of 2.36 ha (Ahmed et al., 2016) and has a fully replicated ($n = 4$) planting design of one, two and three species mixtures of alder (*Alnus glutinosa* L.), birch (*Betula pendula* Roth.), beech (*Fagus sylvatica* L.) and oak (*Quercus robur* L.) at a density of 10,000 stems ha⁻¹ planted in 92 plots (Gunina et al., 2017), and has a fully replicated ($n = 4$) planting design of one, two and three species mixtures of alder (*Alnus glutinosa* L.), birch (*Betula pendula* Roth.), beech (*Fagus sylvatica* L.) and oak (*Quercus robur* L.) at a density of 10,000 stems ha⁻¹. BangorDIVERSE plots are approximately 13-18 m above sea level and 700 m from the high tide mark, with a relatively exposed aspect (53°24 N, 4°01 W). The soil is a sandy loam and classified as a Eutric Cambisol (FAO) (Rheidol series) found over alluvial gravel (Smith et al., 2013) or Fluventric Dystrocherept and Dystric Eutrudepts (US Soil Taxonomy classification system) (Glanville et al., 2012). Soil texture in the 0-10 cm layer was 48.2 ± 1.3 % sand, 33.6 ± 0.9 % silt and 18.2 ± 2.1 % clay, determined by laser diffraction (Coulter LS particle size analyser) (Ahmed et al., 2016). The climate is classified as hyper-oceanic and average rainfall is 1250 mm annually and the annual mean soil temperature at 10 cm depth is 11 °C (Campbell Scientific Ltd., Shepshed, UK) (Glanville et al., 2016).

2.2. Trees studied

The two temperate forest tree species were selected from trees existent in the experimental forestry plot, on the basis of their contrasting traits. Explicitly, *A. glutinosa* is actinorhizal, with both arbuscular and ectomycorrhizal fungal association, tolerates waterlogged soils and has intermediate shade tolerance, whereas *C. sativa* is exclusively an ectomycorrhizal, late successional, shade tolerant and relatively long-lived tree species. The trees studied were planted in 2004 and had previously been coppiced (cut at ground level) multiple times, as they were part of the experimental plot which was located below power cables supplying electricity to farm buildings. Three individuals of the two species were selected for being an appropriate and uniform size for ¹⁴CO₂ pulse labelling. In the case of the *A. glutinosa*, the mean height was

175 ± 14 cm and diameter at breast height (DBH; 1.3 m) was 8 ± 1 cm, whilst the mean height of *C. sativa* was 178 ± 6 cm with a DBH of 6 ± 1 cm. Initially a brush cutter was used to clear an area of unwanted and interfering vegetation from a 5 m diameter around each treatment tree. Prior to this intervention, the dominant ground vegetation species had included: stinging nettle (*Urtica dioica*), bramble (*Rubus fruticosus*), thistle (*Cirsium vulgare*), rosebay willowherb (*Chamaenerion angustifolium*), and grasses including Yorkshire fog (*Holcus lanatus*), dock (*Rumex obtusifolius*), chickweed (*Cerastium fontanum*) and regeneration or regrowth of tree species including: sycamore (*Acer pseudoplatanus*), *A. glutinosa* and *C. sativa*.

2.3. Soil chemical properties

To determine if the basic chemical properties of the soil under the canopy of the two tree species studied exhibited any major differences prior to pulse-labelling the soil chemical properties were analysed. Four soil samples were collected using a 7 cm diameter soil corer from under the tree canopy approximately 25 - 35 cm from the tree stem. The soil samples were placed directly into gas permeable polythene bags and stored at 5 °C in a cool box immediately after returning from the field. The soil samples were then passed through a 2 mm sieve prior to analysis. In order to quantify total elemental C and N, a subsample of the soil from under each tree was dried at 105 °C and ball milled before analysis by dry combustion using a CN-2000 analyser (LECO Corp, St Joseph, MI, USA). Soil ammonium (NH₄⁺) and nitrate (NO₃⁻) concentrations were determined following extraction from soil in 1 M K₂SO₄ in a 1:5 (w/v) slurry and horizontal shaking for 1 hour at 200 rpm, before centrifugation at 4000 rpm for 10 min. Colourimetric determination of nitrate was undertaken (λ = 540 nm) by the vanadium chloride reduction method (Miranda et al., 2001). Ammonium was determined by the salicylate-hypochlorite photometric method (λ = 667 nm) of Mulvaney (1996). Plant available phosphorus was determined by horizontal shaking of fresh soil in 0.5 M acetic acid (CH₃CO₂H) at a ratio of 1:5 (w/v) and determined colourimetrically as molybdate-reactive P (Murphy and Riley, 1962). Moisture content was determined gravimetrically by oven drying at 105 °C (Rowell, 1994). The Smith and Doran (1996) method was utilised to measure soil pH and electrical conductivity (EC) in distilled water in a 1:2.5 v/v slurry. Soil dissolved organic carbon (DOC) and total dissolved N (TDN) were quantified using a Shimadzu TOC-V-TN analyser (Shimadzu Corp., Kyoto, Japan) following extraction (1:5 w/v) in 0.5 M K₂SO₄ (Jones and Willett, 2006). The soil chemical and physical properties are shown in Table 1.

2.4. Pulse labelling technique

A single ^{14}C radioisotopic pulse-label was applied in late summer (8th August 2017) by covering the tree with a large gas-proof bag and then sealing the stem at the bottom to prevent $^{14}\text{CO}_2$ gaseous loss. A reaction vessel containing 2 ml of 3 M HCl (namely a 30 mm diameter centrifuge tube lid), was secured inside the bag into which 200 μl of 3 MBq $\text{NaH}^{14}\text{CO}_3$ was added (Amersham International, Amersham, UK) leading to the formation of a $^{14}\text{CO}_2$ enriched atmosphere inside the bag. Each tree was exposed to the same activity (3 MBq) for 4 hours to allow for photo-assimilation in full ambient light with a minimum photosynthetic photon flux density of $800 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The day was meticulously chosen to ensure favourable photosynthetically active radiation and ambient temperature conducive to photoassimilation to maximise the efficacy of the ^{14}C pulse.

2.5. Non-plant tissue

2.5.1. ^{14}C activity of fungal hyphae

To determine the transfer of ^{14}C (as a proxy for recently fixed C), to the extramatrical hyphae, hyphal in-growth mesh bags were manufactured with the dimensions $10 \times 5 \times 2$ cm using 40 μm mesh (Normesh Ltd, Lancashire, UK) by melting the edges together using a plastic bag sealer using a modified methodology from Wallander et al. (2001). The pore size in the mesh enabled the fungal hyphae to penetrate the bag but excluded roots of the trees and ground vegetation. The bags were then filled with 120 g of acid-washed horticultural grade sand mixed with 20 g of bone-meal to encourage the hyphal colonisation of the bags. The mesh bags were then placed horizontally into the upper-most 10 cm of soil (Ah Horizon) at specified points in two concentric circles 0.5 m and 1 m from the base of the each of the 6 trees, the experimental design is shown in Figure 1. The mesh bags were placed in the soil 1008 hours (42 days) prior to the pulse label was completed to allow for the penetration and colonisation of the sand medium.

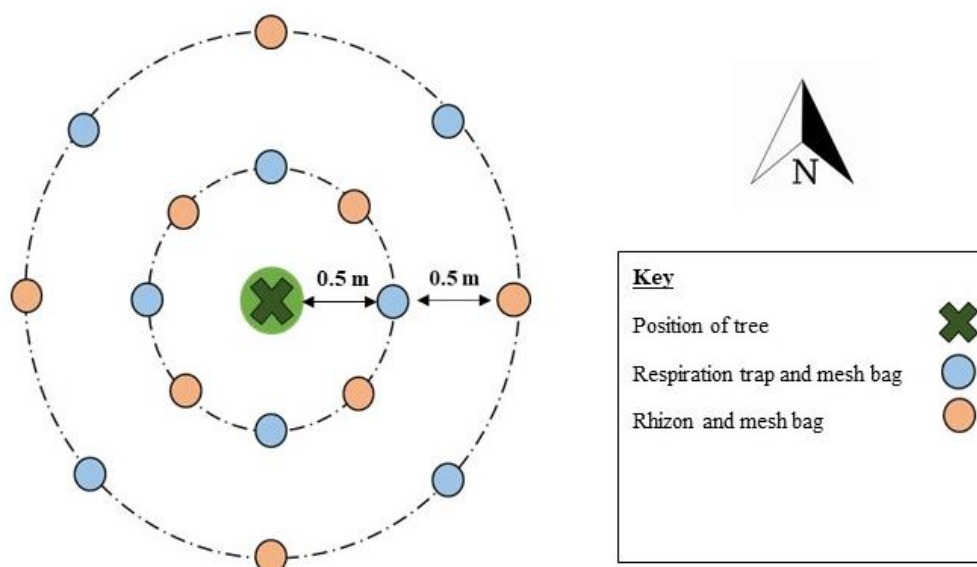


Figure 1: Configuration of hyphal in-growth mesh bags, Rhizons and soil sampling positions relative to each tree, using magnetic North as a datum to locate the first sampling point. Mesh bags were left *in-situ* for a minimum of 42 days to allow for colonisation of mycorrhizal hyphae, then carefully removed at pre-determined time intervals (24, 72, 168, 354 hours after the pulse-label was completed).



Plate 1: *In-situ* and relative positions of the sampling locations in relation to the tree (in centre of plate). Sampling positions for Rhizons, soil sampling, respiration traps and hyphal in-growth mesh bags were protected by plant pots between sampling time intervals (24, 72, 168, 354 hours after the pulse-label was completed).

At the specified time intervals post pulse label, the mesh bags were carefully harvested using a trowel, placed in zip lock bags in a freezer at -20 °C until analysis could commence. The sand was then homogenised prior to a 0.4 g sub-sample being analysed for ^{14}C activity by combusting the organic material in the sample with a Harvey Instruments Biological Oxidiser OX400 (R.J. Harvey Instrument Corp., Hillsdale, NJ, USA). The evolved $^{14}\text{CO}_2$ was bubbled through and dissolved into Oxysolve C-400 (Zinsser Analytic GmbH, Frankfurt, Germany) scintillation fluid prior to determination of ^{14}C content using a Wallac 1409 liquid scintillation counter (Wallac EG and G, Milton Keynes, UK).

2.5.2. Soil respiration

To trace the ^{14}C pulse throughout the plant-soil continuum, the total soil respiration (heterotrophic and autotrophic) was measured as $^{14}\text{CO}_2$ efflux from soil using a 28 mm diameter CO_2 trap containing 1 ml of 3 M NaOH that was suspended above the soil to allow free passage of gases from the soil surface. Each CO_2 trap was collected and replaced at the 0, 24, 72, 168, 354 hours after the initial pulse had been completed.

2.5.3. Soil pore water

^{14}C dissolved in soil solution was sampled following 0, 24, 73, 168, 354 hours after the pulse-label was completed using mini-rhizon suction sampling devices (Rhizosphere Research Products B.V., Wageningen, The Netherlands) (Jones et al., 2016). The H^{14}CO_3 content was determined by liquid scintillation counting, in disintegrations per minute, using a Wallac 1409 scintillation counter (Wallac EG and G, Milton Keynes, UK) with automatic quench correction and Optiphase Hisafe 3[®] (Perkin Elmer, UK) alkali compatible scintillation fluid.

2.5.4. Spatial and temporal dynamics of ^{14}C translocation

Soil respiration and soil pore water was collected at five-time intervals (0, 24, 72, 168, 354 hours) immediately following the ^{14}C pulse-label and hyphal in-growth bags (four time points; 24, 72, 168, and 354), from the sampling positions shown in Figure and Plate 1. All samples were analysed for ^{14}C activity by oxidation and liquid scintillation spectrometry. These sampling positions provided the spatial dimension to the ^{14}C was translocated through the soil.

2.6. Plant biomass

Trees were destructively harvested after 192 hours and separated into four biomass pools (foliage, branch and stem, roots). Additionally, soils were collected from under each replicate tree using a 4 cm diameter soil auger. The foliage, branch and stem material were immediately oven dried at 105 °C until constant mass, and soil oven dried at 105 °C to prevent loss of ^{14}C by microbial respiration. Roots were carefully washed using a sieve stack to remove the adhered soil and stones before being oven-dried at 105 °C until constant mass. The mass of the plant biomass partitions was determined and then each biomass partition was homogenised, subsampled and ground to a fine powder using a MM200 laboratory mixer ball mill (Retsch GmbH, Hann, Germany). A subsample of each ground and homogenised tree partition was combusted using a Harvey Instruments Biological Oxidiser OX400 as described above.

2.7. Ground vegetation sampling

Twenty-four hours prior to the pulse labelling event the vegetation was removed from a circular area, approximately 5 m radius, from the stem of each of the experimental trees. The ground vegetation that had regrown since the pulse was examined and species present identified and recorded. Ground vegetation species which occurred in all the species replicates were then selected from the species present to allow for comparison between treatments. Then, 192 hours (8 days) following the ^{14}C pulse-label, all ground vegetation within the 5 m radius circle was destructively harvested, placed into paper bags and oven-dried at 105 °C until constant mass. The ground vegetation biomass was divided into above and belowground biomass and weighed and analysed for ^{14}C activity independently. The biomass of each of the tree's partitions were weighed and recorded before they were then homogenised, ground to a fine powder using a MM200 laboratory mixer ball mill (Retsch GmbH, Hann, Germany). A subsample of each ground and homogenised biomass sample was combusted as described previously to determine their ^{14}C content.

2.8. Statistical analysis

Prior to analysis all data were tested for homogeneity of variance using Levene's test. The independent variable was the species treatment (*A. glutinosa* and *C. sativa*) and time. The dependant data (total plant biomass and partition biomass, soil chemical properties, ^{14}C activity of biomass pools and ^{14}C labelled respiration efflux) were tested for normality prior to analysis using the Shapiro-Wilk Test. The statistical analysis was conducted using ANOVA (non-

parametric) and Tukey's posthoc test with SPSS® Statistics version 25.0 (IBM Corp, Chicago). Main and interactive effects were considered significant at $P \leq 0.05$.

3. Results

3.1. Soil chemical properties

The biogeochemical properties of the soil under the *A. glutinosa* and *C. sativa* treatments were analysed in order to determine if the soil had been differentially altered by the tree species. The results from the laboratory analysis of the soils are shown in Table 1. Overall, the results show that the species had a very small effect on soil quality. Soils under the actinorhizal tree *A. glutinosa* had slightly elevated concentrations of NO_3^- and NH_4^+ , but these results were not significantly different from the soils under the *C. sativa* trees ($P = 0.60$ and 0.72 for NO_3^- and NH_4^+ respectively). The only soil property which was found to have significantly different results between the two species was electrical conductivity ($P = 0.03$) with the soil under the *C. sativa* treatments being higher than under *A. glutinosa* (167 ± 14 versus $245 \pm 19 \mu\text{S cm}^{-1}$, respectively).

Table 1: Soil chemical properties under *A. glutinosa* or *C. sativa* prior to the ^{14}C pulse being initiated. Data are mean \pm SE ($n = 3$). Significant differences are highlighted in bold and the level of significance is denoted by * $P < 0.05$

Tree species	Soil chemical properties								
	NO_3^- (mg/kg)	NH_4^+ (mg/kg)	Available P (mg/kg)	pH	EC ($\mu\text{S}/\text{cm}$)	C:N ratio	TDN (mg/kg)	TC (mg/kg)	Gravimetric H ₂ O Content (%)
<i>A. glutinosa</i>	3.71 \pm 0.38	3.14 \pm 0.64	1.44 \pm 0.26	5.86 \pm 0.05	167 \pm 14	10.02 \pm 0.67	8.22 \pm 0.91	37.64 \pm 3.60	22.8 \pm 0.1
<i>C. sativa</i>	3.26 \pm 0.48	2.76 \pm 0.39	1.89 \pm 0.21	5.80 \pm 0.03	245 \pm 19	9.87 \pm 0.84	7.43 \pm 0.68	34.81 \pm 3.24	24.5 \pm 0.7
<i>P-value</i>	0.60	0.72	0.30	0.34	0.03*	0.89	0.55	0.81	0.09

EC, electrical conductivity; TDN, total dissolved N; TC, total soluble C.

3.2. Hyphal in-growth mesh bags

Twenty-four hours after the initial ^{14}C pulse label, the ^{14}C activity of hyphae within the in-growth bags was $2.53 \pm 1.21 \text{ Bq g}^{-1}$ and $1.44 \pm 0.10 \text{ Bq g}^{-1}$ for *A. glutinosa* and *C. sativa*, respectively (Figure 2). Throughout the 348-hour experiment, the ^{14}C activity of hyphae under *C. sativa* remained relatively unchanged. In the case of the hyphae of *A. glutinosa*, the values were not different to *C. sativa* until 168 h, while by 336 h the activity of *A. glutinosa* was $14.3 \pm 1.3 \text{ Bq g}^{-1}$, 12-fold more activity than found in *C. sativa* ($1.28 \pm 0.01 \text{ Bq g}^{-1}$). However, the high degree of variability indicated that the difference was not significant ($P = 0.36$).

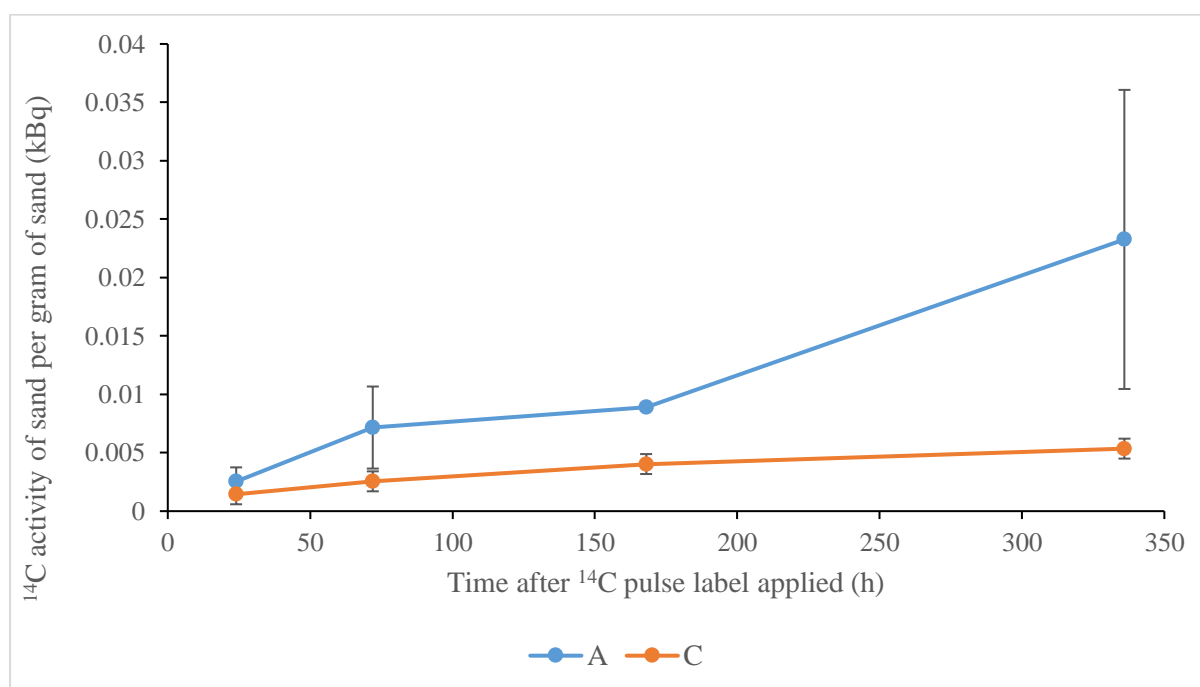


Figure 2- Mean accumulated ^{14}C activity of hyphae collected from hyphal in-growth bags under both species *A. glutinosa* (A) and *C. sativa* (C), during the duration of the experiment. Data is mean ($n = 3$) \pm SE.

The accumulated ^{14}C activity of the sandbags suggests a difference in accumulated ^{14}C activity over the period of the experiment; the differences between species treatments appear to diverge 170 hours after the pulse label was completed. Overall, there were no significant difference between the accumulated ^{14}C activity found in the hyphal in-growth mesh bags under the two species treatments ($P = 0.07$). When the time points were statistically analysed separately, they were not significantly different from each other (with $P = 0.42, 0.37, 0.45$ and 0.37 for samples taken at 24, 72, 168 and 336 hours after the pulse label was completed).

3.3. Soil respiration

The cumulative production of $^{14}\text{CO}_2$ by the belowground community under the two species is shown in Figure 3. Results show that the mean *C. sativa* treatments had consistently more $^{14}\text{CO}_2$ respired immediately post pulse-label event. However, no significant difference between the ^{14}C activity of soil respiration was observed under the two different species treatments ($P = 0.55$).

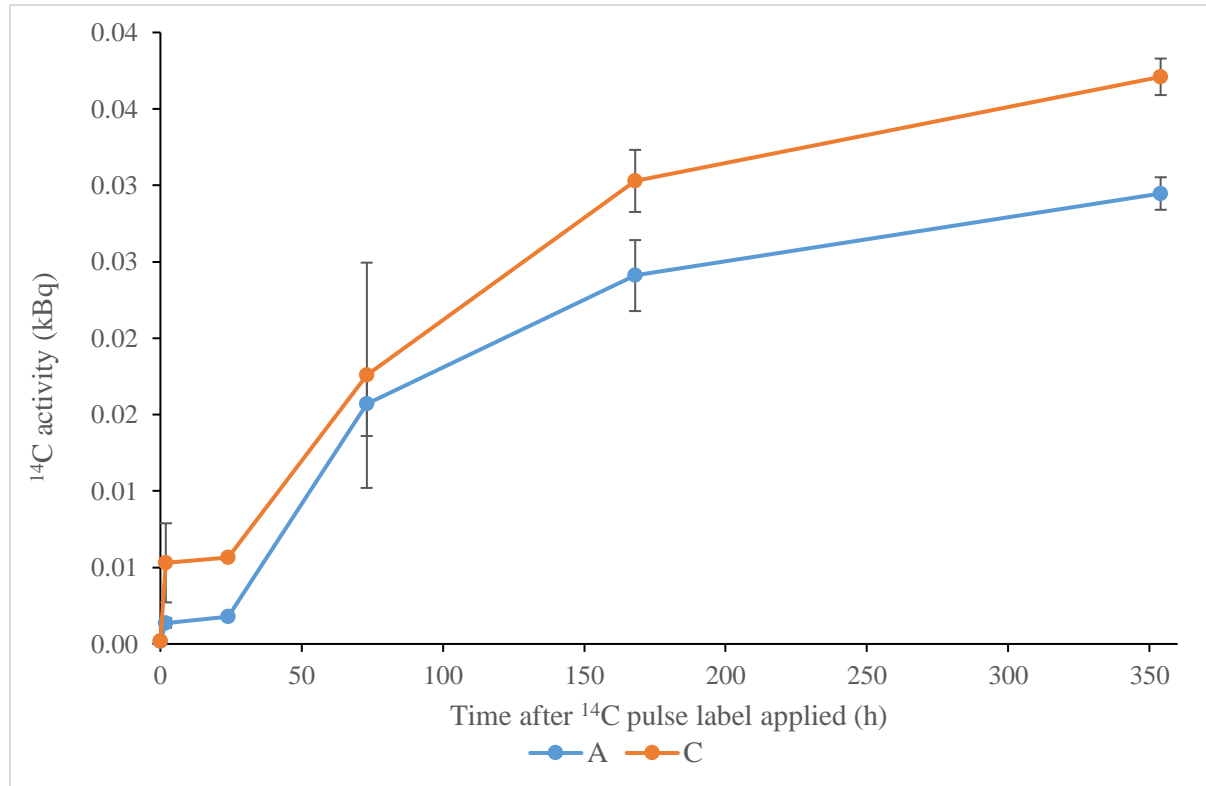


Figure 3- Mean accumulated ^{14}C activity counted in the respiration traps under *A. glutinosa* (A) and *C. sativa* (C) during the duration of the experiment. Data are mean ($n = 3$) \pm SE.

3.4. ^{14}C activity of soil pore water

The ^{14}C activity of the soil pore water was also plotted cumulatively over time (Figure 4) to give an idea of the temporal dynamics of this variable. The results show a similar trend to the soil respiration data plotted in Figure 3, namely that initially the mean ^{14}C activity appears higher in the *A. glutinosa* treatments only to be overtaken by the *C. sativa* treatments approximately 162 hours after the pulse label was completed. Although these trends do suggest slight differences in the speed of transfer or mechanisms underlying transfer to the soil between species, there was no significant difference between the mean ^{14}C activity in the soil solution under the two different tree species [$F_{(1,28)} = 0.75$, $P = 0.79$].

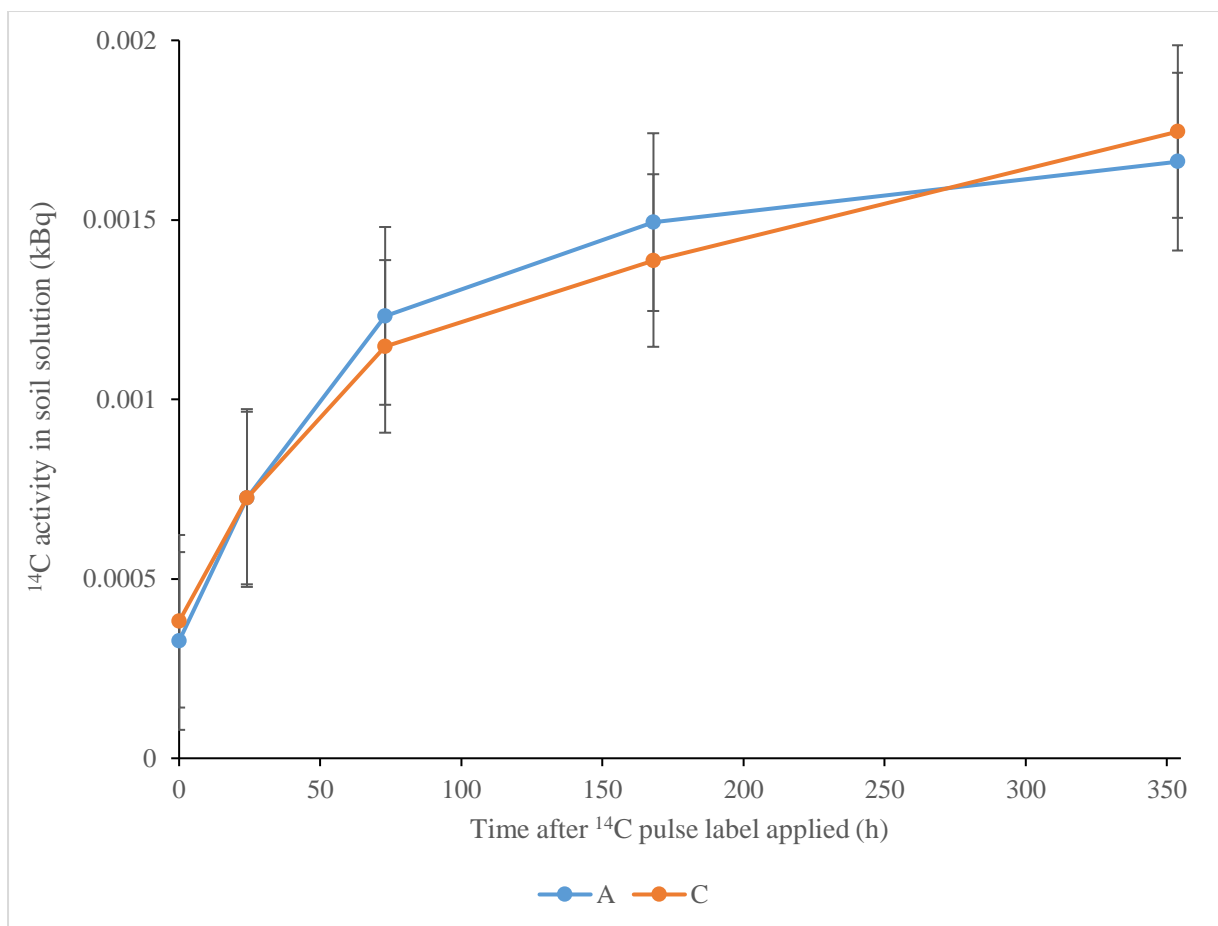


Figure 4- Mean accumulated ^{14}C activity counted in the soil water under *A. glutinosa* (A) and *C. sativa* (C), during the duration of the experiment. Data are mean ($n = 3$) \pm SE.

3.5. Tree partition biomass

Mass of the post-destructively harvested tree partitions are shown in Table 2. Although it appeared that there are differences between the dry weight of the tree partitions between the two species, statistical analysis revealed that the only significant difference between the two species was the coarse root biomass ($P = 0.01$) (Table 2).

Table 2- Mean dry weight biomass (grams) \pm SE ($n = 3$) of each of the tree species and partitions.

Tree species	<i>A. glutinosa</i>	<i>C. sativa</i>	<i>P value</i>
Tree partition			
Leaves	85.0 \pm 19.5	48.2 \pm 16.9	0.49
Stem	57.4 \pm 12.1	37.2 \pm 8.8	0.23
Branches	324.7 \pm 119.6	187.4 \pm 80.3	0.25
Total aboveground biomass	467.1 \pm 144.5	272.8 \pm 104.9	0.39
Coarse roots	112.6 \pm 9.3	37.9 \pm 16.6	0.01*
Fine roots (E)	1367.9 \pm 397.2	1249.2 \pm 184.8	0.48
Total belowground biomass	1480.5 \pm 406.5	1287.1 \pm 201.4	0.53

3.6 ^{14}C activity as a percentage of total ^{14}C activity

Our results show that there are clear differences in ^{14}C allocation between the two species in the field. Aboveground partitions in the *A. glutinosa* treatment (leaf, branch and stem) were larger than the *C. sativa* treatments (Table 3) although the results were not significantly different ($P = 0.63$, 0.35 and 0.11 for leaf, branch and stem, respectively). The total ^{14}C belowground showed the opposite trend with the *C. sativa* treatments having almost twice as much ^{14}C relative to the total activity ($P = 0.02$). This significant difference was driven by the root results which revealed that the relative amount of ^{14}C in *C. sativa* treatment was over twice that of *A. glutinosa* ($P = 0.02$).

Table 3- Mean ^{14}C activity as a percentage of total ^{14}C activity in each tree \pm SE ($n = 3$)

Mean % of total ^{14}C	Leaf	Branch	Stem	Total above- ground	Total root	Soil respiration	Soil solution	Soil	Sand	Total below- ground
<i>A. glutinosa</i>	16.76 \pm 5.00	6.70 \pm 2.92	44.98 \pm 9.69	68.44 \pm 5.86	30.98 \pm 5.85	< 0.01	< 0.01	0.58 \pm 0.33	< 0.01	31.56 \pm 5.86
<i>C. sativa</i>	14.11 \pm 1.09	3.44 \pm 0.99	22.73 \pm 4.75	40.28 \pm 4.28	58.86 \pm 4.8	< 0.01	< 0.01	0.86 \pm 0.54	< 0.01	59.72 \pm 4.28
<i>Probability</i>	0.63	0.35	0.11	0.28	0.02*	0.22	0.29	0.68	0.28	0.02*

The ^{14}C activity when calculated as a percentage of the total ^{14}C activity for each partition, revealed significantly differences in the root activity ($P = 0.02$) and as a result of this significant data the total belowground activity as a percentage of total activity was also significant ($P = 0.02$).

3.6.1. ^{14}C activity above and belowground

A graphical representation of the total above- and below-ground ^{14}C allocation emphasises the differences between the species treatment (Figure 5). The total aboveground ^{14}C activity was not found to be significantly different by treatment ($P = 0.28$), however, the belowground data did show a clear difference ($P = 0.02$).

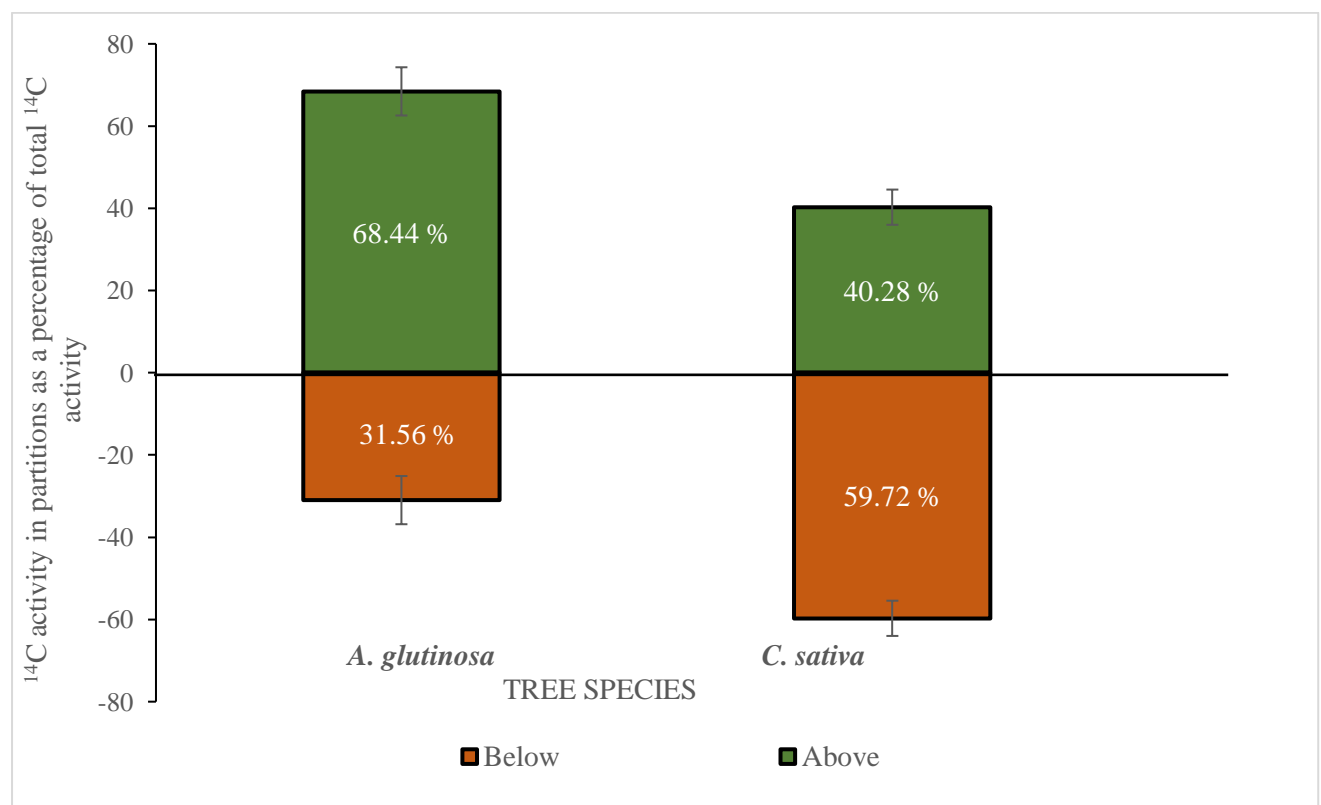


Figure 5- Differences in relative ^{14}C allocation (expressed as a percentage of total ^{14}C activity located in the tree) to both above and belowground tree tissues in the two species treatment (*A. glutinosa* and *C. sativa*). Data is mean ($n = 4$) \pm SE.

3.7.1. ^{14}C activity as kBq per gram

The mean ^{14}C activity as kBq per gram was calculated to normalise allocation of ^{14}C activity to the total size of the species' biomass partitions as shown in Table 4. The ^{14}C activity concentration was found to be roughly the same between the two species in the leaves, branch, stem and coarse root (Table 4). However, the mean fine root data showed that the ^{14}C activity concentration was significant different ($P = 0.01$) and four times higher for *A. glutinosa* than *C. sativa*.

Table 4- ^{14}C activity as kBq per gram for five biomass compartments of *A. glutinosa* and *C. sativa* following a ^{14}C pulse label and destructive harvesting after 14 days (336 hours). Data shown are means \pm SE ($n = 3$). Statistically significant differences are denoted by * $P < 0.05$.

Species	Leaves	Branch	Stem	Coarse root	Fine root
<i>A. glutinosa</i>	22.98 \pm 7.50	18.64 \pm 12.90	12.84 \pm 8.11	2.64 \pm 0.98	47.01 \pm 15.93
<i>C. sativa</i>	28.73 \pm 10.16	12.90 \pm 5.51	8.11 \pm 3.44	3.74 \pm 0.67	11.19 \pm 0.56
<i>P-value</i>	0.67	0.53	0.46	0.41	0.01*

3.7.2. Above and belowground allocation

The mean ^{14}C activity allocated aboveground (as kBq per dried weight gram) was 54.5 ± 9.5 kBq/g for *A. glutinosa* and 49.7 ± 6.4 kBq/g for *C. sativa*, whereas the belowground ^{14}C activity (as kBq per dried weight gram) was 49.7 ± 8.5 kBq/g for *A. glutinosa* and 14.9 ± 0.6 kBq/g for *C. sativa*. Although aboveground allocation was similar in both species' treatments, the belowground allocation was three times the ^{14}C activity per gram of biomass in *A. glutinosa* than *C. sativa* (Figure 6). Statistical analysis revealed that the above and belowground ^{14}C allocation was significantly different between the two tree species ($P = 0.02$).

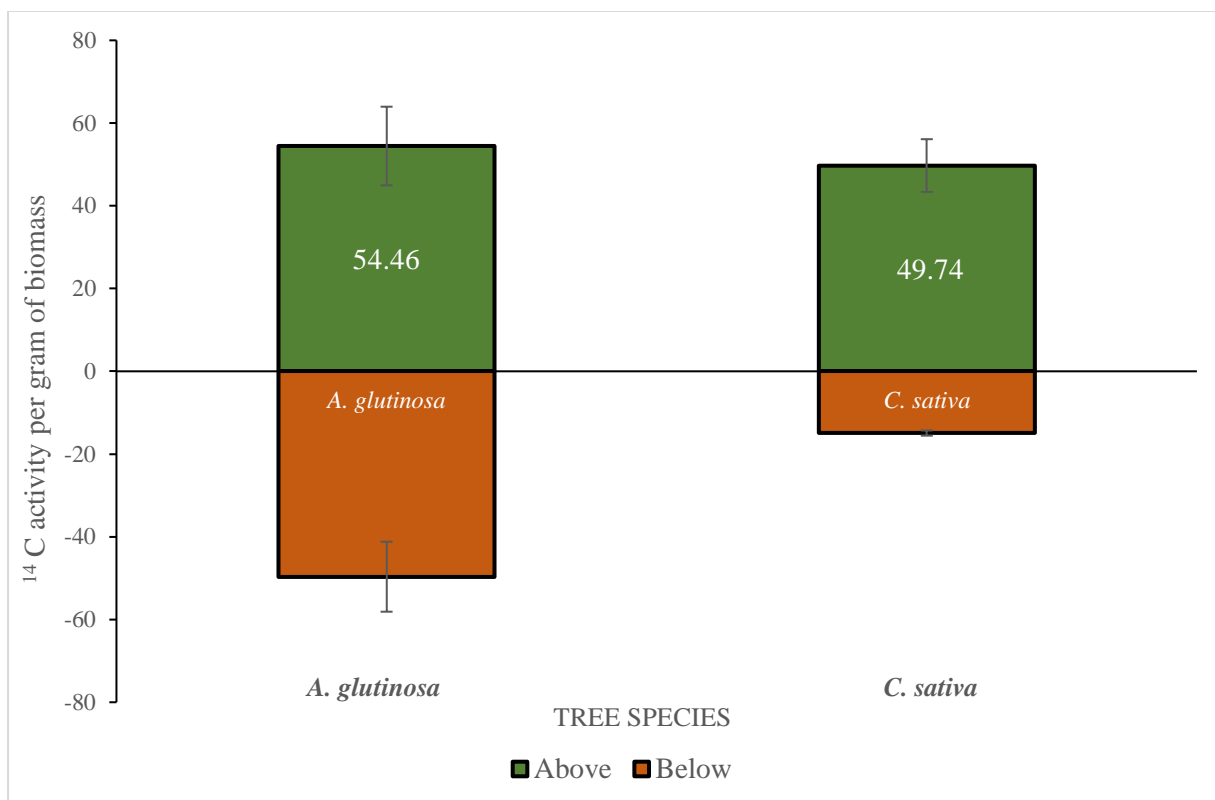


Figure 6: Comparison of the above and belowground allocation of ^{14}C activity expressed as kBq per gram of dried biomass weight (grams) in the two species studied. Data are mean ($n=3$) \pm SE.

3.8. Ground vegetation

The ^{14}C activity which was detected in the ground vegetation was also divided into above and belowground components (Figure 7). Surprisingly, no obvious differences or trends in ^{14}C content were observed in the weed species growing around the trees ($P = 0.55$).

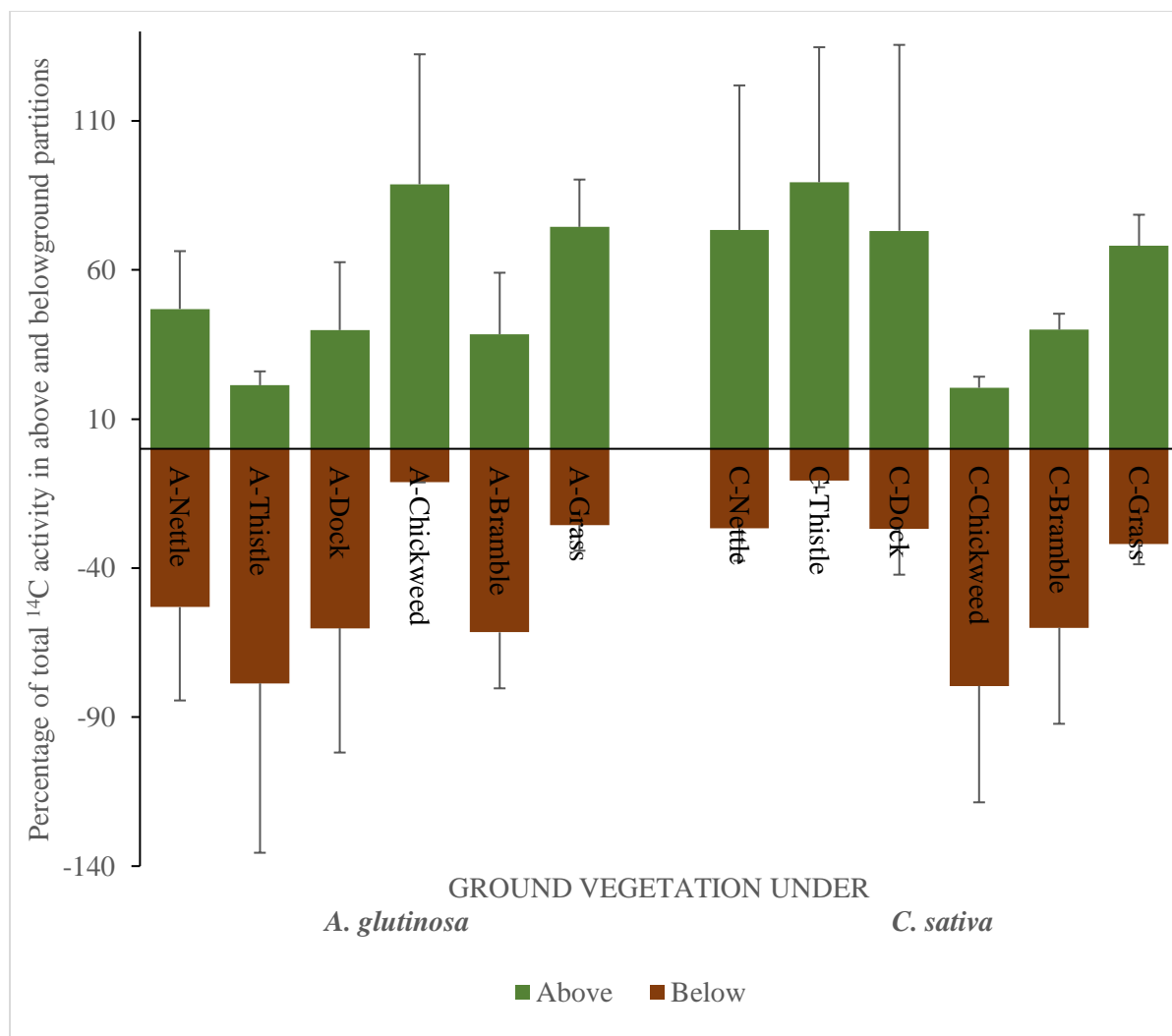


Figure 7- Percentage ^{14}C activity in the above and belowground biomass partitions of the ground vegetation located under the trees of the two species treatments. Data are mean ($n = 4$) \pm SE.

To establish if there were differences in above and belowground ^{14}C allocation to the understorey weed species between the two species treatments, we decided to compare the species treatments as an above/ belowground activity ^{14}C % allocation ratio (Table 5). Although the species treatment appeared to have different above-belowground allocation trends (see Figure 9), the statistical analysis revealed no significant differences in ^{14}C allocation. The P values of the statistical analysis are also tabulated in Table 5.

Table 5- The above and belowground allocation as a percentage of total ^{14}C in the understorey plant species represented as a ratio (aboveground/ belowground). Data are mean ($n= 3$) \pm SE.

Weed species	Aboveground-to-belowground ^{14}C activity ratio		<i>P</i> -value
	<i>A. glutinosa</i>	<i>C. sativa</i>	
<i>Urtica dioica</i> (Stinging nettle)	2.47 \pm 0.47	2.64 \pm 1.08	0.90
<i>Cirsium vulgare</i> (common thistle)	1.98 \pm 1.05	11.25 \pm 7.64	0.27
<i>Rumex obtusifolius</i> (broadleaved dock)	1.09 \pm 0.54	1.95 \pm 0.92	0.29
<i>Cerastium fontanum</i> (mouseear chickweed)	1.81 \pm 1.04	0.74 \pm 0.54	0.87
<i>Rubus fruticosus</i> (bramble)	15.12 \pm 11.94	1.07 \pm 0.46	0.41
<i>Holcus lanatus</i> (grass spp.)	3.54 \pm 1.95	3.26 \pm 1.35	0.58

4. Discussion

H₁ Transfer of C to ground vegetation will be greatest via the CMN of *A. glutinosa*, that has a symbiosis with both ecto- and arbuscular mycorrhizae, rather than *C. sativa* an obligate ectomycorrhizal species.

Although it appeared that there were differences in below- and above-ground C allocation to the weeds under the canopy of the two species treatments, statistical analysis revealed that there were no significant difference in either the total, above- or below-ground allocation to the ground vegetation. The hypothesis was therefore rejected.

The key results from this study include the results of the allocation of C (as activity per gram) for the two species. These data showed that the belowground allocation i.e. the allocation of C from the source (leaves) to the sink (roots) was higher in *C. sativa* than *A. glutinosa*. We expected that the belowground allocation in *A. glutinosa* would be greater than that of *C. sativa* as a result of the additional energetic demands of the dual mycorrhizal symbiosis (both arbuscular and ectomycorrhizal) and the bacterial symbiosis with *Frankia alni*. Many previous studies have suggested a positive relationship between the mycorrhizal/ bacterial symbiotic diversity and belowground allocation (Hobbie, 2006; Rygielwicz and Andersen, 1994;

Robinson, 2004). This suggests that the belowground C allocation in the *A. glutinosa* treatments probably further allocates the C to the fungal and bacterial partners (Schiestl-Aalto et al., 2019), whereas in *C. sativa* this C remains in the root biomass, predominantly the fine roots.

This hypothesis is supported by the allocation to hyphae in *A. glutinosa* treatments which shows that C transfer to hyphae in *A. glutinosa* is faster and more prolonged than *C. sativa*. This could therefore go some way to explaining the lack of C in the roots of *A. glutinosa* when compared to *C. sativa*. Interestingly, however, if there was increased C transfer to the hyphae in the case of *A. glutinosa* then it appears that the C must have been utilised by the fungi for hyphal growth as there were no visible differences in the ^{14}C activity recovered in soil respiration. The soil respiration data did not distinguish the heterotrophic (fungal) from the autotrophic (plant) respiration, which may have skewed the data. We would also expect that total belowground respiration would be proportionally more in the *A. glutinosa* treatments. Contrastingly, we would expect a greater proportion of the total; belowground respiration to be autotrophic (from the tree roots). Another interesting result is that you can see the huge differences in biomass allocation in the ground flora. The differences in the results will now be discussed in more detail.

4.1. ^{14}C activity in hyphal in-growth mesh bags

Biological oxidation and subsequent liquid scintillation spectrometry suggested that there was more ^{14}C activity transferred to the soil and hyphae under the *A. glutinosa* trees, however, due to large differences between replicates this trend was not found to be significant. The end of the summer/ beginning of autumn was chosen as the optimal timing period for the experiment to be undertaken as a result of the previous studies that noted as much mycorrhizal colonisation of in-growth mesh bags occurred at the end of the temperate tree growing season (July-September) as for the whole growing season from March (Wallander et al., 2001). In the same experiment Wallander et al. (2001) also noted that mesh bags placed in the ground during the temperate non-growing winter period (December-March) showed no visible signs of mycorrhizal colonisation. This is more obvious as temperate senesced trees are not allocating C belowground during periods of low photosynthetic activity (Royer et al., 2003). The autumn as the most significant period for mycorrhizal growth is supported by the presence at the time of fruiting bodies and the soils chitin: ergosterol ratio (Wallander et al., 1997). It has been

hypothesised that maximal mycorrhizal growth is concurrent with maximal fine root production (Stober et al., 2000).

Some previous studies have shed doubt on the efficacy of the in-growth mesh bag methodology (Wallander et al., 2001) in accurately estimating the biomass and ^{14}C content of the mycorrhizal fungi. Firstly, it is not known how much the mesh bag restricts the normal growth of mycorrhizal hyphae and this would be very challenging to quantify accurately (Wallander et al., 2001). Previous studies have proven that the presence of sand as a substrate in in-growth mesh bags is not a significant impediment to fungal colonisation of mesh bags as tested by the presence of similar levels of ergosterol both inside and outside the mesh bag (Ekblad et al., 1999; Ekblad, 1997). Secondly, we do not know the effect of using sand rather than soil as a substrate and if this discourages or encourages hyphal in-growth. Thirdly, the addition of phosphorus into the sand mix used in the mesh bags may have encouraged the penetration through the mesh of saprophytic fungi. If saprophytic fungi had in fact grown into the bag then ^{14}C measured and assumed to have originated for mycorrhizal fungi could have been from decomposing organic matter, bacterial microbial biomass that fed on ^{14}C enriched root exudates and then decomposed by fungi. Although the sampling technique used in this experiment was consistent and considered a standard sampling technique, it is possible that a portion of the ecto- or arbuscular-mycorrhizal community remained un-sampled and this has been previously reported as a potentially confounding factor (Taylor, 2002; Pickles and Pither, 2014).

4.2. Tree biomass data

The biomass of the tree organs showed only significant differences in the coarse root data between the two species treatments. Sampling techniques including the soil cores which 7 cm in diameter and taken within 30 cm of the soil surface and in-growth mesh bags which were located a maximum of 10 cm, concentrate on the mycorrhizal activity in the forest soil surface. The mean root weight for each tree species per soil core was calculated and then multiplied by the estimated diameter spread of the tree roots. This then gave a total tree root estimation that could be used with the other biomass calculation to investigate the percentages of ^{14}C activity in each of the tree partitions. However, it is known that many mycorrhizal species are active deeper than was sampled (Jenkins et al., 1988; Dalpe et al., 2000; Bornyasc et al., 2005). The specific soil conditions of a field trial are known to alter the root biomass, architecture, spatial heterogeneity and rooting depth of trees (Schenk, 2005; Dickie et al.,

2013). It has been found that even within individuals of the same species rooting depths can vary hugely (Stone and Kalisz, 1991; Canadell et al., 1996). The root core samples, due the experimental timing, were unfortunately taken during the autumn (the end of the growing season), which has been reported to be an inaccurate time to conduct this type of analysis (Anderson and Ingram, 1994; Böhm, 2012). The soils at Henfaes experimental farm are notoriously stony as a result of being derived from glacial till (deposited ca. 10,000 years ago) (Marston et al., 2017). Methods for fine root sampling, storing and washing, however carefully undertaken, will inevitably result in some loss of biomass. It was decided that in this field experiment this had likely happened and as a result a loss correction factor should be applied to the final belowground biomass data. The literature suggests a correction factor of between 1.25-2 depending on estimated losses due to the sampling and processing (Bohm, 1979; Anderson and Ingram, 1994). It was decided in this instance a loss correction factor of 1.5 was most appropriate, based on similar experiments and this was added to the fine root data after the soil cores calculations were completed.

Calculations of the percentage of ^{14}C which at the time of destructive harvesting reside in the various plant structures and below-ground C pools require reliable estimations of the biomass of each compartment. All the aboveground biomass was successfully removed, and by loosening the soil with a spade, some of the coarse roots were extracted from the soil matrix. However, much of the fine roots were not sampled due to the fact that the studied trees had been previously coppiced (cut at just above ground level) on several occasions it was decided that the allometric relationship between above and belowground biomass, or traditional root: shoot or above: belowground biomass relationships found in the literature would be unreliable methods to determine belowground biomass in this instance. It has been reported by some studies that soil cores can underestimate coarse root biomass (Jackson et al., 2009, Major et al., 2012), due to rarely encountering large coarse roots (Taylor et al., 2013) and as a result of limited soil volume being sampled (Levillain et al., 2011). Others, however, report no difference between this methodology and other ways of estimating such as excavated soil pits (Rau et al., 2009, Smith et al., 2013). On balance, due to its simplicity and relative ease of the calculations, the soil core methodology was chosen (Rau et al., 2009). It is hoped the methodology increased the root biomass data obtained from the root cores by reducing the distance between the tree and the sampling point using a previously described method (Sochacki et al., 2017).

4.3. ^{14}C activity above and belowground

The above and belowground allocation data (as a percentage of total ^{14}C activity) emphasises the differences in relative allocation between the two species in the field. Total aboveground ^{14}C activity was larger in the *A. glutinosa* treatments although this was not found to be significant. The total belowground data was found to be significant, with almost half the ^{14}C activity found in the *A. glutinosa* treatments than the *C. sativa* treatments. This is roughly in line with other studies. For example, Gertrudix et al. (2012) found that *C. sativa* had almost twice the C stored belowground in roots than *A. glutinosa*. This could be due to the *A. glutinosa* tripartite symbiotic partners being allocated a large percentage of the belowground C which has been experimentally demonstrated in other species (Kafle et al., 2019; Ossler et al., 2015; Wang et al., 2016).

4.4. ^{14}C activity as kBq per gram

A. glutinosa fine roots had four times as much ^{14}C activity per gram of biomass than the *C. sativa* fine roots. This could be as a result of the soil core methodology which assumes that all fine tree roots located under the canopy of trees originated from that species. As the results were mean data ($n=4$) we can assume that this is unlikely to have made that much difference and therefore that either the belowground allocation is greater in *A. glutinosa* or the fact that the *A. glutinosa* treatments have less fine root biomass and therefore the relative concentration is higher. After revisiting the fine root biomass estimations, it is clear that the former is not the case, as mean fine root biomass was similar between *A. glutinosa* and *C. sativa*. Therefore, this suggests that belowground allocation of recently fixed C is more in *A. glutinosa* or that the allocation is either quicker or slower and that as we only had a single destructive harvesting event, we are unable to rule-out temporal variations between species studied.

4.5. Transfer to ground vegetation

This was perhaps the most surprising result of this experiment, we were expecting the ground vegetation to show more ^{14}C enrichment under the *A. glutinosa* than under the *C. sativa* as a result of *A. glutinosa* having both arbuscular and ecto-mycorrhizal associations and the ground vegetation being thought to be predominantly arbuscular. The ^{14}C activity was found to be quite similar and with no significant differences between species treatments. Recent studies suggest that trees not only interact with distinct mycorrhizal associations, such as arbuscular and ecto-mycorrhizal symbionts, but also a profusion of diverse and disparate

fungus species located in both the rhizosphere and endosphere (Mandyam and Jumpponen, 2005; Newsham, 2011). These findings question the previous assumption that arbuscular and ectomycorrhizae form distinct fungal networks between which trading is not possible (Toju, Sato, 2018) and that the two groups are sufficiently taxonomically and functionally different to make hyphal fusion unlikely (He et al., 2006). Toju and Sato postulate that the generalist endophytic fungus from the ascomycete order Helotiales, which have known associations with both arbuscular and ecto mycorrhizal plants may have a role in mediating feedbacks between the different mycorrhizal types.

The C transfer from the pulsed trees donor roots demonstrated by these results, could be explained by the following possible processes: (1) root grafting. A root graft is when two or more trees, normally of the same or phylogenetically similar species (Graham, 1966) fuse together, allowing the transfer of water and carbohydrates (Fraser et al., 2006), this has been demonstrated experimentally with both dyes (Graham, 1960), and radio-isotopes (Fraser et al., 2006; Woods and Brock, 1964). It is possible that *A. glutinosa* and *C. sativa* form root grafts as both are in the order *Fagales*, *A. glutinosa* is in the family *Betulaceae* whereas *C. sativa* is part of the family *Fagaceae*, but they are relatively close phylogenetically. (2) The movement of C from mycorrhizal mycelia to soil (Johnson et al., 2002); (3) the rapid turnover of mycorrhizal hyphae within the soil (Staddon et al., 2003); (4) the effect of soil fauna (Klironomos and Hart, 2001; Perez-Moreno and Read, 2001; Johnson et al., 2005); (5) root exudation (Jones et al., 2004); and (6) recapture of C of rhizodeposits from either the roots of hyphae (Rangel-Castro et al., 2002; Sun et al., 1999, Toljander et al., 2007; Walker et al., 2003) processes which are mediated by both plants and mycorrhizas (Jones et al., 2004). Unfortunately, our experimental design did not allow us to determine which of these mechanisms was responsible for the transfer of C, recently fixed by the tree, to the ground vegetation.

Another possible explanation of the lack of difference in transfer to the ground vegetation from the two tree species is that the soil was recently afforested land that was previously managed as an agricultural field, which may have influenced the availability of AM fungi propagules. Arbuscular mycorrhizal propagules although fast to accumulate in vegetated soils (Gould et al., 1996), can become sparse under intensive agricultural management (Manharon et al., 2017) and as a result of fragmentation by agriculture, and lack of continuity of land-use, some temperate forest ecosystems are known to be limited by lack of fungal reproductive material (Veresoglou et al., 2017) and dispersal limitation (Zobel and Öpik, 2014).

The co-existence of multiple layers of vegetation are a unique feature of forest ecosystems and the vegetative strata are almost certainly interacting in ways not yet explicit (Sutherland et al., 2013). The interactions between vertical layers of temperate forests are postulated to be asymmetrical because although the canopy level directly influences the understorey by competition for light interception (Barbier et al., 2008). It is believed unlikely that the ground vegetation can directly alter the health of the canopy or its ability to reproduce (Grünfeld et al., 2019). It is thought that canopy trees may facilitate the formation of diverse understoreys (Veresoglou et al., 2017). The woody perennial over-storey may also act as propagules for the establishment of CMN with the AM ground vegetation species (Van der Heijden et al., 2004), although experimental data to confirm this is lacking (Grünfeld et al., 2019). Radić et al. (2012) found that in the case of a grape (*Vitis vinifera* L.) vineyard that the presence of AM weed species in the understorey significantly altered the fungal community with more diversity and different fungal species dominating. The more frequent and diverse herbaceous plants may be contributing by featuring unique and fundamentally useful functional traits and subsequently could be ecologically disproportionately important (Reiss et al., 2009) and may yet be found to interact directly with the canopy species (Gilliam, 2007). It is believed that in some cases the higher quality of understorey litter could be priming essential cycling processes in the soil, such as decomposition, N-mineralisation and nitrification (Van der Krift and Berendse, 1997; Vereoglou et al., 2011; Luo et al., 2016). The interactions between vertical layers in temperate forest ecosystems are highly complex, but it is reasonable to suggest that dual mycorrhizal trees such as *A. glutinosa* increase AMF propagules in the soil and therefore assist with the diversity, health and growth of the understorey plant species (Azcón-Aguilar and Barea, 1997; Barbier et al., 2008; Van der Heijden et al., 1998).

4.6. Spatial and temporal dynamics

The temporal and spatial dynamics appeared as measured by soil respiration traps, soil pore water, and hyphal in-growth mesh bags seemed to suggest that the plant: microbe symbiosis was pulsing carbohydrates to specific regions of soil at different times. Others studies have found similar results using a range of methodologies including high-efficiency Geiger-Müller tubes (Brownlee and Jennings, 1982), *in vivo* imaging of radioisotopes using a β -scanner (Timonen et al., 1997; Olsson and Gray, 1998), and an autoradiography system to image phosphorus transfer (Lindahl et al., 1999; Leake et al., 2001). Nutrient translocation through rhizomorphs and hyphae is known to occur at rates faster than diffusion, so some form of pumping mechanism is assumed, although not yet fully understood (Tlalka et al., 2002). It

has been suggested that the bi-directional flow of nutrients takes place across the symbiotic interfaces surrounded by the plant and fungal membranes passively whereby it is actively transported into the receiver organism (Smith and Read, 2010). The transport proteins involved in this process have not yet been isolated, however, and more research in this field is needed (Ferrol et al., 2002).

5. Conclusions

We used ^{14}C as a proxy for photo-assimilated C allocation to different parts of the trees, their symbiotic partners and the ground vegetation that was under the canopy of the two-tree species studied. The results suggest that trees possessing dual mycorrhizal symbiotic relationships (i.e., ecto- and arbuscular mycorrhizae), or tripartite symbiosis that includes N-fixing bacteria are ecologically important for the cycling and storage of C in temperate forest ecosystems. However, to further understand the C allocation dynamics of tripartite symbiosis and in particular to identify the specific mechanisms which mediate C allocation to the nitrogen-fixing bacteria arbuscular and ectomycorrhizal symbionts, further analyses would be required. Combining N-fixing species with other temperate forest species is not often considered when selecting temperate forest species in commercial and non-commercial afforestation practice. As a result, many of the potential benefits to the diversity and functionality of temperate forest ecosystems and agroforestry systems are not being exploited. Furthermore, the bilateral resource sharing between trees and the ground vegetation under their canopy has not been considered both in terms of the effect it could have of C storage and priming of leaf litter and the implications for the population and competition dynamics of temperate forest ecosystems.

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7. References

- Adjoud-Sadadou, D. and Halli-Hargas, R., 2017. Dual mycorrhizal symbiosis: an asset for eucalypts out of Australia? *Canadian Journal of Forest Research*, 47(4), pp.500-505.
- Ahmed, I.U., Smith, A.R., Jones, D.L. and Godbold, D.L., 2016. Tree species identity influences the vertical distribution of labile and recalcitrant carbon in a temperate deciduous forest soil. *Forest Ecology and Management*, 359, pp.352-360.
- Allen, M.F., O'Neill, M.R., Crisafulli, C.M. and MacMahon, J.A. (2018). Succession and Mycorrhiza on Mount St. Helens. In: Crisafulli C., Dale V. (eds) *Ecological Responses at Mount St. Helens: Revisited 35 years after the 1980 Eruption*. Springer, New York, NY. pp 199-215.
- Anderson, J.M. and Ingram, J.S.I., 1994. Tropical soil biology and fertility: a handbook of methods. *Soil Science*, 157(4), p.265.
- Azcón-Aguilar, C. and Barea, J.M., 1997. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens—an overview of the mechanisms involved. *Mycorrhiza*, 6(6), pp.457-464.
- Barbier, S., Gosselin, F. and Balandier, P., 2008. Influence of tree species on understory vegetation diversity and mechanisms involved—a critical review for temperate and boreal forests. *Forest Ecology and Management*, 254(1), pp.1-15.
- Bellei, M., Garbaye, J. and Gil, M., 1992. Mycorrhizal succession in young Eucalyptus viminalis plantations in Santa Catarina (southern Brazil). *Forest Ecology and Management*, 54(1-4), pp.205-213.
- Bennett, J.A., Maherali, H., Reinhart, K.O., Lekberg, Y., Hart, M.M. and Klironomos, J., 2017. Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science*, 355(6321), pp.181-184.
- Berendsen, R.L., Pieterse, C.M. and Bakker, P.A., 2012. The rhizosphere microbiome and plant health. *Trends in Plant Science*, 17(8), pp.478-486.
- Bever, J.D., 2015. Preferential allocation, physio-evolutionary feedbacks, and the stability and environmental patterns of mutualism between plants and their root symbionts. *New Phytologist*, 205(4), pp.1503-1514.

- Bhandari, P. and Garg, N., 2017. Dynamics of arbuscular mycorrhizal symbiosis and its role in nutrient acquisition: an overview. In: *Mycorrhiza-nutrient uptake, biocontrol, ecorestoration*. Springer, Cham, pp 21–43.
- Billings, S.A., 2015. ‘One physical system’: Tansley's ecosystem as Earth's critical zone. *New Phytologist*, 206(3), pp.900-912.
- Böhm, W., 2012. *Methods of studying root systems* (Vol. 33). Springer Science and Business Media, Dordrecht.
- Bornyas, M.A., Graham, R.C. and Allen, M.F., 2005. Ectomycorrhizae in a soil-weathered granitic bedrock regolith: linking matrix resources to plants. *Geoderma*, 126(1-2), pp.141-160.
- Brownlee, C. and Jennings, D.H., 1982. Long distance translocation in *Serpula lacrimans*: velocity estimates and the continuous monitoring of induced perturbations. *Transactions of the British Mycological Society*, 79(1), pp.143-148.
- Brundrett, M., Bougher, N., Dell, B., Grove, T. and Malajczuk, N., 1996. *Working with mycorrhizas in forestry and agriculture* (No. 435-2016-33680). Canberra: Australian Centre for International Agricultural Research.
- Brundrett, M. and McGonigle, T., 1994. Estimation of root length and colonization by mycorrhizal fungi. In *Practical Methods in Mycorrhiza Research*. pp 51-59. Mycologue Publications, Guelph, Ontario.
- Brundrett, M., Murase, G. and Kendrick, B., 1990. Comparative anatomy of roots and mycorrhizae of common Ontario trees. *Canadian Journal of Botany*, 68(3), pp.551-578.
- Brundrett, M.C. and Tedersoo, L., 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist*, 220(4), pp.1108-1115.
- Bryla, D.R. and Eissenstat, D.M., 2005. Respiratory costs of mycorrhizal associations. In *plant respiration* (pp. 207-224). Springer, Dordrecht.
- Canadell, J., Jackson, R.B., Ehleringer, J.B., Mooney, H.A., Sala, O.E. and Schulze, E.D., 1996. Maximum rooting depth of vegetation types at the global scale. *Oecologia*, 108(4), pp.583-595.

- Chen, Y.L., Brundrett, M.C. and Dell, B., 2000. Effects of ectomycorrhizas and vesicular–arbuscular mycorrhizas, alone or in competition, on root colonization and growth of *Eucalyptus globulus* and *E. urophylla*. *The New Phytologist*, 146(3), pp.545-555.
- Chilvers, G.A., Lapeyrie, F.F. and Horan, D.P., 1987. Ectomycorrhizal vs endomycorrhizal fungi within the same root system. *New Phytologist*, 107(2), pp.441-448.
- Chilvers GA, Gust LW. 1982. Comparison between the growth rates of mycorrhizas, uninfected roots and a mycorrhizal fungus of *Eucalyptus st-johnii* R. T. Bak. *New Phytologist*, 91, pp.453 – 466.
- Chilvers, G.A. and Gust, L.W., 1982. Comparisons between the growth rates of mycorrhizas, uninfected roots and a mycorrhizal fungus of eucalyptus ST -JO HNII RT BAK. *New Phytologist*, 91(3), pp.453-466.
- Dalpé, Y., Diop, T.A., Plenchette, C. and Gueye, M., 2000. Glomales species associated with surface and deep rhizosphere of *Faidherbia albida* in Senegal. *Mycorrhiza*, 10(3), pp.125-129.
- Dickie, I.A., Martínez-García, L.B., Koele, N., Grelet, G.A., Tylianakis, J.M., Peltzer, D.A. and Richardson, S.J., 2013. Mycorrhizas and mycorrhizal fungal communities throughout ecosystem development. *Plant and Soil*, 367(1-2), pp.11-39.
- Dominik, T., 1961. *Mycotrophy of poplars in their natural associations in Poland*. Centralny Instytut Informacji Naukowo-Technicznej i Ekonomicznej.
- Epron, D., Bahn, M., Derrien, D., Lattanzi, F.A., Pumpanen, J., Gessler, A., Högberg, P., Maillard, P., Dannoura, M., Gérant, D. and Buchmann, N., 2012. Pulse-labelling trees to study carbon allocation dynamics: a review of methods, current knowledge and future prospects. *Tree Physiology*, 32(6), pp.776-798.
- Ek, H., 1997. The influence of nitrogen fertilization on the carbon economy of *Paxillus involutus* in ectomycorrhizal association with *Betula pendula*. *New Phytologist*, 135(1), pp.133-142.
- Ekblad, A.L.F., Wallander, H., Carlsson, R. and Huss-Danell, K., 1995. Fungal biomass in roots and extramatrical mycelium in relation to macronutrients and plant biomass of ectomycorrhizal *Pinus sylvestris* and *Alnus incana*. *New Phytologist*, 131(4), pp.443-451.

- Ferrol, N., Barea, J.M. and Azcon-Aguilar, C., 2002. Mechanisms of nutrient transport across interfaces in arbuscular mycorrhizas. In *Diversity and Integration in Mycorrhizas*, pp. 231-237. Springer, Dordrecht.
- Francis, R. and Read, D.J., 1994. The contributions of mycorrhizal fungi to the determination of plant community structure. *Plant and Soil*, 159(1), pp.11-25.
- Fraser, E.C., Lieffers, V.J. and Landhäusser, S.M., 2006. Carbohydrate transfer through root grafts to support shaded trees. *Tree Physiology*, 26(8), pp.1019-1023.
- Gardner, J.H. and Malajczuk, N., 1988. Recolonisation of rehabilitated bauxite mine sites in Western Australia by mycorrhizal fungi. *Forest Ecology and Management*, 24(1), pp.27-42.
- Graham, B.F. and Bormann, F.H., 1966. Natural root grafts. *The Botanical Review*, 32(3), pp.255-292.
- Graham, B.F., 1960. Transfer of dye through natural root grafts of *Pinus strobus* L. *Ecology*, 41(1), pp.56-64.
- Gertrudix, R.R.P., Montero, G. and Del Rio, M., 2012. Biomass models to estimate carbon stocks for hardwood tree species. *Forest Systems*, 21(1), pp.42-52.
- Gilliam, F.S., 2007. The ecological significance of the herbaceous layer in temperate forest ecosystems. *BioScience*, 57(10), pp.845-858.
- Glanville, H.C., Hill, P.W., Schnepf, A., Oburger, E. and Jones, D.L., 2016. Combined use of empirical data and mathematical modelling to better estimate the microbial turnover of isotopically labelled carbon substrates in soil. *Soil Biology and Biochemistry*, 94, pp.154-168.
- Glanville, H., Rousk, J., Golyshin, P. and Jones, D.L., 2012. Mineralization of low molecular weight carbon substrates in soil solution under laboratory and field conditions. *Soil Biology and Biochemistry*, 48, pp.88-95.
- Gosling, P., Hodge, A., Goodlass, G. and Bending, G.D., 2006. Arbuscular mycorrhizal fungi and organic farming. *Agriculture, Ecosystems and Environment*, 113(1-4), pp.17-35.
- Gould, A.B., Hendrix, J.W. and Ferriss, R.S., 1996. Relationship of mycorrhizal activity to time following reclamation of surface mine land in western Kentucky. I. Propagule and spore population densities. *Canadian Journal of Botany*, 74(2), pp.247-261.

- Grimoldi, A.A., Kavanová, M., Lattanzi, F.A., Schäufele, R. and Schnyder, H., 2006. Arbuscular mycorrhizal colonization on carbon economy in perennial ryegrass: quantification by $^{13}\text{CO}_2/^{12}\text{CO}_2$ steady-state labelling and gas exchange. *New Phytologist*, 172(3), pp.544-553.
- Grünfeld, L., Wulf, M., Rillig, M.C., Manntschke, A. and Veresoglou, S.D., 2019. Neighbours of arbuscular-mycorrhiza associating trees are colonized more extensively by arbuscular mycorrhizal fungi than their conspecifics in ectomycorrhiza dominated stands. *New Phytologist*. (in press).
- Gunina, A., Smith, A.R., Godbold, D.L., Jones, D.L. and Kuzyakov, Y., 2017. Response of soil microbial community to afforestation with pure and mixed species. *Plant and Soil*, 412(1-2), pp.357-368.
- He, X., Bledsoe, C.S., Zasoski, R.J., Southworth, D. and Horwath, W.R., 2006. Rapid nitrogen transfer from ectomycorrhizal pines to adjacent ectomycorrhizal and arbuscular mycorrhizal plants in a California oak woodland. *New Phytologist*, 170(1), pp.143-151.
- Helgason, T., Daniell, T.J., Husband, R., Fitter, A.H. and Young, J.P.W., 1998. Ploughing up the wood-wide web? *Nature*, 394(6692), pp.431-431.
- Hobbie, E.A., 2006. Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. *Ecology*, 87(3), pp.563-569.
- Jackson, R.B., Cook, C.W., Phippen, J.S. and Palmer, S.M., 2009. Increased belowground biomass and soil CO_2 fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest. *Ecology*, 90(12), pp.3352-3366.
- Jakobsen, I. and Rosendahl, L., 1990. Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. *New Phytologist*, 115(1), pp.77-83.
- Jenkins, M.B., Virginia, R.A. and Jarrell, W.M., 1988. Depth distribution and seasonal populations of mesquite-nodulating rhizobia in warm desert ecosystems. *Soil Science Society of America Journal*, 52(6), pp.1644-1650.
- Johnson, D.J., Clay, K. and Phillips, R.P., 2018. Mycorrhizal associations and the spatial structure of an old-growth forest community. *Oecologia*, 186(1), pp.195-204.
- Johnson, D., Krsek, M., Wellington, E.M., Stott, A.W., Cole, L., Bardgett, R.D., Read, D.J. and Leake, J.R., 2005. Soil invertebrates disrupt carbon flow through fungal networks. *Science*, 309(5737), pp.1047-1047.

- Johnson, D., Leake, J.R., Ostle, N., Ineson, P. and Read, D.J., 2002. In situ $^{13}\text{CO}_2$ pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. *New Phytologist*, 153(2), pp.327-334.
- Jones, D.L., Hodge, A. and Kuzyakov, Y., 2004. Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist*, 163(3), pp.459-480.
- Jones, D.L. and Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology and Biochemistry*, 38(5), pp.991-999.
- Jones, M.D., Durall, D.M. and Tinker, P.B., 1998. A comparison of arbuscular and ectomycorrhizal *Eucalyptus coccifera*: growth response, phosphorus uptake efficiency and external hyphal production. *New Phytologist*, 140(1), pp.125-134.
- Jones, T.G., Evans, C.D., Jones, D.L., Hill, P.W. and Freeman, C., 2016. Transformations in DOC along a source to sea continuum; impacts of photo-degradation, biological processes and mixing. *Aquatic Sciences*, 78(3), pp.433-446.
- Kafle, A., Garcia, K., Wang, X., Pfeffer, P.E., Strahan, G.D. and Bücking, H., 2019. Nutrient demand and fungal access to resources control the carbon allocation to the symbiotic partners in tripartite interactions of *Medicago truncatula*. *Plant, Cell and Environment*, 42(1), pp.270-284.
- Klironomos, J.N. and Hart, M.M., 2001. Food-web dynamics: animal nitrogen swap for plant carbon. *Nature*, 410(6829), p.651.
- Konvalinková, T. and Jansa, J., 2016. Lights off for arbuscular mycorrhiza: on its symbiotic functioning under light deprivation. *Frontiers in Plant Science*, 7, p.782.
- Körner, C., Asshoff, R., Bignucolo, O., Hättenschwiler, S., Keel, S.G., Peláez-Riedl, S., Pepin, S., Siegwolf, R.T. and Zotz, G., 2005. Carbon flux and growth in mature deciduous forest trees exposed to elevated CO_2 . *Science*, 309(5739), pp.1360-1362.
- Laliberté, E., Lambers, H., Burgess, T.I. and Wright, S.J., 2015. Phosphorus limitation, soil-borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands. *New Phytologist*, 206(2), pp.507-521.

- Lapeyrie, F.F. and Chilvers, G.A., 1985. An endomycorrhiza-ectomycorrhiza succession associated with enhanced growth of *Eucalyptus dumosa* seedlings planted in a calcareous soil. *New Phytologist*, 100(1), pp.93-104.
- Leake, J.R., Donnelly, D.P., Saunders, E.M., Boddy, L. and Read, D.J., 2001. Rates and quantities of carbon flux to ectomycorrhizal mycelium following ^{14}C pulse labeling of *Pinus sylvestris* seedlings: effects of litter patches and interaction with a wood-decomposer fungus. *Tree Physiology*, 21(2-3), pp.71-82.
- Leake, J.R. and Read, D.J., 2017. Mycorrhizal symbioses and pedogenesis throughout Earth's history. In *Mycorrhizal mediation of soil* (pp. 9-33). Elsevier.
- Lendenmann, M., Thonar, C., Barnard, R.L., Salmon, Y., Werner, R.A., Frossard, E. and Jansa, J., 2011. Symbiont identity matters: carbon and phosphorus fluxes between *Medicago truncatula* and different arbuscular mycorrhizal fungi. *Mycorrhiza*, 21(8), pp.689-702.
- Levillain, J., Thongo M'Bou, A., Deleporte, P., Saint-André, L. and Jourdan, C., 2011. Is the simple auger coring method reliable for below-ground standing biomass estimation in *Eucalyptus* forest plantations?. *Annals of Botany*, 108(1), pp.221-230.
- Lindahl, B., Stenlid, J., Olsson, S. and Finlay, R., 1999. Translocation of ^{32}P between interacting mycelia of a wood-decomposing fungus and ectomycorrhizal fungi in microcosm systems. *New Phytologist*, 144(1), pp.183-193.
- Lodge, D.J., 2000. Ecto-or arbuscular mycorrhizas—which are best? *New Phytologist*, 146(3), pp.353-354.
- Lodge, D.J. and Wentworth, T.R., 1990. Negative associations among VA-mycorrhizal fungi and some ectomycorrhizal fungi inhabiting the same root system. *Oikos*, 57, pp.347-356.
- Luo, Z., Wang, E. and Sun, O.J., 2016. A meta-analysis of the temporal dynamics of priming soil carbon decomposition by fresh carbon inputs across ecosystems. *Soil Biology and Biochemistry*, 101, pp.96-103.
- Machón, P.J.A.P., Pajares, J.A., Diez, J.J. and Alves-Santos, F.M., 2009. Influence of the ectomycorrhizal fungus *Laccaria laccata* on pre-emergence, post-emergence and late damping-off by *Fusarium oxysporum* and *F. verticillioides* on Stone pine seedlings. *Symbiosis*, 49(2), p.101.

- Major, J.E., Johnsen, K.H., Barsi, D.C. and Campbell, M., 2012. Total belowground carbon and nitrogen partitioning of mature black spruce displaying genetic× soil moisture interaction in growth. *Canadian Journal of Forest Research*, 42(11), pp.1939-1952.
- Mandyam, K. and Jumpponen, A., 2005. Seeking the elusive function of the root-colonising dark septate endophytic fungi. *Studies in Mycology*, 53, pp.173-189.
- Manoharan, L., Rosenstock, N.P., Williams, A. and Hedlund, K., 2017. Agricultural management practices influence AMF diversity and community composition with cascading effects on plant productivity. *Applied Soil Ecology*, 115, pp.53-59.
- Marsden, K.A., Jones, D.L. and Chadwick, D.R., 2017. DMPP is ineffective at mitigating N₂O emissions from sheep urine patches in a UK grassland under summer conditions. *Agriculture, Ecosystems and Environment*, 246, pp.1-11.
- Miranda, K.M., Espey, M.G. and Wink, D.A., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*, 5(1), pp.62-71.
- Molina, R. and Horton, T.R., 2015. Mycorrhiza specificity: its role in the development and function of common mycelial networks. In *Mycorrhizal Networks* (pp. 1-39). Springer, Dordrecht.
- Mulvaney, R.L., 1996. Nitrogen—inorganic forms. *Methods of soil analysis part 3—Chemical methods*, (methodsofsoilan3), pp.1123-1184.
- Murphy, J. and Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27, pp.31-36.
- Newsham, K.K., 2011. A meta-analysis of plant responses to dark septate root endophytes. *New Phytologist*, 190(3), pp.783-793.
- Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S. and Kennedy, P.G., 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, 20, pp.241-248.
- Olsson, S. and Gray, S.N., 1998. Patterns and dynamics of ³²P-phosphate and labelled 2-aminoisobutyric acid (¹⁴C-AIB) translocation in intact *basidiomycete* mycelia. *FEMS Microbiology Ecology*, 26(2), pp.109-120.

Ossler, J.N., Zielinski, C.A. and Heath, K.D., 2015. Tripartite mutualism: Facilitation or trade-offs between rhizobial and mycorrhizal symbionts of legume hosts. *American Journal of Botany*, 102(8), pp.1332-1341.

Perez-Moreno, J. and Read, D.J., 2001. Nutrient transfer from soil nematodes to plants: a direct pathway provided by the mycorrhizal mycelial network. *Plant, Cell and Environment*, 24(11), pp.1219-1226.

Pickles, B.J. and Pither, J., 2014. Still scratching the surface: how much of the 'black box' of soil ectomycorrhizal communities remains in the dark? *New Phytologist*, 201(4), pp.1101-1105.

Pickles, B.J., Wilhelm, R., Asay, A.K., Hahn, A.S., Simard, S.W. and Mohn, W.W., 2017. Transfer of ^{13}C between paired Douglas-fir seedlings reveals plant kinship effects and uptake of exudates by ectomycorrhizas. *New Phytologist*, 214(1), pp.400-411.

Prasad, R., Bhola, D., Akdi, K., Cruz, C., Sairam, K.V.S.S., Tuteja, N. and Varma, A., 2017. Introduction to mycorrhiza: historical development. In *Mycorrhiza-Function, Diversity, State of the Art* (pp. 1-7). Springer, Cham.

Radić, T., Hančević, K., Likar, M., Protega, I., Jug-Dujaković, M. and Bogdanović, I., 2012. Neighbouring weeds influence the formation of arbuscular mycorrhiza in grapevine. *Symbiosis*, 56(3), pp.111-120.

Rangel-Castro, J.I., Danell, E. and Pfeffer, P.E., 2002. A ^{13}C -NMR study of exudation and storage of carbohydrates and amino acids in the ectomycorrhizal edible mushroom *Cantharellus cibarius*. *Mycologia*, 94(2), pp.190-199.

Rau, B.M., Johnson, D.W., Chambers, J.C., Blank, R.R. and Lucchesi, A., 2009. Estimating root biomass and distribution after fire in a Great Basin woodland using cores and pits. *Western North American Naturalist*, 69(4), pp.459-469.

Reiss, J., Bridle, J.R., Montoya, J.M. and Woodward, G., 2009. Emerging horizons in biodiversity and ecosystem functioning research. *Trends in Ecology and Evolution*, 24(9), pp.505-514.

Řezáčová, V., Konvalinková, T. and Jansa, J., 2017. Carbon fluxes in mycorrhizal plants. In *Mycorrhiza-eco-physiology, secondary metabolites, nanomaterials* (pp. 1-21). Springer, Cham.

- Rillig, M.C., 2004. Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecology Letters*, 7(8), pp.740-754.
- Robinson, D., 2004. Scaling the depths: below-ground allocation in plants, forests and biomes. *Functional Ecology*, 18(2), pp.290-295.
- Rowell, D.L., 1994. *Soil science: methods and applications*. Department of Soil Science, University of Reading.
- Royer, D.L., Osborne, C.P. and Beerling, D.J., 2003. Carbon loss by deciduous trees in a CO₂-rich ancient polar environment. *Nature*, 424(6944), p.60.
- Rygiewicz, P.T. and Andersen, C.P., 1994. Mycorrhizae alter quality and quantity of carbon allocated below ground. *Nature*, 369(6475), pp.58-60.
- Schenk, H.J. and Jackson, R.B., 2005. Mapping the global distribution of deep roots in relation to climate and soil characteristics. *Geoderma*, 126(1), pp.129-140.
- Schiestl-Aalto, P., Ryhti, K., Mäkelä, A., Peltoniemi, M., Bäck, J. and Kulmala, L., 2019. Analysis of the NSC storage dynamics in tree organs reveals the allocation to belowground symbionts in the framework of whole tree carbon balance. *Frontiers in Forests and Global Change*, 2, p.17.
- Simard, S.W., Beiler, K.J., Bingham, M.A., Deslippe, J.R., Philip, L.J. and Teste, F.P., 2012. Mycorrhizal networks: mechanisms, ecology and modelling. *Fungal Biology Reviews*, 26(1), pp.39-60.
- Simard, S.W. and Durall, D.M., 2004. Mycorrhizal networks: a review of their extent, function, and importance. *Canadian Journal of Botany*, 82(8), pp.1140-1165.
- Slavíková, R., Püschel, D., Janoušková, M., Hujslová, M., Konvalinková, T., Gryndlerová, H., Gryndler, M., Weiser, M. and Jansa, J., 2017. Monitoring CO₂ emissions to gain a dynamic view of carbon allocation to arbuscular mycorrhizal fungi. *Mycorrhiza*, 27(1), pp.35-51.
- Smith, A.R., Lukac, M., Bambrick, M., Miglietta, F. and Godbold, D.L., 2013. Tree species diversity interacts with elevated CO₂ to induce a greater root system response. *Global Change Biology*, 19(1), pp.217-228.

- Smith, A.R., Lukac, M., Hood, R., Healey, J.R., Miglietta, F. and Godbold, D.L., 2013. Elevated CO₂ enrichment induces a differential biomass response in a mixed species temperate forest plantation. *New Phytologist*, 198(1), pp.156-168.
- Smith, J.L. and Doran, J.W., 1996. Measurement and use of pH and electrical conductivity for soil quality analysis. In *Methods for assessing soil quality (Vol. 49)*. Soil Science Society of America Madison, WI.
- Smith, S.E. and Read, D.J., 2010. *Mycorrhizal Symbiosis*. Academic press. London.
- Sochacki, S.J., Ritson, P., Brand, B., Harper, R.J. and Dell, B., 2017. Accuracy of tree root biomass sampling methodologies for carbon mitigation projects. *Ecological Engineering*, 98, pp.264-274.
- Staddon, P.L., Ramsey, C.B., Ostle, N., Ineson, P. and Fitter, A.H., 2003. Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of ¹⁴C. *Science*, 300(5622), pp.1138-1140.
- Stober, C., George, E. and Persson, H., 2000. Root growth and response to nitrogen. In *Carbon and nitrogen cycling in European forest ecosystems* (pp. 99-121). Springer, Berlin, Heidelberg.
- Stone, E.L. and Kalisz, P.J., 1991. On the maximum extent of tree roots. *Forest Ecology and Management*, 46(1-2), pp.59-102.
- Sun, Y.P., Unestam, T., Lucas, S.D., Johanson, K.J., Kenne, L. and Finlay, R., 1999. Exudation-reabsorption in a mycorrhizal fungus, the dynamic interface for interaction with soil and soil microorganisms. *Mycorrhiza*, 9(3), pp.137-144.
- Sutherland, W.J., Freckleton, R.P., Godfray, H.C.J., Beissinger, S.R., Benton, T., Cameron, D.D., Carmel, Y., Coomes, D.A., Coulson, T., Emmerson, M.C. and Hails, R.S., 2013. Identification of 100 fundamental ecological questions. *Journal of Ecology*, 101(1), pp.58-67.
- Talbot, J.M., Allison, S.D. and Treseder, K.K., 2008. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. *Functional Ecology*, 22(6), pp.955-963.
- Taylor, A.F., 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. In *Diversity and integration in Mycorrhizas* (pp. 19-28). Springer, Dordrecht.

- Taylor, B.N., Beidler, K.V., Cooper, E.R., Strand, A.E. and Pritchard, S.G., 2013. Sampling volume in root studies: the pitfalls of under-sampling exposed using accumulation curves. *Ecology Letters*, 16(7), pp.862-869.
- Timonen, S., Finlay, R.D., Olsson, S. and Söderström, B., 1996. Dynamics of phosphorus translocation in intact ectomycorrhizal systems: non-destructive monitoring using a β -scanner. *FEMS Microbiology Ecology*, 19(3), pp.171-180.
- Tlalka, M., Watkinson, S.C., Darrah, P.R. and Fricker, M.D., 2002. Continuous imaging of amino-acid translocation in intact mycelia of *Phanerochaete velutina* reveals rapid, pulsatile fluxes. *New Phytologist*, 153(1), pp.173-184.
- Toju, H. and Sato, H., 2018. Root-associated fungi shared between arbuscular mycorrhizal and ectomycorrhizal conifers in a temperate forest. *Frontiers in Microbiology*, 9, p.433.
- Toljander, J.F., Lindahl, B.D., Paul, L.R., Elfstrand, M. and Finlay, R.D., 2007. Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. *FEMS Microbiology Ecology*, 61(2), pp.295-304.
- Van Der Heijden, M.G. and Horton, T.R., 2009. Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology*, 97(6), pp.1139-1150.
- Van Der Heijden, M.G., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A. and Sanders, I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396(6706), pp.69-72.
- Van Der Krift, T.A. and Berendse, F., 2001. The effect of plant species on soil nitrogen mineralization. *Journal of Ecology*, 89(4), pp.555-561.
- Vannette, R.L. and Rasmann, S., 2012. Arbuscular mycorrhizal fungi mediate below-ground plant–herbivore interactions: a phylogenetic study. *Functional Ecology*, 26(5), pp.1033-1042.
- Veresoglou, S.D., Sen, R., Mamolos, A.P. and Veresoglou, D.S., 2011. Plant species identity and arbuscular mycorrhizal status modulate potential nitrification rates in nitrogen-limited grassland soils. *Journal of Ecology*, 99(6), pp.1339-1349.
- Walker TS, Bais HP, Grotewold E, Vivanco JM. 2003. Root exudation and rhizosphere biology. *Plant Physiology*, 132, pp.44–51.

- Wallander, H., Massicotte, H.B. and Nylund, J.E., 1997. Seasonal variation in protein, ergosterol and chitin in five morphotypes of *Pinus sylvestris* L. ectomycorrhizae in a mature Swedish forest. *Soil Biology and Biochemistry*, 29(1), pp.45-53.
- Wallander, H., Nilsson, L.O., Hagerberg, D. and Bååth, E., 2001. Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. *New Phytologist*, 151(3), pp.753-760.
- Wang, G., Sheng, L., Zhao, D., Sheng, J., Wang, X. and Liao, H., 2016. Allocation of nitrogen and carbon is regulated by nodulation and mycorrhizal networks in soybean/maize intercropping system. *Frontiers in Plant Science*, 7, p.1901.
- Wężowicz, K., Rozpądek, P. and Turnau, K., 2017. Interactions of arbuscular mycorrhizal and endophytic fungi improve seedling survival and growth in post-mining waste. *Mycorrhiza*, 27(5), pp.499-511.
- Wilson, G.W., Rice, C.W., Rillig, M.C., Springer, A. and Hartnett, D.C., 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. *Ecology Letters*, 12(5), pp.452-461.
- Woods, F.W. and Brock, K., 1964. Interspecific transfer of Ca-45 and P-32 by root systems. *Ecology*, 45(4), pp.886-889.
- Wright, D.P., Scholes, J.D. and Read, D.J., 1998. Effects of VA mycorrhizal colonization on photosynthesis and biomass production of *Trifolium repens* L. *Plant, Cell and Environment*, 21(2), pp.209-216.
- Zobel, M. and Öpik, M., 2014. Plant and arbuscular mycorrhizal fungal (AMF) communities—which drives which? *Journal of Vegetation Science*, 25(5), pp.1133-1140.

8. Appendices

8.1 ^{14}C activity as kBq per partition

Table S1- kBq per partition

Tree partition	Species treatment		<i>P</i> value
	<i>A. glutinosa</i>	<i>C. sativa</i>	
Leaves	1680.91 ± 301.02	1092.73 ± 123.08	0.15
Stem	629.21 ± 201.11	291.89 ± 105.77	0.21
Branch	5155.74 ± 1745.86	1690.85 ± 183.08	0.12
Root	3758.64 ± 1579.72	4729.91 ± 971.23	0.63
Soil respiration	0.06 ± 0.01	0.08 ± 0.02	0.47
Soil H ₂ O	<0.01	<0.01	0.57
Soil	52.91 ± 25.27	56.17 ± 27.42	0.93
Sand	0.02 ± 0.01	0.01 ± 0.00	0.22
Total	3811.63 ± 1571.45	4786.17 ± 944.42	0.62

Chapter 7- General discussion and future work

7.1. Overarching aims and objectives

The overarching aim of this research was to investigate the importance of plant: microbe symbioses, commonly found in temperate forest ecosystems, to the belowground cycling and storage of C in soils. Specifically, the objectives were:

1. To investigate the differences in belowground C allocation between early and late-successional temperate tree species (Chapter 3 and Chapter 6)
2. To investigate the intra and interspecific transfer of C via CMNs in temperate forest ecosystems (Chapter 4)
3. To investigate the effect of trees with tripartite mutualistic symbiotic relationships on the magnitude and allocation of C transferred via CMN (Chapter 5)
4. To investigate the differences in belowground allocation between glasshouse-based and field-based experiments (Chapter 6)

In the final chapter, I will discuss and synthesise the empirical findings presented in the thesis and relate them to overarching aims and objectives and discussing possible future research.

Objective 1: *To investigate the differences in belowground C allocation between early and late-successional temperate tree species (Chapter 3 and Chapter 6)*

We tested the hypothesis that the pioneer trees species (*A. glutinosus* and *B. pendula*) allocate more C belowground because they are frequent found as primary colonisers, or colonisers of soils with poor nutritional status. Successful colonisation in these conditions requires a growth strategy that invests in belowground biomass (e.g. roots and mycorrhizal symbionts) for nutrient and water acquisition. Chapter 3 showed that in the three-tree species studied there were no differences in C allocation belowground between *C. sativa* and *A. glutinosa* or *B. pendula*. The life history character traits, and successional status of a species, are thought to be associated with specific morphological and functional traits (Leuschner and Meier, 2018). Namely, that late successional stage trees will normally develop under the shade, or partial shade, of other trees and as a result are generally found to have both a higher leaf area and lower rate of photosynthesis compared to early successional species. However, in the case of *A. glutinosa* a broader range of light tolerance enables establishment under canopy in addition to full light (Coll et al., 2008). In early successional stage trees, the maximum rate of photosynthesis is typically higher than late successional species, which would support a greater

allocation of C to belowground biomass for tree stability and access to resources. In contrast to the traditionally held view, the data presented in Chapter 3 showed that *A. glutinosa* allocated less C belowground than *B. pendula* or *C. sativa*. This unexpected finding could be explained by the differences in the mycorrhizae community structure present in the soil and those that are in symbiosis with the trees. Indeed, fruiting bodies of *Laccaria oblongospora* were observed in the experimental plots and have been reported to prevent colonisation by other species of mycorrhizae by altering the root architecture (Chen et al., 2000). Another explanation could be that this was because the experiments were conducted using seedlings that have been shown to alter their morphological traits and patterns of C allocation as a tree ages (Paz 2003; Delagrangue et al. 2004; Claveau et al. 2005; Kneeshaw et al. 2006).

Smith and Read, (2010) suggest that approximately 2 to 40 % of photosynthetically fixed C is allocated below ground for either; growth, repair, or metabolic processes (e.g. respiration) by the root system. C exuded from roots may also be utilised for growth and respiration of the soil microbial community (Walker et al., 2003), or becomes incorporated into the soil organic matter pool through the process of root, hyphal and microbial turnover (Jones et al., 2009). Belowground allocation of C can have also been shown to promote root exudation and microbial activity (Tang et al., 2009). Although the rhizosphere is a microbial hotspot, comprised of a diverse microbial community, the mycorrhizal fungi are the most ecologically significant (Jones et al., 2009) that is thought, in some cases, to use up to 30 % of the photosynthetically fixed C assimilated by its symbiotic partner (Johnson et al., 2002; Leake et al., 2006). The use of C isotopes presents an opportunity to increase our knowledge of C allocation to the plant- soil system, and are revealing that C fluxes can vary temporally, according to the time of day, season or even annually. For example, during a mast year the allocation of C to the plant biomass partitions may change due to the C demand of producing seed with subsequent impacts on autotrophic and heterotrophic respiration (Brüggemann et al., 2011). The general principles of C partitioning are thought to involve molecular regulation although the mechanisms that underpin this process are not yet fully understood (Slewinski and Braum, 2010). Empirical studies have also shown that C partitioning occurs by the means of transport carbohydrates through phloem using a pressure-driven mass-flow system, whereby it can be delivered to areas of C requirement i.e. from source to sink (Van Bel, 2003). Ultimately, the allocation is not only driven by the delivery mechanism, in this case photosynthesis, but also the C demand (sink strength) of various plant tissues (Wardlaw 1969). Kayler et al., (2010) suggested that the time lag from C fixation to belowground respiration is an important

indication of the size and status of the C pools, nutritional status, biological activity and the hydration status of the plant, and not just the successional stage of the tree species.

Objective 2: *To investigate the intra- and inter-specific transfer of C via CMNs in temperate forest ecosystems (Chapter 4).*

In chapter 4, a slightly larger transfer of C between inter-specific species combinations was observed when compared to intraspecific species combination, however there were no statistically significant differences between the two different groupings. The observed trend suggests that interspecific exchange of C and nutrients (i.e. interspecific facilitation) may be more widespread than previously thought and important to our understanding of plant community competition dynamics. DNA extracted from soil to identify mycorrhizal species identified three species, *Sphaerospora brunnea*, *Inocybe curvipes* and *Laccaria oblongospora* as the likely candidates for the fungi responsible for mediating the transfer between the trees in the treatments. However, no statistically significant differences were found between the diversity and / or abundance of mycorrhizae present in the different tree species combinations. This suggests that C transfer between trees was unlikely to have been limited by an insufficient bank of fungal reproductive propagules present in the soil used in this study. If microbial diversity and abundance were not driving the increased transfer of C between interspecific species combinations then the transfer is most likely mediated by source: sink relationships of the multiple partners, that warrants further study to disentangle. Although it is methodologically difficult to fully quantify the costs and benefits of mycorrhizal symbiosis, particularly because of the challenges in separating root from hyphal respiration in the field (Heinemeyer et al., 2006), and the speed of transfer to belowground symbionts (Johnson et al., 2002). Individual plant species have unique responses to mycorrhizal fungal colonisation (Plett, and Martin, 2018). For this reason, many studies have chosen to compare mycorrhizal with non-mycorrhizal plants of the same species (Norman and Hooker, 2000; Stefania et al., 2018). However, this methodology is not without its own issues (Smith and Smith, 2012), such as the presence of mycorrhizal fungi stimulating root exudation (Kaiser et al., 2015). Some even suggest that it may not be possible to fully compare the responses of the non-mycorrhizal and mycorrhizal plants of the same size and species (Lendenmann et al., 2011), hence the popularity of split root experiments (Douds et al., 2000). The cost benefit analysis of mycorrhizal symbiosis can alter over time depending on climatic conditions, nutrient availability and particularly with the plant and fungal species identity (Péret et al., 2018).

The results from the two CMN experiments, Chapters 4 and 5 demonstrated that transfer between trees via CMN was not only dependant on abundance or diversity of the mycorrhizae present. Chapter 4 suggests that the energetic demands of nitrogen fixation was likely resulting in preferential allocation of donor fixed ^{14}C being allocated to the receiver nodules. Chapter 5 showed a trend whereby more transfer took place in inter- than intraspecific treatments, again suggesting that the growth demands of species at different stages led to bilateral resource sharing. We therefore suggest that the allocation of C to plant: microbe symbiotic partners by the trees appeared to be regulated by source: sink relationships, it is likely that source-sink relationships would be one of the mechanisms involved in the CMN. Another potential mechanism is mycorrhizal mediated trading, whereby nutrient exchange is mediated by the fungi at the plant: microbial interface following the biological market model (Kiers et al., 2011; Fellbaum et al, 2014), or possibly the evolutionary economic game theory (Archetti et al., 2011) whereby uncooperative partners positively selected those that were co-operative.

Objective 3: *To investigate the effect of trees with tripartite mutualistic symbiotic relationships on the magnitude and allocation of C transferred via CMN (Chapter 5)*

The results from Chapter 5 showed that if the trees receiving C via the CMN were actinorhizal, then the C sink strength mediated by the energetic demand of the process of N-fixation was large enough for C to be preferentially translocated to the root nodule via the CMN rather than to other plant organs. This C was transferred through the hyphae of the mycorrhizal hyphae, which were present in the sand tube and forming a CMN connecting the two trees through the 40 μm screen, which covered the tube ends. It is also clear from the results of Chapter 5 and Chapter 6, that plant: microbe symbiosis plays a significant role in not only the belowground transfer and storage of C in temperate ecosystems but also in the formation of plant communities and the mediation of interplant competition (Fonesca et al., 2017).

This may have been an over ambitious objective as investigating belowground root exudation and the abundance and diversity of microbes in the rhizosphere and soil, many of which are currently unculturable (Suyal et al., 2019), has been considered for some time as mission impossible due to the experimental challenges it presents (Oburger and Jones, 2018). Specifically, that analysis of the quality and quantity of rhizodeposits has been hampered by the lack of analytical technology to allow accurate estimates, and that belowground sampling of these minute chemicals into a substrate as heterogeneous as soil also presents multiple sampling challenges (Oburger and Jones, 2018). Another confounding factor is the fact that the

exuded metabolites are simultaneously being released through multiple pathways whilst simultaneously being consumed and cycled during microbial turnover as microbes excrete and respire C, or in some cases, microbial C is assimilated by others when microbes consume each other. Often studies have been conducted in hydroponic (soil less), sterile conditions, to simplify the number of potential variables, this reductionist technique can produce results that further our understanding of C cycling, although how applicable these results are to any real-life situation is still a topic of debate (Sasse et al., 2018; Klein et al., 1988). It is widely accepted that the presence of microbes at the root interface will not only stimulate increased rhizodeposition (Philips et al., 2004) but in some cases can also release compounds that increase root leakiness (Philips et al., 2004; Kudoyarova et al., 2014). To ensure constant benefit for both symbiotic partners in the relationship, these symbiotic relationships are a constant battle between both trading partners, almost akin to predator prey relationships (Oburger and Jones, 2018).

Objective 4: *To investigate the differences in belowground allocation between glasshouse-based and field-based experiments (Chapter 6)*

Our glasshouse experiments were conducted on *A. glutinosa*, *B. pendula* and *C. sativa* selected because they were the species also present in the BangorDIVERSE field experiment. Due to the size of the trees in the field experiment, only coppiced *A. glutinosa* and *C. sativa* trees were available for pulse labelling. Belowground allocation of C for *A. glutinosa* was 32 % of the total ^{14}C activity in both the glasshouse and field-based experiments, whereas for *C. sativa* the belowground C allocation was 60 % of the total ^{14}C assimilated in the glasshouse, but only 45 % of the total ^{14}C activity in the field experiment.

The allocation of C belowground was surprisingly similar for *A. glutinosa* grown in the two different experimental settings, suggesting that the climatic conditions may not influence allocation of C in this species. In contrast, for the *C. sativa* 15 % more C was allocated belowground in the glasshouse experiment. These differences could be explained by the treatment of the soil in the glasshouse experiment, which was sieved to 2 mm altering the soil structure and influencing the soil microbial communities. The mixing and alteration of soil structure is similar to the treatment of soils during tillage by industrial agriculture. Pedology and edaphology both have their origins in agricultural science and as a result, the majority of experimentation of soil biological interactions have been conducted in agricultural soils. However, agricultural soils are altered through various management practices designed to

increase the productivity of a chosen crop species (Thompson, 2017). Agricultural soils are routinely ploughed, mixed and disturbed before the inevitable need for amendment and/or fertilisation to replace the nutrition removed by the harvesting of the agricultural crops and their by-products (Gold, 2016). This processing undoubtedly alters the nutrient dynamics by destroying microsites, which are known to protect C from microbial interactions (Keiluweit et al., 2017), the sieving process replicates this to some extent.

Another possible explanation is that the black plastic plant pots used were in direct sunlight, which may have artificially increased microbial activity influencing microbial metabolic rates and efflux of CO₂. Microbial biomass C turnover rates ranges from 7 to 95 days (Kaštovská and Šantrůčková, 2007) whereas in mycorrhizal fungi it ranges from 5 to 9 days, indicating a comparative expeditious flux of C (Godbold et al., 2006). Turnover times of the soil microbial community can be hard to quantify as often microbial C is consumed by other microbes before being respired (Jones et al., 2009). However, many alternative mechanisms have been postulated in the literature to explain C allocation in different experimental systems (Řezáčová et al., 2017). For example: (i) the choice of model host plant and species of mycorrhizae partner in symbiosis (Pearson and Jakobsen, 1993; Lerat et al., 2003; Lendenmann et al., 2011); (ii) developmental stage of the symbiosis (Wright et al., 1998); (iii) the volume of the pots (if grown in pots); length of the pulse and chase periods (Řezáčová et al., 2017); and (iv) the environmental conditions (Slavíková et al., 2017).

1.2 The importance of plant: microbe symbiosis in shaping temperate forest ecosystems

Plant microbe symbiosis is known, not only to stimulate belowground C allocation and therefore rhizodeposition and C storage in soils, but can also indirectly affect C capture by the photobionts by indirectly shaping their plant communities through many, not fully understood, mechanisms (Kandlikar et al., 2019). This section will briefly discuss the primary ways in which belowground symbiotic microorganisms have and do indirectly affect the temperate terrestrial primary production through modifying and mediating plant community dynamics.

It is widely accepted that 400 million years ago, the first plants left the sea to begin to inhabit the terrestrial ecosystems and that early forms of arbuscular mycorrhizal plant microbe symbiosis were fundamental to facilitating this process (Remy et al., 1994; Brundrett et al., 2002). The majority of terrestrial plants have retained these beneficial symbiotic relationships (Harley and Harley 1987; Wang and Qui 2006; Smith and Read 2010), with current estimates

of between 74 – 82 % of global plants remaining dependant on this relationship (Brundrett 2009). Over the proceeding millennia the plant species without AM symbiosis have developed a suite of alternative survival strategies including; ericoid, ecto-, and orchid mycorrhizal symbiosis, relationships with nitrogen-fixing bacteria, endophytic fungal and bacterial symbiosis, carnivory, cluster roots and parasitism (Brundrett 2009; Lambers et al., 2011, Lambers and Teste 2013). In some cases, the AM relationships crossed the line from beneficial to the plant to antagonistic (Brundrett 2009; Lambers et al., 2011; Lambers and Teste 2013) and in some cases even pathogenic (Hentschel et al., 2000; Lutzoni et al., 2018).

It is known that plant symbiont can alter the rhizosphere by altering; the plant immune responses (Jacott et al., 2017), hormones (Egamberdieva et al., 2017), exudate composition (Ulrich et al., 2019), and defences to herbivory (Rasmann et al., 2017). It has been suggested that the plant symbiont is also fundamental to shaping the rhizosphere and diversity and functionality of the plant-associated microbiome (Uroz et al., 2019). The topic of succession in plant communities has long been a topic considered fundamental to ecology (Walker and Del Moral, 2003; Prach and Walker, 2011). The concept that symbiotic relationships alter successional trajectory is fundamentally important, not only to understanding how plant communities have and may change over time (Knelman et al., 2018; Allen et al., 2018), but also in the restoration of contaminated and disturbed ecosystems (Pulsford et al., 2016) such as the recolonisation of spoil heaps created by past industrial mining (Frouz et al., 2008; Prach and Walker 2011). Previously the role of aboveground and belowground interactions had been largely overlooked particularly in the biogeochemical cycling and storage of fundamental elements such as C (Shri et al., 2019) and N (Moreau et al., 2019).

C flows not only from trees to fungi, but also from fungi to tree although historically as this is a less frequent study subject (Řezáčová et al., 2017). However up to ten percent of global plant species are at least partially mycoheterotrophic, which means they receive a C benefit from the fungi for the majority of their lifecycle (Leake, 2005). C radio-isotope experimentation (^{14}C) has demonstrated a C flux from fungus to plant in green orchids (*Goodyera repens*) in association with mycorrhizal fungi *Ceratobasidium cornigerum* (Cameron et al., 2008). Although probably less common and therefore ecologically significant there is also proof that fungi can supply some plants with C in the form of amino acids (Aduzinadah and Read, 1986). This form of C transfer which is atypical may yet be significant to the C trading of mycorrhizal associations (Taylor et al., 2004) and may yet be discovered in other forms of mycorrhizal association (Johnson, 2008). Recent methodological developments, such as next generating

sequencing and the expansion of online DNA datasets are allowing us to identify previously unknown microbial diversity and inevitably new forms of symbiotic relationships (Balestrini and Lumini, 2018).

The importance of symbiotic microbial relationships to maintaining diverse and healthy plant populations especially under stress conditions in both agricultural systems (Arora, 2018) and forest ecosystems (DeLuca et al., 2019) is now irrefutable (Spinosa, 2008; Pickles et al., 2020). However anthropogenic activities are now threatening the microorganisms that underpin a healthy global ecosystem (Cavicchioli et al., 2019). Threats to microorganisms are complex and varied and include; climate change (Cavicchioli et al., 2019), pollution (Xavier and Germinda, 1999), N deposition (Regus et al., 2017; Lilleskov et al., 2019) and agricultural activities (Nelkner, et al., 2019). For instance, tillage damages the richness of AM fungi and potentially results in the loss of some of the services AM fungi provide to agriculturally desirable plant hosts. The phylogenetic under dispersion in an AM fungal community, indicated by the presence of few AM fungal genera or families, could be used as an indicator of where AM fungal communities may be limited in functional diversity, if phylogeny can be linked with functional complementarity and AM fungal coexistence (Maherali and Klironomos 2007). Finally, the application of niche theory and temporal community dynamics to AM fungal communities would greatly improve the knowledge base as to how environmental heterogeneity may support AM fungal diversity and thus the overall facilitative effects of an AM fungal community. Nonetheless, although the facilitative, and to a lesser extent antagonistic, effects of AM fungi have been long studied, the mechanisms that control them in a predictable fashion for future ecological application have yet to be fully realized.

An idea known as the Hologenome concept has been postulated (Zilber-Rosenberg and Rosenberg, 2008) which argues that advances in both microbiology and discoveries revealed by novel DNA extraction techniques across all biological fields are suggesting that we can no longer consider animals and plants as individuals all are holobionts comprised of a host individual with multiple symbiotic microbes. As we have discussed microbes are not only on the external surfaces of organisms, but within the body fluids (exosymbionts), and also inside many plant and animal cells (endosymbionts). The human microbiota is thought to contain ten times more microbial cells than the number of human cells (Wenner, 2007) and these microbes are now associated with both mental and physical health (Falony et al., 2019). Plants have been found to have 10^8 bacteria per gram of leaf (Lindow and Brandl, 2003), whereas the rhizosphere can contain up to 10^9 bacteria and 10^6 fungal cells per gram of soil (Forster, 1988). In order for

these microbiotas to be involved directly in the evolution of organisms they would need to be passed down over generations and this has been demonstrated through many mechanisms (Rosenberg and Zilber-Rosenberg, 2011). These include vegetative reproduction (Rosenberg and Zilber-Rosenberg, 2016), cytoplasmic inheritance (Dawid and Blackler, 1972), eggs (Baumann et al., 1995), via faeces (Engel and Moran, 2013), contact during and after birth (Gilbert et al., 2014) as well as many other possible vectors.

2. Future research

Suggestions for future research in this field include more radioisotope work with both N and C, to get a greater understanding of how CMN transfer N fixed by N-fixation in roots nodules between trees intra- and inter-specifically to further investigate the most interesting results from Chapter 4, namely that the CMN plant symbiosis preferentially allocated C to the root nodule of the receiver tree. More field-based research is required to further investigate transfer from ectomycorrhizal only trees to arbuscular mycorrhizal ground vegetation to establish which of the potential mechanisms is responsible for the unexpected results measured in Chapter 6. To get a sense for what is happening at a greater scale these experiments could be replicated using other soils in temperate forests both in the UK and abroad. More species could be investigated and common combinations in order to assist in the creation of soil C modelling software which will be essential in working out where the most efficient gains can be made in sequestering more C belowground in temperate forest for the least financial cost.

More research needs to be conducted to quantify C storage under species mixes that occur commonly in temperate forest ecosystems. This will allow the development of more accurate terrestrial C storage models. In the same way as we are improving aboveground yield models to account for the modern drive away from monoculture clear-cut silvicultural systems and towards forms of continuous cover forestry. Continuous cover forestry increases the importance of understanding how species diversity of both primary producing and microbial components of the plan: microbe symbiosis can mitigate anthropogenic C emissions. There is also a need for more integration of research within the field of plant: microbe symbiotic partnerships. Research has historically focussed in on specialised areas at different scales, which has led to an incomplete understanding of the highly complex mechanisms mediating symbiosis (Ferlian et al., 2018) and in particular how these relationships can help us to achieve

sustainable food production and possibly help mitigate some of the detrimental effects of human driven climate change (Pickles et al., 2020).

3. Conclusions

The empirical data presented in this thesis suggests that the presence of plant microbe symbiosis alters the plant community's allocation belowground, root exudation and ultimately C cycling and storage in temperate forest ecosystems. Consistently throughout the experimental chapters, *C. sativa* has shown greater belowground allocation of C than *A. glutinosa* or *B. pendula*. We therefore suggest that of the three species studied *C. sativa* would be best to maximise C sequestration potential belowground. These results also highlight how little is known about tree belowground allocation across the temperate forest biome. If we are to improve C accountancy and global circulatory models more experimentation of this nature is required. A better understanding of how species choices can improve C draw down under anthropogenically driven climatic change is essential. We postulate from our results that the allocation of C to the tree organs is determined by a combination of supply and demand across a source: sink gradient and the interplay of the two or more symbiotic partners constantly vying to ensure continued benefit from the partnership. It is also understood that the source sink strength of relative biomass partitions will be different amongst, or between species, and at different times during the tree's lifecycle. Our studies suggested that although the presence of actinorhizal root nodules did not seem to alter the overall belowground allocation, it was obvious that the C demand of the N-fixation process was modifying the source: sink relationships in those treatment sufficiently for the plant: microbe continuum to preferentially allocate C to the root nodule of *A. glutinosa* via the CMN. Forest ecology has traditionally neglected to fully account for the importance of belowground symbiotic relationships and as we learn more about their ability to facilitate resource sharing amongst and between species we will also have to find new ways to protect them from the pressures the future may hold for them.

4. References

- Abuzinadah, R.A. and Read, D.J., 1986. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants: I. Utilization of peptides and proteins by ectomycorrhizal fungi. *New Phytologist*, 103(3), pp.481-493.
- Allen, M.F., O'Neill, M.R., Crisafulli, C.M. and MacMahon, J.A., 2018. Succession and Mycorrhizae on Mount St. Helens. In *Ecological responses at Mount St. Helens: revisited 35 years after the 1980 eruption* (pp. 199-215). Springer, New York, NY.
- Archetti, M., Scheuring, I., Hoffman, M., Frederickson, M.E., Pierce, N.E. and Yu, D.W., 2011. Economic game theory for mutualism and cooperation. *Ecology Letters*, 14(12), pp.1300-1312.
- Arora, N.K., 2018. Agricultural sustainability and food security. *Environmental Sustainability*, 1(3), pp.217–219.
- Balestrini, R. and Lumini, E., 2018. Focus on mycorrhizal symbioses. *Applied Soil Ecology*, 123, pp.299-304.
- Baumann, P., Baumann, L., Lai, C.Y., Rouhbakhsh, D., Moran, N.A. and Clark, M.A., 1995. Genetics, physiology, and evolutionary relationships of the genus *Buchnera*: intracellular symbionts of aphids. *Annual Review of Microbiology*, 49(1), pp.55-94.
- Brüggemann, N., Gessler, A., Kayler, Z.E., Keel, S., Badeck, F.W., Barthel, M., Boeckx, P., Buchmann, N., Brugnoli, E., Esperschütz, J. and Gavrichkova, O., 2011. Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: a review. *Biogeosciences Discussions*, 8(2), pp.3619-3695.
- Brundrett, M.C., 2002. Coevolution of roots and mycorrhizas of land plants. *New Phytologist*, 154(2), pp.275-304.
- Brundrett, M.C., 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant and Soil*, 320(1-2), pp.37-77.
- Cameron, D.D., Johnson, I., Read, D.J. and Leake, J.R., 2008. Giving and receiving: measuring the carbon cost of mycorrhizas in the green orchid, *Goodyera repens*. *New Phytologist*, 180(1), pp.176-184.

- Cavicchioli, R., Ripple, W.J., Timmis, K.N., Azam, F., Bakken, L.R., Baylis, M., Behrenfeld, M.J., Boetius, A., Boyd, P.W., Classen, A.T. and Crowther, T.W., 2019. Scientists' warning to humanity: microorganisms and climate change. *Nature Reviews Microbiology*, 17(9), pp.569-586.
- Chen, Y.L., Brundrett, M.C. and Dell, B., 2000. Effects of ectomycorrhizas and vesicular–arbuscular mycorrhizas, alone or in competition, on root colonization and growth of *Eucalyptus globulus* and *E. urophylla*. *New Phytologist*, 146(3), pp.545-555.
- Claveau, Y., Messier, C. and Comeau, P.G., 2005. Interacting influence of light and size on aboveground biomass distribution in sub-boreal conifer saplings with contrasting shade tolerance. *Tree physiology*, 25(3), pp.373-384.
- Coll, L., Potvin, C., Messier, C. and Delagrange, S., 2008. Root architecture and allocation patterns of eight native tropical species with different successional status used in open-grown mixed plantations in Panama. *Trees*, 22(4), p.585.
- Dawid, I.B. and Blackler, A.W., 1972. Maternal and cytoplasmic inheritance of mitochondrial DNA in *Xenopus*. *Developmental Biology*, 29(2), pp.152-161.
- Delagrange, S., Messier, C., Lechowicz, M.J. and Dizengremel, P., 2004. Physiological, morphological and allocational plasticity in understory deciduous trees: importance of plant size and light availability. *Tree Physiology*, 24(7), pp.775-784.
- DeLuca, T.H., Pingree, M.R.A. and Gao, S., 2019. Assessing soil biological health in forest soils. In *Developments in Soil Science* (Vol. 36, pp. 397-426). Elsevier.
- Douds, D.D., Pfeffer, P.E. and Shachar-Hill, Y., 2000. Carbon partitioning, cost, and metabolism of arbuscular mycorrhizas. In *Arbuscular Mycorrhizas: Physiology and Function* (pp. 107-129). Springer, Dordrecht.
- Egamberdieva, D., Wirth, S.J., Alqarawi, A.A., Abd_Allah, E.F. and Hashem, A., 2017. Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. *Frontiers in Microbiology*, 8, p.2104.
- Engel, P. and Moran, N.A., 2013. The gut microbiota of insects—diversity in structure and function. *FEMS Microbiology Reviews*, 37(5), pp.699-735.

- Falony, G., Vandeputte, D., Caenepeel, C., Vieira-Silva, S., Daryoush, T., Vermeire, S. and Raes, J., 2019. The human microbiome in health and disease: hype or hope. *Acta Clinica Belgica*, 74(2), pp.53-64.
- Fellbaum, C.R., Mensah, J.A., Cloos, A.J., Strahan, G.E., Pfeffer, P.E., Kiers, E.T. and Bücking, H., 2014. Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants. *New Phytologist*, 203(2), pp.646-656.
- Ferlian, O., Biere, A., Bonfante, P., Buscot, F., Eisenhauer, N., Fernandez, I., Hause, B., Herrmann, S., Krajinski-Barth, F., Meier, I.C. and Pozo, M.J., 2018. Growing research networks on mycorrhizae for mutual benefits. *Trends in Plant Science*, 23(11), pp.975-984.
- Fonseca, M.B., Dias, T., Carolino, M.M., França, M.G.C. and Cruz, C., 2017. Belowground microbes mitigate plant-plant competition. *Plant Science*, 262, pp.175-181.
- Foster, R.C., 1988. Microenvironments of soil microorganisms. *Biology and Fertility of Soils*, 6(3), pp.189-203.
- Frouz, J., Prach, K., Pižl, V., Háněl, L., Starý, J., Tajovský, K., Materna, J., Balík, V., Kalčík, J. and Řehouňková, K., 2008. Interactions between soil development, vegetation and soil fauna during spontaneous succession in post mining sites. *European Journal of Soil Biology*, 44(1), pp.109-121.
- Gilbert, S.F., 2014. A holobiont birth narrative: the epigenetic transmission of the human microbiome. *Frontiers in Genetics*, 5, p.282.
- Godbold, D.L., Hoosbeek, M.R., Lukac, M., Cotrufo, M.F., Janssens, I.A., Ceulemans, R., Polle, A., Velthorst, E.J., Scarascia-Mugnozza, G., De Angelis, P. and Miglietta, F., 2006. Mycorrhizal hyphal turnover as a dominant process for carbon input into soil organic matter. *Plant and Soil*, 281(1-2), pp.15-24.
- Gold, M.V., 2016. *Sustainable Agriculture: The Basics* (p. 1). Apple Academic Press, Oakville.
- Harley, J.L. and Harley, E.L., 1987. A check-list of mycorrhiza in the British flora. *New Phytologist*, 105(2), pp.1-102.
- Heinemeyer, A., Ineson, P., Ostle, N. and Fitter, A.H., 2006. Respiration of the external mycelium in the arbuscular mycorrhizal symbiosis shows strong dependence on recent photosynthates and acclimation to temperature. *New Phytologist*, 171(1), pp.159-170.

- Hentschel, U., Steinert, M. and Hacker, J., 2000. Common molecular mechanisms of symbiosis and pathogenesis. *Trends in Microbiology*, 8(5), pp.226-231.
- Jacott, C., Murray, J. and Ridout, C., 2017. Trade-offs in arbuscular mycorrhizal symbiosis: disease resistance, growth responses and perspectives for crop breeding. *Agronomy*, 7(4), p.75.
- Johnson, D., 2008. Resolving uncertainty in the carbon economy of mycorrhizal fungi. *New Phytologist*, 180(1), pp.3-5.
- Johnson, D., Leake, J.R., Ostle, N., Ineson, P. and Read, D.J., 2002. In situ $^{13}\text{CO}_2$ pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. *New Phytologist*, 153(2), pp.327-334.
- Jones, D.L., Nguyen, C. and Finlay, R.D., 2009. Carbon flow in the rhizosphere: carbon trading at the soil–root interface. *Plant and Soil*, 321(1-2), pp.5-33.
- Kaiser, C., Kilburn, M.R., Clode, P.L., Fuchslueger, L., Koranda, M., Cliff, J.B., Solaiman, Z.M. and Murphy, D.V., 2015. Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation. *New Phytologist*, 205(4), pp.1537-1551.
- Kandlikar, G.S., Johnson, C.A., Yan, X., Kraft, N.J. and Levine, J.M., 2019. Winning and losing with microbes: how microbially mediated fitness differences influence plant diversity. *Ecology Letters*, 22(8), pp.1178-1191.
- Kaštovská, E. and Šantrůčková, H., 2007. Fate and dynamics of recently fixed C in pasture plant–soil system under field conditions. *Plant and Soil*, 300(1-2), pp.61-69.
- Kayler, Z., Gessler, A. and Buchmann, N., 2010. What is the speed of link between aboveground and belowground processes? *New Phytologist*, 187(4), pp.885-888.
- Keiluweit, M., Wanzek, T., Kleber, M., Nico, P. and Fendorf, S., 2017. Anaerobic microsites have an unaccounted role in soil carbon stabilization. *Nature Communications*, 8(1), pp.1-10.
- Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J.A., Franken, O., Verbruggen, E., Fellbaum, C.R., Kowalchuk, G.A., Hart, M.M., Bago, A. and Palmer, T.M., 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*, 333(6044), pp.880-882.

Klein, D.A., Frederick, B.A., Biondini, M. and Trlica, M.J., 1988. Rhizosphere microorganism effects on soluble amino acids, sugars and organic acids in the root zone of *Agropyron cristatum*, *A. smithii* and *Bouteloua gracilis*. *Plant and Soil*, 110(1), pp.19-25.

Kneeshaw, D.D., Kobe, R.K., Coates, K.D. and Messier, C., 2006. Sapling size influences shade tolerance ranking among southern boreal tree species. *Journal of Ecology*, 94(2), pp.471-480.

Knelman, J.E., Graham, E.B., Prev  y, J.S., Robeson, M.S., Kelly, P., Hood, E. and Schmidt, S.K., 2018. Interspecific plant interactions reflected in soil bacterial community structure and nitrogen cycling in primary succession. *Frontiers in Microbiology*, 9, p.128.

Kudoyarova, G.R., Melentiev, A.I., Martynenko, E.V., Timergalina, L.N., Arkhipova, T.N., Shendel, G.V., Kuz'mina, L.Y., Dodd, I.C. and Veselov, S.Y., 2014. Cytokinin producing bacteria stimulate amino acid deposition by wheat roots. *Plant Physiology and Biochemistry*, 83, pp.285-291.

Lambers, H., Brundrett, M.C., Raven, J.A. and Hopper, S.D., 2011. Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant and Soil*, 348(1-2), p.7.

Lambers, H. and Teste, F.P., 2013. Interactions between arbuscular mycorrhizal and non-mycorrhizal plants: do non-mycorrhizal species at both extremes of nutrient availability play the same game? *Plant, Cell and Environment*, 36(11), pp.1911-1915.

Leake, J.R., 2005. Plants parasitic on fungi: unearthing the fungi in myco-heterotrophs and debunking the 'saprophytic' plant myth. *Mycologist*, 19(3), pp.113-122.

Leake, J.R., Ostle, N.J., Rangel-Castro, J.I. and Johnson, D., 2006. Carbon fluxes from plants through soil organisms determined by field ¹³CO₂ pulse-labelling in an upland grassland. *Applied Soil Ecology*, 33(2), pp.152-175.

Lendenmann, M., Thonar, C., Barnard, R.L., Salmon, Y., Werner, R.A., Frossard, E. and Jansa, J., 2011. Symbiont identity matters: carbon and phosphorus fluxes between *Medicago truncatula* and different arbuscular mycorrhizal fungi. *Mycorrhiza*, 21(8), pp.689-702.

- Lerat, S., Lapointe, L., Gutjahr, S., Piché, Y. and Vierheilig, H., 2003. Carbon partitioning in a split-root system of arbuscular mycorrhizal plants is fungal and plant species dependent. *New Phytologist*, 157(3), pp.589-595.
- Leuschner, C. and Meier, I.C., 2018. The ecology of Central European tree species: Trait spectra, functional trade-offs, and ecological classification of adult trees. *Perspectives in Plant Ecology, Evolution and Systematics*, 33, pp.89-103.
- Lilleskov, E.A., Kuyper, T.W., Bidartondo, M.I. and Hobbie, E.A., 2019. Atmospheric nitrogen deposition impacts on the structure and function of forest mycorrhizal communities: a review. *Environmental Pollution*, 246, pp.148-162.
- Lindow, S.E. and Brandl, M.T., 2003. Microbiology of the phyllosphere. *Applied Environmental Microbiology*, 69(4), pp.1875-1883.
- Lutzoni, F., Nowak, M.D., Alfaro, M.E., Reeb, V., Miadlikowska, J., Krug, M., Arnold, A.E., Lewis, L.A., Swofford, D.L., Hibbett, D. and Hilu, K., 2018. Contemporaneous radiations of fungi and plants linked to symbiosis. *Nature Communications*, 9(1), p.5451.
- Maherali, H. and Klironomos, J.N., 2007. Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science*, 316(5832), pp.1746-1748.
- Moreau, D., Bardgett, R.D., Finlay, R.D., Jones, D.L. and Philippot, L., 2019. A plant perspective on nitrogen cycling in the rhizosphere. *Functional Ecology*, 33(4), pp.540-552.
- Nelkner, J., Henke, C., Lin, T.W., Pätzold, W., Hassa, J., Jaenicke, S., Grosch, R., Pühler, A., Sczyrba, A. and Schlüter, A., 2019. Effect of long-term farming practices on agricultural soil microbiome members represented by Metagenomically Assembled Genomes (MAGs) and their predicted plant-beneficial genes. *Genes*, 10(6), p.424.
- Norman, J.R. and Hooker, J.E., 2000. Sporulation of *Phytophthora fragariae* shows greater stimulation by exudates of non-mycorrhizal than by mycorrhizal strawberry roots. *Mycological Research*, 104(9), pp.1069-1073.
- Oburger, E. and Jones, D.L., 2018. Sampling root exudates—mission impossible?. *Rhizosphere*, 6, pp.116-133.
- Paz, H., 2003. Root/Shoot allocation and root architecture in seedlings: variation among Forest Sites, Microhabitats, and Ecological Groups1. *Biotropica*, 35(3), pp.318-332.

- Pearson, J.N. and Jakobsen, I., 1993. Symbiotic exchange of carbon and phosphorus between cucumber and three arbuscular mycorrhizal fungi. *New Phytologist*, 124(3), pp.481-488.
- Péret, B., Svistoonoff, S. and Laplaze, L., 2018. When plants socialize: symbioses and root development. *Annual Plant Reviews online*, pp.209-238.
- Phillips, D.A., Fox, T.C., King, M.D., Bhuvaneswari, T.V. and Teuber, L.R., 2004. Microbial products trigger amino acid exudation from plant roots. *Plant Physiology*, 136(1), pp.2887-2894.
- Pickles, B.J., Truong, C., Watts-Williams, S.J. and Bueno, C.G., 2020. Mycorrhizas for a sustainable world. *New Phytologist*, 225(3), pp.1065-1069.
- Plett, J.M. and Martin, F.M., 2018. Know your enemy, embrace your friend: using omics to understand how plants respond differently to pathogenic and mutualistic microorganisms. *The Plant Journal*, 93(4), pp.729-746.
- Prach, K. and Walker, L.R., 2011. Four opportunities for studies of ecological succession. *Trends in Ecology and Evolution*, 26(3), pp.119-123.
- Pulsford, S.A., Lindenmayer, D.B. and Driscoll, D.A., 2016. A succession of theories: purging redundancy from disturbance theory. *Biological Reviews*, 91(1), pp.148-167.
- Rasman, S., Bennett, A., Biere, A., Karley, A. and Guerrieri, E., 2017. Root symbionts: Powerful drivers of plant above-and belowground indirect defenses. *Insect Science*, 24(6), pp.947-960.
- Regus, J.U., Wendlandt, C.E., Bantay, R.M., Gano-Cohen, K.A., Gleason, N.J., Hollowell, A.C., O'Neill, M.R., Shahin, K.K. and Sachs, J.L., 2017. Nitrogen deposition decreases the benefits of symbiosis in a native legume. *Plant and Soil*, 414(1-2), pp.159-170.
- Remy, W., Taylor, T.N., Hass, H. and Kerp, H., 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences of the United States of America*, 91(25), pp.11841-11843.
- Řezáčová, V., Konvalinková, T. and Jansa, J., 2017. Carbon fluxes in mycorrhizal plants. In *Mycorrhiza-eco-physiology, secondary metabolites, nanomaterials* (pp. 1-21). Springer, Cham.

- Rosenberg, E. and Zilber-Rosenberg, I., 2011. Symbiosis and development: the hologenome concept. *Birth Defects Research Part C: Embryo Today: Reviews*, 93(1), pp.56-66.
- Rosenberg, E. and Zilber-Rosenberg, I., 2016. Microbes drive evolution of animals and plants: the hologenome concept. *MBio*, 7(2), e01395-15.
- Sasse, J., Martinoia, E. and Northen, T., 2018. Feed your friends: do plant exudates shape the root microbiome? *Trends in Plant Science*, 23(1), pp.25-41.
- Slavíková, R., Püschel, D., Janoušková, M., Hujšlová, M., Konvalinková, T., Gryndlerová, H., Gryndler, M., Weiser, M. and Jansa, J., 2017. Monitoring CO₂ emissions to gain a dynamic view of carbon allocation to arbuscular mycorrhizal fungi. *Mycorrhiza*, 27(1), pp.35-51.
- Slewinski, T.L. and Braun, D.M., 2010. Current perspectives on the regulation of whole-plant carbohydrate partitioning. *Plant Science*, 178(4), pp.341-349.
- Shi, M., Fisher, J.B., Phillips, R.P. and Brzostek, E.R., 2019. Neglecting plant–microbe symbioses leads to underestimation of modeled climate impacts. *Biogeosciences*, 16(2), pp.457-465.
- Smith, S.E. and Read, D.J., 2010. *Mycorrhizal Symbiosis*. Academic press. London.
- Smith, S.E. and Smith, F.A., 2012. Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia*, 104(1), pp.1-13.
- Spinosa, R., 2008. Fungi and Sustainability. *Fungi*, 1(1), pp.38-43.
- Stefania, S., Chialva, M., Novero, M., Bonfante, P. and Lanfranco, L., 2018. Performances of mycorrhizal tomatoes under water and nutrient stress conditions. In *V International Plant Science Conference (IPSC)* (pp. 86-86).
- Suyal, D.C., Joshi, D., Debbarma, P., Soni, R., Das, B. and Goel, R., 2019. Soil Metagenomics: Unculturable Microbial Diversity and Its Function. In *Mycorrhizosphere and Pedogenesis* (pp. 355-362). Springer, Singapore.
- Tang, Y.J., Martin, H.G., Myers, S., Rodriguez, S., Baidoo, E.E. and Keasling, J.D., 2009. Advances in analysis of microbial metabolic fluxes via ¹³C isotopic labeling. *Mass Spectrometry Reviews*, 28(2), pp.362-375.

- Taylor, A.F., Gebauer, G. and Read, D.J., 2004. Uptake of nitrogen and carbon from double-labelled (^{15}N and ^{13}C) glycine by mycorrhizal pine seedlings. *New Phytologist*, 164(2), pp.383-388.
- Thompson, P.B., 2017. *The spirit of the soil: Agriculture and environmental ethics*. Routledge. New York.
- Ulrich, D.E., Sevanto, S., Peterson, S., Ryan, M. and Dunbar, J., 2019. Effects of soil microbes on functional traits of loblolly pine (*Pinus taeda*) seedling families from contrasting climates. *Frontiers in Plant Science*, 10, p.1643.
- Uroz, S., Courty, P.E. and Oger, P., 2019. Plant Symbionts Are Engineers of the Plant-Associated Microbiome. *Trends in Plant Science*, 24(10), pp.895-916.
- Van Bel, A.J., 2003. The phloem, a miracle of ingenuity. *Plant, Cell and Environment*, 26(1), pp.125-149.
- Walker, T.S., Bais, H.P., Grotewold, E. and Vivanco, J.M., 2003. Root exudation and rhizosphere biology. *Plant Physiology*, 132(1), pp.44-51.
- Wang, B. and Qiu, Y.L., 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*, 16(5), pp.299-363.
- Wardlaw, I.F., 1969. The effect of water stress on translocation in relation to photosynthesis and growth. *Australian Journal of Biological Sciences*, 22(1), pp.1-16.
- Wenner, M., 2007. Humans carry more bacterial cells than human ones. *Scientific American*, 30. pp.34-56.
- Wright, D.P., Read, D.J. and Scholes, J.D., 1998. Mycorrhizal sink strength influences whole plant carbon balance of *Trifolium repens* L. *Plant, Cell and Environment*, 21(9), pp.881-891.
- Xavier, L.J.C. and Germida, J.J., 1999. Impact of human activities on mycorrhizae. In *Proceedings of the 8th International Symposium on Microbial Ecology* Bell CR, Brylinsky M, Johnson-Green P (eds) Atlantic Canada Society for Microbial Ecology, Halifax, Canada.
- Zilber-Rosenberg, I. and Rosenberg, E., 2008. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Reviews*, 32(5), pp.723-735.

8. Appendices

This section will provide some background photographs of the experimental design and construction.

1. Experimental design and construction photos of Chapter 3



Plate 1: Memorial roof glasshouse 3 with experimental Chapter 3 and 4, in progress (taken 18/05/16).



Plate 2: Chapter 3 bisected plant pot with 39 micron screen secured to exclude tree roots from half the pot volume.



Plate 3: 72 pots completed for the experimental Chapter 3.



Plate 4: An *A. glutinosa* (common alder) tree potted in the bisected pot in air-dried soil that had been sieved to 4 mm to be used in Chapter 3.



Plate 5: A *C. sativa* (sweet chestnut) tree potted in the bisected pot in air-dried soil that had been sieved to 4 mm to be used in Chapter 3.



Plate 6: *C. sativa* (sweet chestnut) potted into a bisected pot for experimental Chapter 3 (taken 18/05/16)

2. Experimental design and construction photos of an experiment which does not appear in the thesis

This Perspex slab experiment was a failed attempt to visualise ^{14}C activity being exuded, using a phosphor imaging machine to photograph the fine roots after a ^{14}C pulse-chase had been applied. Unfortunately after constructing the Perspex slabs and growing the three species for 6 months, it was discovered the phosphor imaging machine wasn't working.



Plate 7: Perspex cut up ready for slab construction

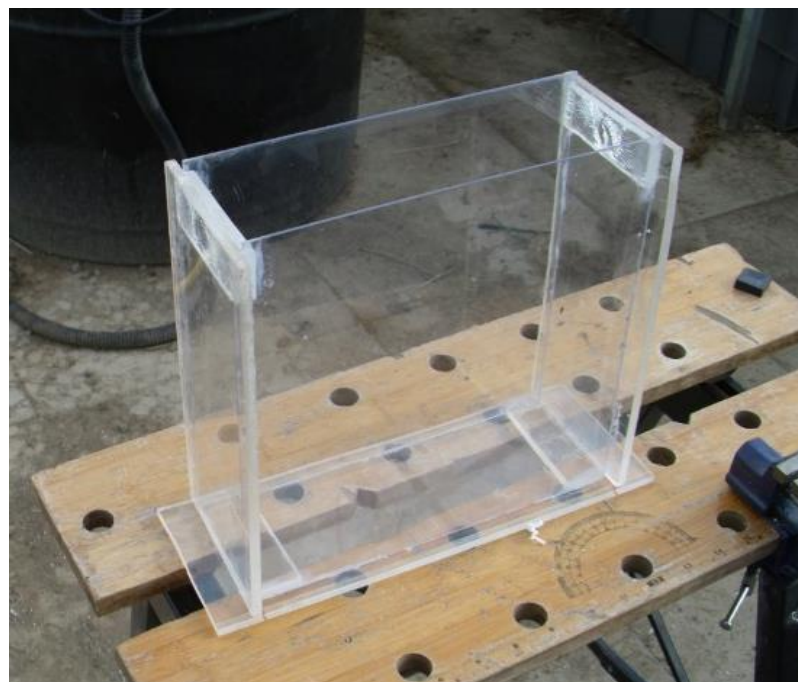


Plate 8: First Perspex slab constructed



Plate 9: reinforced Perspex slabs



Plate 10: stones were placed in the bottom of the slabs to allow drainage and prevent anaerobic conditions rom damaging the roots



Plate 11: three species of tree potted up in the slabs



Plate 12: Growth of trees in slabs (taken 18/05/2016)

3. Experimental design and construction photos of Chapter 4 showing the Perspex tubes interconnecting pots (transfer via. common hyphal networks)

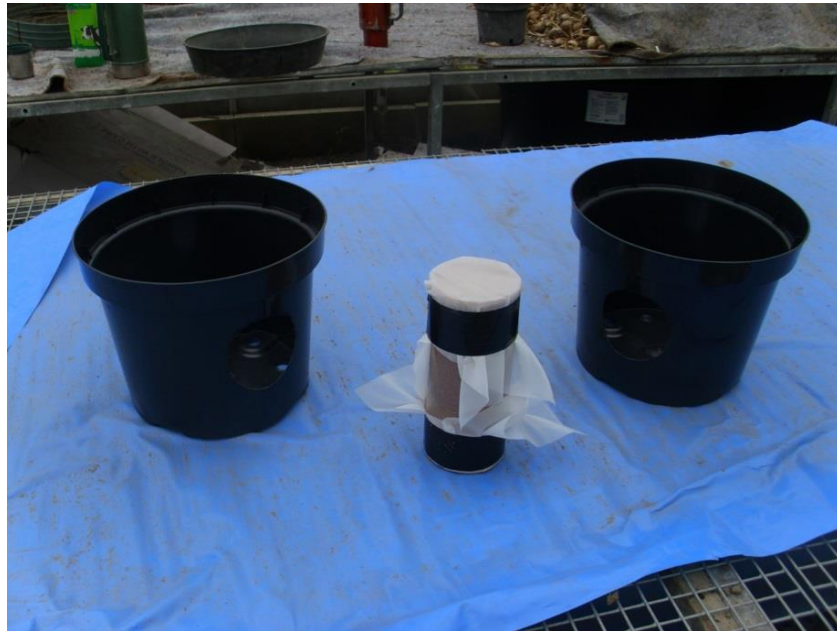


Plate 11: 48 pots had elliptical holes which matched the diameter of the interconnecting tubing



Plate 12: Interconnecting tubing was each filled with 2kg of sand and 20g of bone-meal, then the ends were covered in 40 micron screen and secured with fabric tape



Plate 13: pots were then stapled together with wooded supporting struts to prevent mycorrhizal hyphal from being damaged in transit during the experiment



Plate 14: Interconnecting tubing was each filled with 2kg of sand and 20g of bone-meal, then the ends were covered n 40 micron screen



Plate 15: Pots were then planted with the trees and left to grow and develop mycorrhizal