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DOCTOR OF PHILOSOPHY

An evaluation of perennial mobile green manures for climate change mitigation in agriculture

Ward, Chloe

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**An evaluation of perennial mobile green manures
for climate change mitigation in agriculture**

A thesis submitted to Bangor University by
Chloe Ward

In candidature for the degree of:
Philosophiae Doctor
September 2020

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Abstract

Much of the climate change impact of agriculture is due to the addition of nitrogen (N) to soil to increase crop yields. The production of mineral N fertiliser from atmospheric N₂ causes carbon dioxide (CO₂) emissions, and once added to soil, fertiliser N is easily transformed to other forms and lost as leached nitrate (NO₃⁻), ammonia gas (NH₃) and the greenhouse gas nitrous oxide (N₂O). An alternative to fertiliser N is the use of green manures, plants grown on agricultural land, which after incorporation into soil provide N to the following crop. N fixing green manures gain atmospheric N by a symbiosis with microbes, therefore adding N without CO₂ emissions, and providing beneficial organic matter to soil. Counteracting these advantages is a lower land use efficiency due to the space required for green manure cultivation. Green manure additions can result in carbon (C) sequestration, however soil C dynamics are complex and soil C can also be lost by a stimulation of respiration known as priming.

N pollution from soil is generally higher when mineral N concentrations are high, which can result from poor synchrony of N supply with crop demand. The incorporation of green manures offers limited scope for effective targeting of N. This synchrony of N supply with demand could be improved by, instead of incorporating green manures, harvesting the plant material and adding it to soil to meet crop demand. Negating the requirement for incorporation, allows use of a wider range of species including shrubs and trees. Growing N fixers suited to lower quality land e.g. wet or exposed areas, could increase land use efficiency by reducing the demand for prime agricultural land. We chose three such N fixing species and refer to these as Perennial Mobile Green Manures (PMGMs): *Alnus glutinosa* (Alder), *Gunnera manicata* (Gunnera), and *Ulex europaeus* (Gorse). This thesis investigated N provision to a crop, and potential for N pollution by these PMGMs, compared to the conventional green manure, *Trifolium pratense* (red clover) and ammonium nitrate (NH₄NO₃) fertiliser, in a one year pot experiment, and field experiments over two seasons. A six week incubation investigated soil C dynamics by use of ¹⁴C isotopes.

In both pot and field experiments, the PMGMs supplied N to crops at a slower rate than clover or NH₄NO₃ but by the end of the pot experiment resulted in equal or more biomass and N uptake than from clover. Potential for N pollution from PMGMs was considerably lower than from clover or NH₄NO₃, with mean NO₃⁻ concentrations in the soil solution of the pot experiment reaching only 25 mg N L⁻¹ compared to over 130 mg N L⁻¹ from clover and NH₄NO₃ additions. Emissions of N₂O from PMGMs were considerably lower than those from clover and NH₄NO₃, in the pot trial, with applied N lost as N₂O-N from gunnera and alder being 0.34 %, and 0.61 %, respectively compared to 5.3 % from clover. N₂O emissions from all treatments in the field experiment were low which is likely to be due to dry weather conditions. Data from the incubation study indicated that after one year, gunnera and alder additions could result in a net loss of C due to priming. These predictions, however, do not consider factors of cropping soil e.g. roots, and meso and macro fauna and require further investigation. We conclude that PMGMs could improve N use efficiency and reduce NO₃⁻ leaching and N₂O emissions compared to incorporation of clover, and have potential for a more favourable C balance than NH₄NO₃. Thus, PMGMs have strong potential for inclusion into a more sustainable agricultural landscape.

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Centre for Alternative Technology
Canolfan y Dechnoleg Amgen

List of Abbreviations

BNF	Biological Nitrogen Fixation
CDD	Cumulative Day Degree
CEC	Cation Exchange Capacity
DM	Dry Matter
EF	Emission Factor
FE	Fertiliser Equivalent
DOC	Dissolved Organic Carbon
DON	Dissolved Organic Nitrogen
DW	Dry Weight
GHG	Greenhouse Gas
IPCC	Intergovernmental Panel on Climate Change
NRE	Nitrogen Recovery Efficiency
NUE	Nitrogen Use Efficiency
PMGM	Perennial Mobile Green Manure
SM	Secondary metabolite
SEM	Standard Error of the Mean
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
TGW	Thousand grain weigh
TN	Total Nitrogen
WFPS	Water Filled Pore Space

Chapter 1. Introduction and Thesis Aims

1.1. Introduction

The agriculture we rely on for human survival is itself contributing to the threat to that survival by destruction of the ecological and planetary systems which sustain us (IPCC 2019). Agriculture is one of the major contributors to biodiversity loss (Hayhow et al. 2019, IPBES 2019), and an estimated 23 % of global greenhouse gas emissions are due to land use, principally agriculture (IPCC 2019). The increasingly unstable climate and the weakening of the ecosystem services necessary for the functioning of agriculture is threatening crop productivity (IPCC 2019, FAO 2019, Pilling et al. 2020). To avoid an unprecedented humanitarian crisis, we depend on mitigating climate change, and biodiversity loss while also maintaining agricultural production (Ripple et al. 2020).

Nitrogen (N) is the most commonly limiting nutrient for crop growth (Olson and Kurtz 1982, Hirsch and Mauchline 2015, Moreau et al. 2015). High agricultural yields are made possible by the supply of plant available N compounds which have been converted from N₂ gas either by an industrial process powered by fossil fuels or by the growing leguminous plants which, by association with bacteria, fix N using sunlight energy (Battye et al. 2017). This has enabled huge increases in food production, which is now relied on to feed the world's population (Erisman et al. 2008). As a consequence, it is estimated that 70 % of reactive N in the terrestrial ecosystems is now due to anthropogenic activity (Battye et al. 2017).

That climate change and agricultural production are both principally determined by the exchange of carbon (C) and N between non-gaseous forms in the soil and gaseous forms in the air, gives agriculture a key role as both a driver of climate change and a solution to it. Our success, or otherwise, in both, will be determined in part by our ability to efficiently fix N, and retain it in the agricultural system until the N contained within proteins is consumed by people. In tandem to this is the necessity to fix more C than that respired, whether that respiration is by microbes, larger life forms, or power stations, and retain the fixed C in living or dead organic matter. What this amounts to in agricultural terms is high N use efficiency, while limiting losses of C, by, for example fossil fuel use for fertiliser manufacture, or loss of soil C caused by agricultural practices.

1.2. Impacts of mineral and organic N

N fertiliser production uses in the region of 1 to 2 % of the world's energy supply, causing associated CO₂ emissions from fossil fuel use (Houlton et al. 2019). High concentrations of mineral N in soil, can lead to leaching of nitrate (NO₃⁻) causing pollution of waterways, or emission of the potent greenhouse gas nitrous oxide (N₂O) (Galloway et al. 2003, Fowler et al. 2013, Battye et al. 2017). N₂O has around

300 times the global warming potential of CO₂ over a 100 year period (Giles et al. 2012, Benckiser, Schartel, and Weiske 2015) and agriculture, forestry and other land use accounts for 81 % of its emissions (IPCC 2019).

Biologically fixed N is often supplied to arable soil by N fixing green manure plants which are sown on the land where the N is to be added. Once N has been fixed and accumulated in the green manure biomass, it is incorporated into the soil, and the following crop uptakes N from the decomposed plant matter (Blanco-Canqui et al. 2015, Carr et al. 2020). Production of green manures does not cause the CO₂ emissions associated with N fertiliser manufacture and the incorporation of plant matter to the soil can improve soil health, and sequester C (Kibblewhite et al. 2008, Lehtinen et al. 2014, Poeplau and Don 2015)

N supplied to soil within plant matter, however, once converted to mineral forms, is also prone to loss, by leaching (Neeteson and Carton 2001, Campiglia et al. 2011) and as N₂O (Chen et al. 2013, Muhammad et al. 2019). NO₃⁻ leaching and production of N₂O generally increase when the N supplied is in excess of that needed by crops due to the accumulation of mineral N in soil (Crews and Peoples 2005, Moller 2018, Hansen et al. 2019). Fertiliser N is often added in several applications to synchronise with crop demand (AHDB 2018), and techniques of precision agriculture continue to be developed to further target supply and demand, (Norton and Ouyang 2019, Rees et al. 2020). Green manures added by incorporation, however, do not offer this flexibility and therefore, synchrony can be poor (Crews and Peoples 2005, Chen et al. 2014). Another drawback of supply of N by green manures is the requirement for cropland to be set aside for N fixing, which reduces the land available for crop production, and so reduces overall yields from such systems (Smith et al. 2018), limiting the amount of land available for preservation of natural habitats for biodiversity and C sequestration.

1.3. Perennial Mobile Green Manures

A method used to improve the synchrony of N supply by green manures is the practice of cutting green manures and applying them at times and locations to better match with crop demand, which are known as “cut and carry” (Van der Burgt et al. 2013, Benke et al. 2017) or “mobile green manures” (Sorensen and Thorup-Kristensen 2011). This thesis explores a novel strategy, which combines the technique of mobile green manures with that of supplying N via long lived perennial N fixing plants which we refer to as Perennial Mobile Green Manures (PMGMs). These N fixing plants can be chosen to be suited to growing on lower quality land, such as on exposed or flood-prone sites, and therefore increase land use efficiency by reducing the area required for N fixing high quality cropping land. The N fixing areas could simultaneously act as biodiversity reserves and sequester C in unploughed soil and woody parts of plants. Successful use of PMGMs could increase both the land use efficiency and

the nitrogen use efficiency (NUE) over that of traditional green manures, while retaining the advantages over mineral N of N fixing without CO₂ emissions and supply of organic matter to soil. We research the use of PMGMs for their N supply to a crop, and effects on climate change mitigation.

1.4 Aims and thesis plan

For the use of PMGMs to be viable, perennial N fixing plants which can grow on low quality land, and produce leaf matter which can supply N to crops need to be identified. Here we investigated three plant species which could be potentially used as PMGMs: *Alnus glutinosa* (Alder), *Gunnera manicata* (Gunnera), and *Ulex europaeus* (Gorse) for their performance as an N source to crops and their environmental impacts. The literature review (Chapter 2) summarises the current knowledge on the use of green manures, their functioning in agricultural systems, and how they impact N and C dynamics in soil to affect climate change impact.

Chapters 3 to 6 report laboratory, pot and field experiments assessing different aspects of the effect of PMGMs. The PMGMs were trialled in comparison to N rich products of the two other commonly used methods of fixing N for temperate agriculture, that of mineral fertiliser, the product of Haber Bosch N fixing and of traditional green manure leaf tissue, the product of leguminous N fixing. Chapter 3 reports a pot experiment which assessed the N fertilisation value of the PMGMs, when leaves were mixed into soil and plants grown in the leaf-soil mix. The N uptake of plants growing in PMGM fertilised soil was compared to that of plants growing in soil treated with leaves of the traditional green manure red clover (*Trifolium pratense*) and additions of the mineral fertiliser ammonium nitrate (NH₄NO₃). In addition, the N₂O emissions were measured as well as various soil properties to gain an understanding of N and C dynamics.

The pot experiment is followed by a laboratory incubation experiment (Chapter 4) to measure the C dynamics as affected by the PMGMs, again with comparison with red clover and NH₄NO₃. Chapters 5 and 6 report two years of testing the PMGMs in a field experiment measuring the same outputs as the pot experiment, with an additional measurement of loss of N from soil as ammonia (NH₃). During the first year the effects of drying and mulching PMGM material were compared with those of using fresh material or incorporation into soil. The second year gave data on the effect of repeated additions of PMGMs giving an insight into their longer term effects. In Chapter 7, the results across all experiments are considered and recommendations given for future research.

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Chapter 2. Literature Review: Climate change impact of green manure use in agriculture and a novel approach to mitigation.

Contributions: CW wrote the chapter with advice and edits from PWH.

2.1. Comparative effects of provision of N via green manures and mineral N

2.1.1 Mineral N fertiliser

To produce today's high crop yields, large scale agriculture commonly relies on supplementing soil N with industrially produced N supplied as soluble fertiliser, most often as ammonium nitrate (NH_4NO_3), urea or urea-ammonium nitrate (AHDB 2018, Defra 2020). Application rates of mineral N fertiliser in the UK are typically between 100 and 200 kg N ha⁻¹ to arable crops, and between 50 and 100 kg N ha⁻¹ to grassland (Brown et al. 2019), with an average of 137 and 54 kg N ha⁻¹ applied to tillage crops and grassland respectively in the UK in 2019 (Defra 2020). Industrial N fixing uses the Haber-Bosch process, commonly using energy from natural gas to break the strong triple bond between the paired N atoms in atmospheric dinitrogen (N_2) and converting it to NH_3 (Wood and Cowie 2004, Smil 2011). Non fossil fuel methods to reduce or eliminate the CO_2 emissions in industrial N fixing are becoming more feasible, such as use of hydrogen generation from renewable electricity (Houlton et al. 2019), or transition-metal-catalysed reduction of N gas (Ashida et al. 2019, Bezdek and Chirik 2019). However, at present N fertiliser production uses an estimated 1 to 2 % of the world's fossil fuel energy with the associated CO_2 emissions (Houlton et al. 2019).

2.1.2 Biological nitrogen fixation

Before the commercialisation of the Haber Bosch process in 1913, agricultural production depended on the management of biological nitrogen fixation (BNF) (Smil, 2011). Leguminous crops such as soya beans gain N through a symbiosis with the N_2 -fixing bacteria Rhizobia which in return gain fixed C in sugars from plant photosynthesis (Smil 1999, Marschner 2011). Other N fixing and non-N fixing plants are also grown and used in a variety of ways with the purpose of supplying N to crops, rather than being harvested for any saleable part. The term cover crop is generally used to refer to a crop sown on land which would otherwise be left bare, often over winter, with the purposes of protecting soil from erosion by wind and water, and taking up N which could otherwise be lost via leaching (Blanco-Canqui et al. 2015). Catch crops are used similarly but often for shorter periods of time, for example in summer between the harvest of one crop and the sowing of the next. Green manures, however, are used not just for prevention of leaching on un-used land, but are often grown instead of an economically saleable crop with the purpose of increasing the N supply to the following crop (Snapp et al. 2005, Cherr, et al. 2006, Blanco-Canqui et al. 2015, Carr et al. 2020, Tei et al. 2020). A green

manure fertility building phase can cover a full year or more and can be referred to as a ley, especially where the green manures are grazed by animals (Stockdale et al. 2019, Hansen et al. 2019).

Commonly used leguminous green manures for temperate regions include red clover (*Trifolium pratense*), white clover (*Trifolium repens*), hairy vetch (*Vicia sativa*), and alfalfa (*Medicago sativa*) (Snapp et al. 2005, Cherr et al. 2006, Rosenfeld and Rayns 2011). Non N fixing species are also used as cover crops or green manures, and sometimes referred to as “N lifters”, due to their role in preventing N leaching further down the soil profile, and bringing it up into the plant leaves from which it is re-added to soil (Philipps et al. 2003). Typically, a mix of N fixers and N lifters is recommended, with deep rooted N lifters to prevent leaching, and N fixers to fix N when sunlight energy is plentiful. Non-legumes which are often grown in combination with legumes include perennial ryegrass (*Lolium perenne*) and cocksfoot (*Dactylis glomerata*) (Rosenfeld and Rayns 2011). Cover crops and green manures are usually incorporated into soil by tillage (Abdalla et al. 2019). However, they are also used in no till systems by termination by herbicides, and other methods are being developed by direct drilling into a mulch of cover crop, which has been killed in situ by roller-crimper machinery, or by cold winter temperatures (Halde et al. 2014, Cornelius and Bradley 2017, Frascioni et al. 2019). Plant N is also incorporated into soil as crop residues, crop parts which are left in the field after harvest (De Neve et al. 2004, Coppens et al. 2007, Chen et al. 2014, Kan et al. 2020).

2.1.3 Plant organic matter to increase soil C

Addition of organic plant material to soil, can result in C sequestration and is an important tool in climate change mitigation (Blanco-Canqui et al. 2015, Minasny et al. 2017). Increased soil organic matter (SOM) content has many reported advantages for soil health including increased soil stability, soil porosity and improved drainage, water holding capacity, and increased microbial biomass which promotes nutrient cycling (Whalen and Sampedro 2010, Blanco-Canqui et al. 2015, Bhattacharya et al. 2016, Abdalla et al. 2019). Conversely, a reliance on inorganic N fertilisation can reduce the size and diversity of the microbiome (Stockdale et al. 2002, Ramirez, Craine, and Fierer 2012, Ma et al. 2018). Green manures and soil organic C are explored further in section 2.5.

2.1.4 Land use efficiency

Counteracting the positive impacts of supplying N via BNF within plant matter is the disadvantage of the land area required for N fixing. Agriculture is at present estimated to cover 49% of the world’s ice-free land surface (IPCC 2019), and the efficiency of crop production determines the land area left available for habitats to provide essential ecosystem services and C sequestration in soils and biomass (Lamb et al. 2016, Balmford et al. 2018). Though the provision of fixed N via green manures negates the need for fossil fuels, it is effectively swapping fossil fuel energy (the product of historical

photosynthesis), for the use of energy supplied by present day photosynthesis and therefore the present day use of land area for sunlight energy capture.

Though leguminous cover crops during winter can provide some N, it is green manures, grown during the summer months which fix the largest quantity of N. Green manures are typically reported to fix annual rates in the region of 150 kg N ha^{-1} , though this can vary considerably (Rosenfeld and Rayns 2011, Lupwayi and Soon 2015, Carr et al. 2020). The land area required per unit of crop production in systems where all the crop's N is supplied by BNF fixed on the farm, is necessarily more than that required in fertiliser N systems (Williams et al. 2006, Powlson et al. 2011, Smith et al. 2018). It is difficult to measure the amount of land would be needed for green manures to fix the total amount of N required for arable cropping, as present day organic arable farming often relies partly on inputs of animal manure N originally fixed by legumes such as clovers on grazing land. However, with green manure N fixing rates of around 150 kg N ha^{-1} and typical fertiliser requirements being on average 137 kg N ha^{-1} applied for arable crops (Defra 2020) it is likely to be a substantial proportion of land. Though other factors, both social and industrial, impact heavily on the quantity of land required for agriculture, notably the proportion of plant verses animal protein in the diet, and the quantities of food wasted (Foley et al. 2011, Galloway et al. 2017, Houlton et al. 2019), the extra land required for provision of N by BNF is impactful on agricultural efficiency.









2.1.5 Nitrogen use and recovery efficiency

Another important driver of agricultural efficiency is the proportion of the supplied N which is taken up by the crop, referred to as N recovery efficiency (NRE) or N uptake efficiency (Fageria and Baligar 2005, Conant et al 2013, Salim and Raza 2020). As with mineral fertilisers, N from green manures can be lost via leaching as NO_3^- causing eutrophication of rivers, and as N gases including the greenhouse gas N_2O (Zarabi and Jalai 2012, Chen et al. 2013, Pugesgaard et al. 2017). An important technique for reducing N loss is that of synchronising the supply with demand (Crews and Peoples, 2005). The use of green manures by incorporation, however, cannot benefit from techniques to increase NRE through precision agriculture which are being applied to use of mineral fertilisers (Tei et al. 2020, Rees et al. 2020). The overall efficiency of N use is influenced by more than just the NRE and includes the retention of N in agricultural systems and the efficiency of plant N use and is referred to as N use efficiency (NUE) (Cassman et al. 2002, Perchlik and Tegeder 2017, Kubota et al. 2018). The issue of NUE in green manure use, and N_2O emissions are explored further in sections 2.3 and 2.4.

2.1.6 Comparison of benefits and drawbacks

Table 1.1 summarises the environmental benefits and drawbacks of supplying N via industrial or biological fixation. The supply of C and N are fundamentally linked, as fixed C is required to supply the energy to fix N, and fixed N is required as a component of the organic materials necessary for photosynthesis. The aim of increasing and preserving both N and C in organic forms, and reducing N and C in gaseous forms, would seem to fundamentally favour the use of BNF. However, the high demands on land and the difficulties in increasing NUE are disadvantages for organic systems. An increase in the efficiencies of both land use and NUE in such systems would contribute greatly to food security and climate change mitigation. A challenge is to use BNF in such a way that it also has the advantages afforded by mineral N of flexibility and targeting of application. In addition, the inefficiencies of devoting agricultural land to BNF need to be addressed.

Table 1.1 Summary of advantages and disadvantages of provision of N via green manures and mineral fertiliser.

Method of N fixation	Biologically fixed N supplied via green manures	Industrially produced N fertiliser via the Haber Bosch process.
CO ₂ emissions in N fixing	 Carbon neutral by use of photosynthetic energy	 N fertiliser production emits 1 to 2 % of the world's CO ₂ (Houlton et al. 2019).
Addition of organic matter	 Addition of organic matter can improve soil functioning (Blanco-Canqui et al. 2015) and sequester C (Minasny et al. 2017)	 Does not provide organic matter.
Effect on land use efficiency	 Takes agricultural land out of productive cropping, reducing land use efficiency	 Use of mineral N has resulted in huge increases in crop yields (Erisman et al. 2008)
Synchrony of N supply and demand	 Difficult to achieve, which can result in low NUE and emissions of N ₂ O (Crews and Peoples 2005)	 Targeted N application offers increasing N use efficiency (Rees et al. 2020)

2.2 N cycling in agriculture

In 2009, Rockstrom et al. identified the global amount of reactive N (comprising a range of N compounds) to be exceeding our “planetary boundaries”, having breached safe limits for human survival (Rockstrom et al. 2009). Whereas diatomic N_2 which makes up most of our atmosphere is in a very stable form, other compounds of the N cycle are highly reactive, and easily converted to other forms which become pollutants such as NO_3^- and N_2O (Smil 1999, Battye et al. 2017). The N cycle is a complex system of N transformations, in which there are many routes of loss of reactive N as pollutants, or back to atmospheric N_2 . Anthropogenic activity has increased the levels of reactive N so that currently in the region of 80 Mt N yr^{-1} is fixed by the Haber-Bosch process, and about 40 Mt N yr^{-1} by cultivation of legumes in agriculture, in addition to approximately 30 Mt N yr^{-1} of ammonia production caused by fossil fuel combustion and biomass burning (Rockstrom et al. 2009). To return to a safe level of reactive N in global systems, Rockstrom et al. (2009) recommended reducing N fixation down to 35 Mt N yr^{-1} . This is a huge challenge to agriculture. At present about 50% of N supplied to crops is lost to the environment by leaching or as gaseous emissions (Coskun et al. 2017). Reducing the inputs of reactive N to agricultural systems, and prevention of loss of N as polluting forms, while maintaining food security, requires strategies to influence N transformations, and increase N use efficiency (NUE) much more successfully than at present. The following gives an overview of the major processes which make up the N cycle in agricultural systems. This is represented in simplified form in Fig 2.1.

2.2.1 Biological Nitrogen Fixation

Plants are unable to directly use the stable N_2 which makes up 78 % of the atmosphere and therefore rely on N to be “fixed” into reactive forms (Peoples et al. 1995, Bloom 2015). BNF is the reduction of N_2 to NH_3 , mediated by N fixing micro-organisms which can be free living, or live in association with plants (Bloom 2015). In symbiosis with some species of plants, notably legumes, of the *Fabaceae* family, N fixing bacteria use energy from the respiration of C provided by plant photosynthesis to break the triple bond within N_2 , while the plant symbiont gains biologically available N (Galloway et al., 2003, Whalen and Sampedro, 2010). N fixation is enabled by two enzymes: dinitrogenase reductase, an Fe containing protein which gathers electrons, and dinitrogenase, an MoFe containing protein which uses the electrons to produce NH_3 (Whalen and Sampedro 2010). Leguminous crops benefit from N fixing directly; the N gained allowing agricultural production of high protein seeds such as soya and fava beans (Bloom 2015, Battye et al. 2017). Other agricultural crops benefit indirectly from legume fixed N after decomposition of crop residues or leguminous green manures in soil, or via animal manure N from livestock grazing on leguminous plants as shown in Fig 2.1 (Berry et al. 2002, Carr et al. 2020).

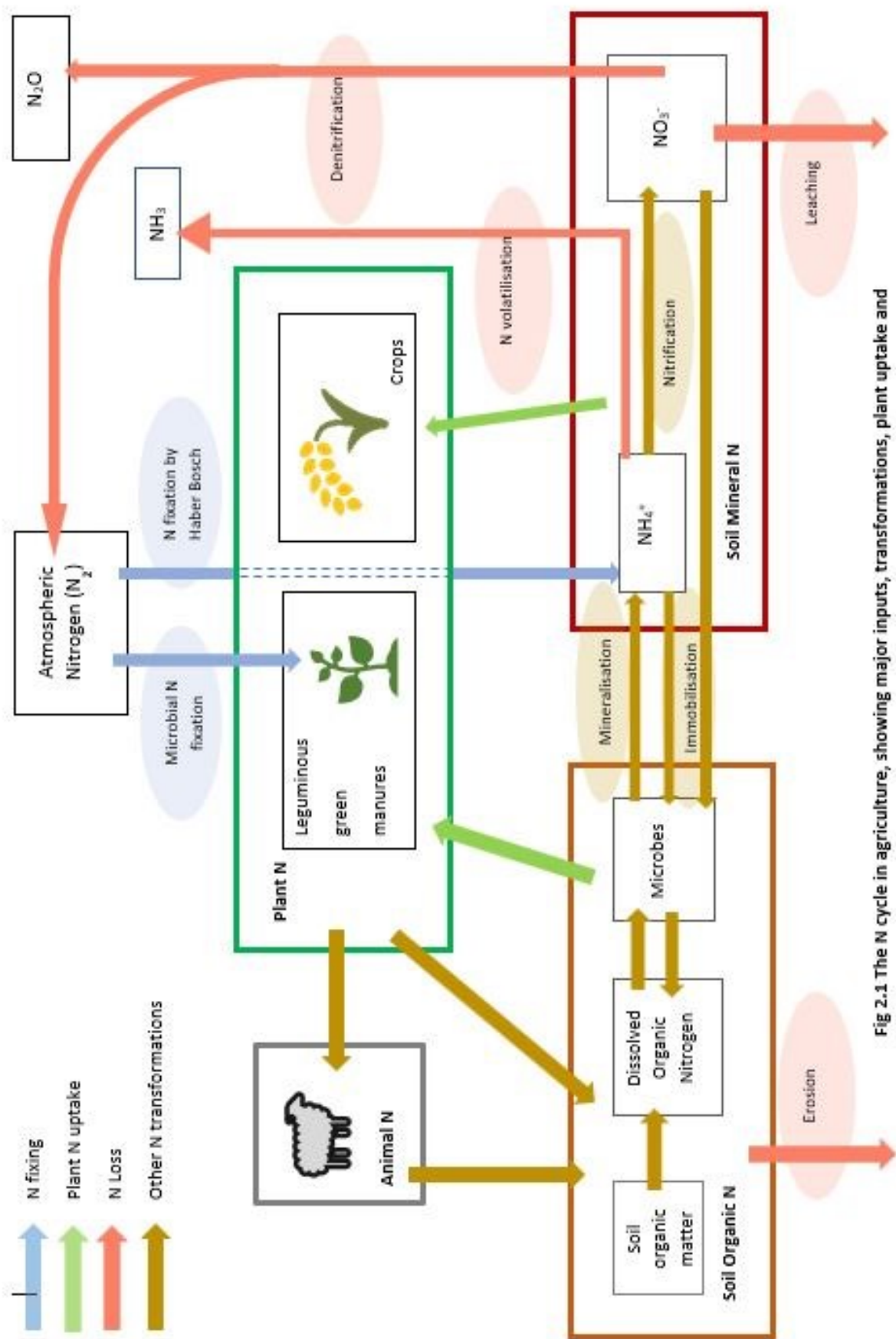


Fig 2.1 The N cycle in agriculture, showing major inputs, transformations, plant uptake and losses

Non leguminous plants also form associations with N fixing microbes. Plants in a range of families including the Betulaceae, Eleagnaceae and Rhamnaceae form associations with the actinobacteria *Frankia*, and are therefore referred to as actinorhizal plants (Santi et al. 2013, Pawlowski and Demchenko 2012). Plants in the Gunneraceae, Salviniaceae and Cycadaceae families form associations with the cyanobacteria *Nostoc* (Vessey et al. 2005; Santi et al. 2013). The *Azolla* fern, in the Salviniaceae family is grown and used as a green manure to supply N in rice cultivation (Lumpkin and Plucknett 1980, Yadav et al. 2014). As *Nostoc* is a non-obligate symbiont, a plant partner is not a necessity, and methods used in some rice paddy systems encourage the proliferation of free-living *Nostoc* to provide fixed N to the rice crop (Vaishampayan et al. 2001, Pawlowski and Demchenko 2012).

2.2.2 Decomposition and mineralisation of organic matter

When plant material is in contact with soil, mesofauna such as collembola, millipedes and earthworms ingest, fragment and mix the material with soil, and excrete the products of digestion, so increasing surface area available to microbes for further degradation (Whalen 2014, Sauvadet et al. 2016). The plant matter itself may contain enzymes which, when released through the breakdown of cell membranes, catalyse autolytic reactions to degrade material into simpler sub-units (Whalen and Sampedro, 2010). The breakdown of large polymers begins outside of cells, by the excretion of extracellular enzymes (Geisseler et al. 2010). Proteins are broken down by hydrolysis (adding H_2O), a reaction which is catalysed by proteolytic enzymes, producing simpler molecules, such as smaller peptides and amino acids, which can be taken up by cells (Mengel 1996, Robertson and Groffman 2007, Geisseler et al. 2010). Peptides and amino acids are further degraded to NH_3 both within cells and by extracellular enzymes in soil solution, a process known as mineralisation (Jackson et al. 2008, Geisseler et al. 2010). NH_3 within microbes which is excess to requirement is released into soil solution and protonated to NH_4^+ (Geisseler et al., 2010). At this point NH_4^+ can follow several pathways, including loss as NH_3 gas, nitrification to NO_3^- or uptake by plants as described below.

2.2.3 NH_3 Volatilisation

N can be lost from soil at this point in the N cycle by volatilisation in which NH_4^+ in solution is converted to NH_3 gas and lost to the atmosphere (Cameron et al. 2013, Engel et al. 2013, Niraula et al. 2018). Volatilisation occurs at increased rates with higher concentrations of NH_4^+ in soil. The NH_4^+ concentration is affected by various processes, including the rate of N mineralisation described above. In agricultural soils the conversion of urea to NH_4^+ can play a significant role in increasing NH_4^+ concentrations (Duan and Xiao 2000, Cameron et al. 2013). Urea is often added to soil as a fertiliser, but is also produced as a result of excretions, for example, of urine by mammals and urease by a variety of microbes (Geisseler et al. 2010). NH_4^+ concentrations are also affected by the rate of loss of

NH_4^+ by nitrification to NO_3^- (see below) and conversely the reduction of NO_3^- to back to NH_4^+ by dissimilatory nitrate reduction to ammonia (DNRA) (Rutting et al. 2011, Friedl et al. 2018, Pan et al. 2020).

The rate of volatilisation is also affected by environmental conditions and soil properties, with higher temperatures and windy conditions resulting in increased volatilisation (Sommer et al. 2004, Cameron et al. 2013, Cameira and Mota 2017). Soil qualities impact on volatilisation as a lower soil Cation Exchange Capacity (CEC) reduce the binding of NH_4^+ ions to soil particles leading to increased NH_4^+ in solution which is available for volatilisation (Duan and Xiao 2000, Cameron et al. 2013). The effect of soil moisture levels is complex, as it affects many of the NH_4^+ producing and consuming reactions described above. Decomposition of organic matter can be slowed by a lack of moisture which reduces the production of NH_4^+ . High amounts of rainfall or irrigation can reduce losses by lowering NH_4^+ concentrations and can wash the NH_4^+ lower down the soil profile resulting in lower volatilisation (Sommer et al. 2004, Cameron et al. 2013). Conversely, the drying of soil by evaporation results in increasing NH_4^+ concentrations leading to higher NH_3 volatilisation (Liu, Li, and Alva 2007). NH_3 losses are higher when the N source is applied on the soil surface, due to exposure to air and one effective way of reducing loss is the injection of urea fertilisers or manures deeper into the soil profile (Sommer and Hutchings 2001, Sommer et al. 2004, Rochette et al. 2013).

2.2.4 Nitrification

Nitrification is the oxidation of NH_4^+ , in stages to NO_2^- and NO_3^- , Fig 2.2 (Robertson and Groffman 2007, Norton and Ouyang 2019). The first reaction is the oxidation of NH_3 to hydroxylamine (NH_2OH), which is then further oxidised to nitrite (NO_2^-) (Hayatsu et al. 2008). Both these reactions are enabled by NH_4^+ oxidising organisms including autotrophic and heterotrophic bacteria and archaea (Whalen and Sampedro, 2010). NO_2^- is then oxidised to NO_3^- by nitrifiers which include autotrophic bacteria, heterotrophic bacteria and heterotrophic fungi (Robertson and Groffman, 2007; Whalen and Sampedro, 2010). Nitrification can result in N_2O production through spontaneous decomposition of NH_2OH , and by the action of NH_3 oxidisers, known as nitrifier denitrification (Giles et al. 2012, Henault et al. 2012, Prosser et al. 2020).

The biggest factor affecting the rate of nitrification is the level of NH_4^+ in the soil solution, but environmental factors and soil chemistry also have a strong influence. When soil C is not limiting, microbial N immobilisation (see below) renders NH_4^+ unavailable for nitrification (Robertson and Groffman 2007, Bottomley et al. 2012). Oxygen availability affects the rate of nitrification, as all nitrifiers are obligate aerobes, and therefore, nitrification is much reduced in waterlogged soils (Robertson and Groffman 2007, Jackson et al. 2008). Though a temperature of 25 to 35°C is optimal,

nitrification can occur in cold temperatures, such a soil under snow, albeit at a slower rate (Robertson and Groffman, 2007).

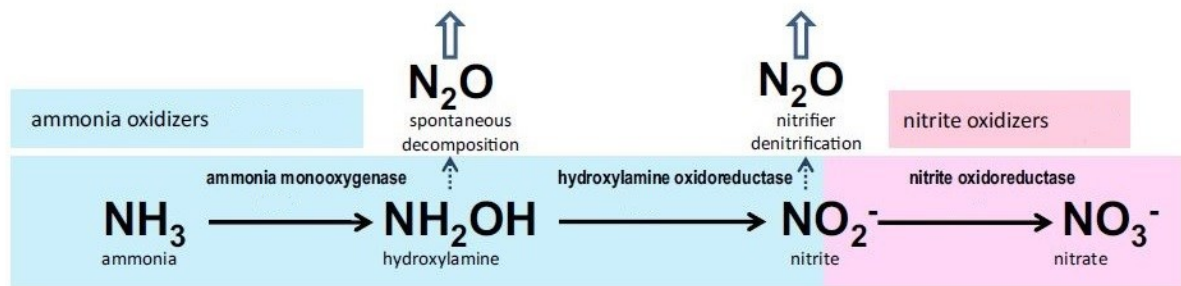


Fig 2.2. The nitrification process, showing the catalysing enzymes, and points of N_2O production. Adapted from Hirsch and Mauchline (2015)

The conversion of N from positively charged NH_4^+ to negatively charged NO_3^- has implications for N mobility and therefore for plant uptake and N losses. Most soils in temperate regions predominantly contain negatively charged particles to which positively charged NH_4^+ can bind, whereas negatively charged NO_3^- is very mobile in soil (Cameron, Di, and Moir 2013). NO_3^- , because of its high mobility, can be more available for plant uptake than NH_4^+ , but is more easily lost through leaching (Di and Cameron 2002, Norton and Ouyang 2019). The CEC of a soil is influenced by the mineral and organic content, for example a higher clay content increases CEC and therefore capacity to retain positively charged nutrients (Robertson and Groffman 2007). In tropical regions soils can have a higher proportion of positively charged particles, therefore changing the relative mobility of NH_4^+ and NO_3^- (Sollins et al. 1988). In temperate regions, as NO_3^- is prone to leaching and conversion to N gases including N_2O , it is advantageous to farmers to reduce the rates of nitrification to that necessary to supply the optimal amount of NO_3^- to crops and therefore limit N losses by leaching or denitrification (see below) (Aarons et al. 2017, Norton and Ouyang 2019).

2.2.5 Plant and microbial N uptake

Plants uptake mineral N as NH_4^+ and NO_3^- , both directly via roots and through mycorrhizal fungi (Marschner 2011). NO_3^- is usually more mobile in soil, and therefore better able to reach the vicinity of plant roots, whereas NH_4^+ is typically less available for plant uptake due to binding to soil and humic particles (Jackson et al. 2008, Hirsch and Mauchline 2015, Norton and Ouyang 2019). Microbes uptake both mineral and organic N, as amino acids and peptides and organic N can contribute significantly to microbial nutrition, with organic molecules having the advantage of providing energy and C for growth (Geisseler et al., 2010).

Until relatively recently, it was thought that the limiting factor for plant N uptake was the speed of mineralisation of organic materials by microbes, producing NH_4^+ as a waste product (Schimel and Bennett, 2004; Geisseler et al., 2010). Recent research has shown that plants also take up organic molecules intact both directly through roots (Jones et al. 2005, Paungfoo-Lonhienne et al. 2008, Hill et al. 2012) and through mycorrhizal fungi (Nasholm et al. 2009, Hodge and Storer 2015). It is now thought that the limiting step in soil N availability is the depolymerisation of polymers to simpler organic molecules (Schimel and Bennett 2004, Hill et al. 2012). In situations where microbes outcompete plants for organic molecules, the effect on plant N nutrition can be short term with microbes serving as a store and slow release of N (Kuzakov and Xu, 2013).

2.2.6 The microbial immobilisation-mineralisation balance

The uptake of N into microbial biomass rendering it unavailable to plants is known as immobilisation (Robertson and Groffman 2007, Chen et al. 2014). The balance between microbial immobilisation and mineralisation is dependent on the ratio of available C to available N in soil relative to the needs of microbes (Geisseler et al. 2010, Kuzakov and Xu 2013). If microbial growth is C limited, microbes use C within organic matter, such as protein hydrolysis products, for growth and respiration, releasing excess N as NH_4^+ into soil solution (Chen et al., 2014). Where C is highly available, microbes take up N from organic matter, immobilising N within microbial biomass, and in circumstances where N is lacking in the substrate, will also take up and immobilise soil dissolved organic N (DON) or mineral N (Nicolardot et al. 2001, Jackson et al. 2008, Chen et al. 2014). Microbial respiration, however, reduces the amount of C in microbial biomass and in soil over time, lowering the available C:N ratio eventually leading to excess N which is mineralised (Robertson and Groffman 2007, Chen et al. 2014). Because soil can be very heterogenous, immobilisation and mineralisation can be occurring at the same time within different microbial subpopulations (Bottomley et al. 2012). The effect of green manures on the immobilisation-mineralisation balance, is explored in relation to green manures in section 2.3.

2.2.7 Denitrification

Another pathway for NO_3^- is that of denitrification, a microbially facilitated process by which reactive N is reduced to inert N_2 , completing the N cycle to our starting point in section 2.2.1 above (Robertson and Groffman 2007). However, denitrification takes place in a series of reactions, as shown in Fig 2.3, and the products of each stage are not always further reduced and can instead be end products (Hayatsu et al. 2008, Giles et al. 2012). Hence denitrification results in a variety of end products including N_2O (Robertson and Groffman 2007, Hirsch and Mauchline 2015). The reactions of denitrification are facilitated by a wide range of heterotrophic bacteria and archaea, with each step requiring a different enzyme (Giles et al. 2012, Hirsch and Mauchline 2015). The prevailing

environmental conditions determine the functioning of the enzymes and microbes involved, and hence the products of denitrification (Cameron et al. 2013).



Fig 2.3. The denitrification process, showing the various products and catalysing enzymes. Adapted from Hirsch and Mauchline (2015).

The rate of denitrification in agricultural soils has implications for the loss of plant available N and the production of N_2O (Hirsch and Mauchline 2015, Moreau et al. 2015, Aarons et al. 2017). Rates of denitrification are affected by the amount of fixed N in agricultural systems, the microbial community, soil C levels, soil water and oxygen levels, pH and soil texture (Butterbach-Bahl et al., 2013). Many of the microbes which facilitate denitrification are facultative aerobes, for which use of oxygen as an electron acceptor is energetically preferable over N compounds, and therefore, reduction of the various forms N is more predominant where oxygen is lacking, such as in soils with a higher water content (Hayatsu et al. 2008, Giles et al. 2012). The amount of denitrification in agricultural soils is poorly understood (Galloway et al 2003) but it is estimated that between 6 and 12 % of fixed N added to agricultural soils is denitrified (Smil, 1999; Galloway et al., 2003). The amount of N lost to denitrification in N fertilised, but not irrigated soils varies widely, but has been calculated to average at $13 \text{ kg N ha}^{-1} \text{ year}^{-1}$ (Barton et al. 2016). Complete denitrification to N_2 does not have the negative environmental effects of N_2O production, but the higher the proportion of reactive N converted back to N_2 the less efficient the agricultural N cycle and the more energy required for N fixation. The factors affecting denitrification and N_2O production in relation to green manure use are further discussed in section 2.4.

2.2.8 Dissimilatory nitrate reduction to ammonia

Dissimilatory nitrate reduction to ammonia (DNRA) also produces N_2O as a by-product and is performed by bacteria and fungi as a respiratory process in which NO_3^- is reduced to NO_2^- and NH_4^+ (Giles et al. 2012, Hirsch and Mauchline 2015). Rates of DNRA have been found to be higher in environments with high organic matter levels, low oxygen and high pH (Rutting et al. 2011, Friedl et al. 2018, Pan et al. 2020). Studies have shown DNRA to be a minor factor in the soil N cycle in agricultural soils (Giles et al., 2012), but research is developing in this area and DNRA may be shown

to be a more significant factor in the production of N_2O than previously thought (Rutting et al. 2011, Hu et al. 2015).

2.3 Nitrogen use efficiency of green manure use

2.3.1 N supply from green manures

The synchrony of N supply and demand, necessary to reduce N losses, can be difficult to achieve with green manures incorporated into soil (Cassman et al. 2002, Crews and Peoples 2005, Finney et al. 2015, Moller 2018). At present, the recovery of manufactured fertiliser in crop plants generally outperforms that which can be achieved with legume N. Research typically shows fertiliser N to be recovered at a rate of between 30 to 50% and legume N at a rate of less than 25 to 30% (Crews and Peoples, 2005). The speed of plant matter decomposition, and the pathways and fate of elements within, depend on a range of factors including temperature and moisture levels, soil chemistry and biota, and the chemical composition of the organic material (Whalen 2014). The chemical composition of green manures affects the speed of transformation to plant available forms and the subsequent N pathways including the proportion of N which is immobilised (Wickings et al. 2012, Whalen 2014). It also affects the soil micro- and macro-fauna, which in turn influence subsequent decomposition and soil chemistry (Garcia-Palacios et al. 2016, Sauvadet et al. 2016). Up to 30% of leafy green matter can be made up of simple water-soluble molecules e.g. organic acids, sugars, and amino acids, which are very labile. Most of the N in plant matter, however is within larger molecules, notably complex proteins, chitin and peptidoglycan, and more recalcitrant materials include cellulose, resin, suberin and phenolics, including lignin (Geisseler et al. 2010, Whalen and Sampedro 2010).

2.3.2 C:N ratio of green manures and N availability

Most fresh organic material contains around 45% C by mass, but the N content varies significantly depending on the species, life stage and growing conditions of the green manure (Kumar and Goh 1999). Research has shown that in agricultural soil, N will typically be immobilised if the C:N ratio of the substrate is over 15 to 20:1 (Chadwick et al. 2000, Whalen and Sampedro 2010). For example, Iritani and Arnold, (1960) showed that tomato seedlings receiving organic matter with a ratio of above 20:1 grew less than those in soil with no addition, and those receiving an addition with a ratio of less than 20:1 grew more than with no addition (in four weeks of growth). As time progresses, however, heterotrophic microbes incorporate C into their biomass, and oxidise it during respiration, thus decreasing the C:N ratio of the remaining substrate to a point where microbes become C limited and N becomes available for plant growth (Whalen 2014, Chen et al. 2014, Hansen et al. 2019).

2.3.3 Secondary metabolites (SMs) and N availability

Although C:N ratio can give a broad indication of the likely decomposition rate, other factors of leaf chemistry such as secondary metabolites (SMs), also play a role (Trinsoutrot et al. 2000, Chomel et al. 2016). SMs are chemicals found in leaf tissue, as well as other parts of the plant, which offer functions

such as protection against sunlight, attracting pollinators or deterring attack by predation (Hattenschwiler and Vitousek 2000, Chomel et al. 2016). SMs can affect the decomposition rate by exhibiting resistance to degradation, binding with proteins to form resistant complexes, having anti-fungal or bacterial properties, being toxic to organisms on a range of trophic levels and inhibiting the action of extracellular enzymes (Garcia-Palacios et al. 2016, Halvorson et al. 2009, Chomel et al. 2016). Several tens of thousands of SM compounds have been isolated which broadly fit into three groups: alkaloids, terpenes and phenolic compounds (Chomel et al., 2016). Correlations have been found between phenolic compounds and speed of decomposition, for example Fox et al. (1990) concluded that lignin content may be a more reliable predictor of mineralisation rate than C:N ratio, and Handayanto et al. (1997) found that N release from tree prunings was correlated with the polyphenol to N ratio. Some phenolic compounds have an allelopathic role. for example, caffeic acid, commonly found in non-woody plants, can inhibit the growth of fungi, bacteria and plants and suppress the growth of young roots (Batish et al. 2008, Chomel et al. 2016). SMs may play a role in the effect of 'home field advantage' in which leaf litter is degraded faster if it enters the soil where it was produced, than if it is transferred to soil of a different environment. This is thought to be due to the decomposer community adapting to the characteristics of local leaf litter. Therefore, leaf matter with a high level of SMs may degrade faster in the environment it was produced, than if it is transferred to another environment (Chomel et al. 2016, Paul 2016). Though N release is necessary for green manures to fertilise crops, some retention by stabilisation into organic matter or undecomposed plant matter is beneficial in agricultural systems for soil functioning, and in building up N stocks which can be later released to supply crops (Crews and Peoples, 2005; Whalen, 2014).

2.3.4 Ammonia loss by volatilisation

After green manures have been decomposed and mineralised to NH_4^+ in soil solution, a pathway of N loss is that of NH_4^+ volatilisation. Loss of NH_4^+ by volatilisation from animal manure and urea fertilisers is well documented and known to be much reduced when the N is incorporated into soil or injected deeper into the profile compared to surface applications (Sommer and Hutchings 2001, Sommer et al. 2004, Cameron et al. 2013, Sanaullah et al. 2020). Research into NH_3 emissions from decomposing plant material however is limited (Hansen et al. 2019, Li et al. 2019, de Ruijter and Huijsmans 2019). Data from a handful of studies record greatly varying levels of NH_3 volatilisation, from minimal amounts of less than 0.1 % of clover N (Harper et al. 1995) and less than 0.5 % of pea plant residue N (Engel et al. 2013), 1.2 % lost of N applied in cauliflower residues (Nett et al. 2016), 5 % of N applied as lentil green manure (Janzen and McGinn 1991), and right up to an estimated 17 to 39 % of plant N (Larsson et al. 1998). Losses as NH_3 have been found to be higher from plant material with lower C:N ratios (de Ruijter et al. 2010). Volatilisation of NH_3 from plant N is likely to be delayed compared to

that from mineral N, due to the time needed for mineralisation to NH_3 . Time periods of high volatilisation have been recorded as ranging from 7 days (Janzen and McGinn 1991), to one month (Nett et al. 2016) to 6 weeks after application (de Ruijter, Huijsmans, and Rutgers 2010).

As with animal manure or fertiliser N, incorporation of plant N into soil has been found to reduce or eliminate loss by volatilisation (de Ruijter and Huijsmans 2019). For example, de Ruijter et al. (2010) found that mixing residues with soil reduced NH_3 emissions to insignificant amounts, compared to a loss of up to 16 % from mulch residue. Moisture levels also affect the amount of volatilisation. Janzen and McGinn (1991) found that volatilisation from mulched material increased after the soil had been wetted. The high emissions recorded by de Ruijter et al. (2010) were in cool temperate conditions in winter in the Netherlands, and the low emissions recorded by Engel et al. (2013) from mulched material ($< 0.5\%$ of pea plant N) were in very dry conditions. Nett et al. (2016) found an interacting effect of soil type and the method of addition in that cauliflower residues which were mulched emitted more NH_3 than when ploughed in a loamy sand, but methods of addition made no difference in a silty loam or sandy clay loam.

2.3.5 Leaching of N from green manures

As with mineral fertilisers, leaching of N can occur when mineralised N from low C:N green manures exceeds that which the crop can take up (Bergstrom and Kirchmann 2004, Abdalla et al. 2019, Hansen et al. 2019). For example, Campiglia et al. (2011) found mulching of a pepper crop with hairy vetch, (C:N ratio of 12.1), at a rate 177 kg ha^{-1} resulted in the leaching of $102.3 \text{ kg ha}^{-1} \text{ NO}_3^- \text{ N}$ during the growing season of April to September, compared to $48.2 \text{ kg ha}^{-1} \text{ NO}_3^- \text{ N}$ from soil with no addition. This produced a nitrate level in the leachate which was over three times the EU permitted level. Similarly, de Ruijter et al. (2010) found that 20 to 60 % of N from leek and broccoli residues was leached after being left in the soil after crop harvesting. Though this last example was of crop residues, not added with the purpose of N fertilisation, the high losses show the importance of considering synchrony of N supply and demand when using plant N sources. Though plant N generally needs to be transformed to NO_3^- before leaching can occur, systems using organic N do not necessarily have lower losses by leaching than those using mineral N, and organic systems have been known to incur leaching losses which are as high as inorganic systems when scaled by crop yield (Stockdale et al. 2001, Stockdale et al. 2002) or even by cropping area (Williams et al. 2006, Smith et al. 2019).

2.3.6 Crop N uptake

The final step in increasing N supply synchronisation is the efficient uptake by the crop, which is influenced by plant health, pest and disease populations, water management, and plant microbe symbiosis such as mycorrhizal fungi (Jackson et al. 2008, Kibblewhite et al. 2008, Whalen 2014).

Uptake of intact organic N from green manures would be advantageous for agricultural N use efficiency and climate change mitigation, as, by-passing nitrification and denitrification, prevents opportunities for loss of N as NO_3^- , NH_3 or N_2O . The uptake of organic N, and therefore C within peptides and amino acids may also give the plant a C bonus (Schmidt et al. 2013, Franklin et al. 2017). Uptake of intact organic N by plants is a fast developing area of research and there is increasing evidence that organic N may be an important source of N for agricultural plants (Hill et al. 2011, Czaban et al. 2016, Ma et al. 2018, Enggrob et al. 2019, Moreau et al. 2019).

2.4. Nitrous oxide emissions from agriculture

N₂O is the dominant greenhouse gas (GHG) from arable soils in temperate climates and intrinsically linked with the supply of N to crops. N₂O has a global warming potential of 298 times that of CO₂ over a 100 year period (Butterbach-Bahl et al. 2013) and agriculture, forestry and other land use accounts for 81 % of its emissions (IPCC 2019). Approximately 4 % of UK agricultural emissions are due to N₂O (DEFRA 2015). N₂O is also the single most important pollutant causing ozone depletion via the production of ozone-depleting nitrogen oxides (NO and NO₂) in the stratosphere (Butterbach-Bahl et al. 2013, Charles et al. 2017). CO₂ loss from arable soil is also an important factor, and the C balance of losses and gains is affected by use of green manures (Oertel et al. 2016, Bailey, et al. 2019), which is explored in section 2.5. Though methane emissions are high from agriculture, this is mostly due to enteric fermentation by ruminant animals (Herrero et al. 2016), or emissions from soils with poor drainage such as paddy rice fields (Jussi et al. 2020), but in temperate arable agriculture CH₄ emission from most agricultural soils is not a major factor (Oertel et al. 2016).

2.4.1 N₂O Emission Factors (EFs)

Emission factors (EFs) are a measure of the amount of N added to soil which is emitted as N₂O N (Henault et al. 2012). This is calculated by subtracting the N₂O N emitted by control soil (with no N addition) from the that emitted by soil which has been given an N containing application. This value is then expressed as a proportion of the N added (Hergoualc'h et al. 2019). The EF is used by the IPCC as a measure of the N₂O impact of N fertilisation, which is organised into a tiered system of increasing levels of precision. Until recently the Intergovernmental Panel on Climate Change (IPCC) used a standard 1 % EF globally from all types of N addition to estimate N₂O emissions, but in 2019 this was updated to take account of climatic variations (Buendia et al. 2019, Myrriotis et al. 2019).

These global EFs are using the most basic methodology referred to as tier 1. Recommended practice now, however is for countries to use tier 2 methodology to develop their own country specific EFs taking into account varying factors giving a range of EFs which may be specific to an N source, climatic region or other factor conditions (Hergoualc'h et al. 2019). For example the UK now use a country specific EF for urea or urea NH₄NO₃ added to arable land of 0.604 % and other mineral N to arable land of 0.791 %, (Wakeling 2017, Myrriotis et al. 2019). Tier 3 methodology uses a modelling approach to estimate emissions for a region, considering many factors including N input, climate, soil type, the effect on soil processes, and their interactions. Though the UK uses country specific EFs for mineral fertiliser, a country specific EF for incorporation of plant residues or cover crops has not yet been developed (DECC 2015). The EF used by the IPCC for residues and cover crops in wet soils is 0.6 % with

an uncertainty range of 0.1 to 1.1 % and in dry soils is, as for any N input, 0.5 % with an uncertainty range of 0 to 1.1 % (Hergoualc'h et al. 2019).

2.4.2 Soil processes producing N₂O

As described in section 2.2, nitrification and denitrification are the dominant N₂O forming processes in soil, though it is also a product of many other biotic and abiotic processes, many of which are poorly understood (Butterbach-Bahl et al. 2013, Giles et al. 2012, Hansen et al. 2019, Prosser et al. 2020, Chalk and Smith 2020). N₂O is produced by denitrification when NO₃⁻ is not fully reduced to N₂ (Robertson and Groffman 2007, Giles et al. 2012). Heterotrophic bacteria are considered to be the most significant denitrifying organisms, but archaea and fungi are also capable of denitrification and may play a greater role than previously thought (Hayatsu et al. 2008). Organisms capable of the reduction of NO₃⁻ are more widespread in soil, than those which perform the later stages of reduction of N₂O to N₂ (Henault et al. 2012). For example, fungal denitrifiers lack N₂O reductase and therefore the ability to convert N₂O to N₂, and therefore a fungal dominated soil could result in higher N₂O emissions (Mothapo et al. 2013, Wei et al. 2014, Chen et al. 2015). Nitrification produces N₂O when NO₂⁻, instead of being fully nitrified to NO₃⁻ is denitrified to N₂O (Hansen et al. 2019, Prosser et al. 2020). Loss of N from farmland by leaching, can cause indirect N₂O production by subsequent denitrification (Parkin et al. 2016) (Butterbach-Bahl et al. 2013). The IPCC estimate that on average 0.75 % of the NO₃-N leached is subsequently transformed to N₂O-N (Parkin et al. 2016, Abdalla et al. 2019). Similarly, N lost by volatilisation as NH₃ can subsequently be converted to N₂O (Hansen et al. 2019).

2.4.3 Factors affecting production of N₂O

2.4.3.1 N input to soil

Rates of N₂O production by nitrification or denitrification are affected by the availability of the substrates and therefore inputs of mineral N fertilisers, and of organic materials which decompose to mineral N (Butterbach-Bahl et al. 2013, Chen et al. 2013, Skiba and Rees 2014). A meta-analysis by Stehfest and Bouwman (2006) showed a linear relationship between fertiliser application and N₂O levels and this has been used by the IPCC to estimate N₂O emissions from soil (Henault et al., 2012). This linear relationship, however, does not take into account other factors such as soil moisture, pH or the type of N source applied which also affect rate of N₂O production (Shan and Yan, 2013), and more recent studies have found that N₂O is not strongly correlated with level of N input (Chirinda et al. 2010, Pugesgaard et al. 2017, Shan and Yan 2013). Correlations of N₂O production with

concentrations of NO_3^- or NH_4^+ in soil can, however, indicate whether the dominant source of N_2O is denitrification or nitrification (Butterbach-Bahl et al. 2013, Thangarajan et al. 2013).

2.4.3.2 Oxygen and soil moisture levels

The soil moisture content affects N_2O production both because it is necessary for microbial activity, and because of its effect on soil oxygen concentrations (section 2.2.7) (Butterbach-Bahl et al. 2013, Thangarajan et al. 2013, Hansen et al. 2019). The highest emissions are generally produced in soils where the water filled pore space (WFPS) is in the range of 70 to 80 %, though this depends on the soil type and its effect on oxygen distribution (Butterbach-Bahl et al. 2013). In wetter soil with a WFPS of over 80 % complete denitrification to N_2 increases so N_2O production has been found to decrease (Firestone and Davidson, 1989; Davidson and Verchot, 2000), but this is also variable according to soil type (Butterbach-Bahl et al. 2013). Oxygen levels influence whether nitrification or denitrification is the dominant N_2O producing process. N_2O produced by incomplete denitrification, is more prevalent in low oxygen conditions as the enzyme nitrous oxide reductase which catalyses the final stage of denitrification is more sensitive to oxygen inhibition than the enzymes that facilitate reduction to N_2O (Giles et al. 2012, Robertson and Groffman 2007, Thangarajan et al. 2013). Therefore, denitrification is typically the dominant N_2O forming process when water filled pore space (WFPS) is above 60 %, with more N_2O produced by nitrification at WFPS below 60 % (Davidson and Verchot 2000, Bateman and Baggs 2005, Thangarajan et al. 2013). It has, however, been shown that bacteria are capable of denitrification in aerobic conditions, and this may be a more significant factor than previously thought (Bateman and Baggs 2005, Hayatsu et al. 2008, Butterbach-Bahl et al. 2013, Chen et al. 2015). In addition, oxygen levels within soil vary greatly over small distances, so even well drained soil can contain many anaerobic micro-sites (Butterbach-Bahl et al., 2013).

2.4.3.3 Carbon

Soil denitrifying organisms require C as an electron donor, for the reduction of NO_3^- and therefore production of N_2O by denitrification is limited by C availability (Giles et al. 2012, Thangarajan et al. 2013). Provision of C also has an indirect effect, by stimulating respiration which in turn decreases oxygen concentrations (Giles et al. 2012). Even when the total soil C content is high, the concentration of labile C can be a more important driver of N_2O emissions by denitrification than NO_3^- availability (Mitchell et al. 2013). Therefore, the type of N supplied to crops, and if organic N, the quality of the C impacts N_2O emissions (Baggs et al. 2002, Mitchell et al. 2013, Hansen et al. 2019).

2.4.3.4 Temperature

In temperate climates both nitrification and denitrification increase at the higher range of soil temperatures due to an increase in enzyme activity (Kamp et al. 1998, Reay et al. 2012) with the

optimal temperature for nitrification being 25 to 35 °C (Thangarajan et al. 2013), but higher temperatures also increase denitrification due to a higher microbial respiration rate reducing soil oxygen levels (Butterbach-Bahl et al., 2013). Increased reaction speed causes a multiplying effect on denitrification with increased substrate available for reactions at each step. However, in cold temperatures which fluctuate around 0 °C denitrification can be increased due to freeze-thaw action releasing C (e.g. in microbial cell lysis) necessary as the electron donor (Butterbach-Bahl et al., 2013).

2.4.4 Mitigation of agricultural N₂O emissions

Spatial variability across field soil causes different rates of N mineralisation from soil SOM which, if fertiliser N is supplied evenly across the field, result in variable concentrations of mineral N in soil, with higher concentrations prone to resulting in N₂O emissions (Rees et al. 2020). Methods to supply N more accurately to match with crop demand are in development, for example using tractor mounted reflectance sensors to monitor crop N status, allowing better targeting of N applications (Rees et al. 2020). Other methods to reduce N₂O emissions include the use of slow release fertilisers, and additions of inhibitors of urease and nitrification, all of which can prevent the build up of high concentrations of NO₃⁻ (Thapa et al. 2016, Norton and Ouyang 2019, Dimkpa et al. 2020, Li and Chen 2020).

2.4.5 Nitrous oxide production by green manure addition

As seen in section 2.3, synchrony of supply and demand of green manure N is not always well matched, which can lead to excess mineral N in soil, and therefore provide the substrates of NH₄⁺ and NO₃⁻ for N₂O production. In addition, if green manure decomposition results in easily available C, coinciding with excess mineral N, a green manure can provide high concentrations of both the necessary C and NO₃⁻ for N₂O production by denitrification (Giles et al. 2012). A green manure with a high C content can also reduce the availability of NO₃⁻ by immobilising mineral N in microbial biomass (section 2.2.6). Because of this, green manures of lower C:N ratios generally result in higher N₂O emissions (Huang et al. 2004, Chen et al. 2013, Hansen et al. 2019).

There is a large amount of spatial and temporal variability in N₂O production with high emissions from small areas and time periods known as “hotspots” and “hot moments” which can contribute disproportionately to the total emissions (Butterbach-Bahl et al. 2013, Henault et al. 2012). With the nature of plant matter being less homogenous than mineral N, these hotspots can be especially important from green manures (Li et al. 2016, Parkin et al. 2016). The IPCC uses an EF for N₂O from plant N of 0.6 % in wet soils and 0.5 % in dry soils with an uncertainty range of up to 1.1 % (Hergoualc’h et al. 2019). However, a wide range of EFs, have been recorded from plant matter N. A review by

Charles et al (2017) found field experiment EFs of organic residues of only 0.19 ± 0.08 , but much higher EFs have been recorded, for example by Huang et al. (2004) of over 2 %, and Velthof et al. (2002) of 6 % from addition of broccoli residue. Organic agriculture has been found on the whole to have lower emissions of N₂O per unit area (Pugesgaard et al. 2017, Hansen et al. 2019), however, due to overall lower yields, the yield scale emissions can be equal or higher than conventional agriculture (Skinner et al. 2014, Smith et al. 2018, Hansen et al. 2019). With such high variability in emissions from green manures, there is likely to be scope for reducing N₂O production by changes in agricultural practice, and though challenging, this is an area which could significantly mitigate the climate change impact of agriculture.

2.5 Carbon in agricultural soil

Soil organic C exists in varied forms including dead organisms, exudates from roots and microbes, faecal matter, lysed cells and humus (Bhattacharya et al. 2016). Up to 80 % of organic matter is comprised of humus, a complex material which is not fully understood, but is made up of materials including phenol-aromatics, lipids, carbohydrates, and protein-derived nitrogenous compounds (Paul 2016). Global soil C stocks are estimated to be around four times that of the world's biomass, at approximately 2344 Gt in the top meter of the world's soils, compared to 560 Gt in the biomass (Stockmann et al. 2013), and interest in soil C has increased in recent years due to its role as a C source and sink in climate change mitigation (Rial-Lovera et al. 2017, Watson 2000, Jussi et al. 2020).

2.5.1 Carbon losses and gains in agricultural soil

Organic C is thought to remain in the soil due to chemical stability, for example as humic acids, and physical inaccessibility, with decomposition also being influenced by the functioning of the soil biota (von Lutzow et al. 2006). Recently, the view that it is the chemical nature of soil C which determines its stability in soil has been challenged, with some studies showing that biological and environmental factors have a greater effect on decomposition rate than the molecular structure of the material (Schmidt et al. 2011). It is now thought that long term stability of soil C is caused by the interaction of products of microbial degradation with the soil mineral component to form organo-mineral complexes (Blanco-Canqui et al. 2015).

On conversion from natural or semi natural vegetation to agricultural land, C has been found to be lost at a rate of up to 1500 g m⁻¹ (Jarecki and Lal 2003) and conversion of native forest to crop, and pasture to crop has been shown to deplete soil C by an average of 42 % and 59 % respectively (Guo et al. 2002). Agricultural land typically has a net C loss (Jarecki and Lal, 2003), and loss of C from UK arable soils has been estimated to be 1.42 t CO₂-C ha⁻¹ year⁻¹ from 1980 to 2000 (Pretty et al. 2000, Bellamy et al. 2005). Intensive management practices such as use of inorganic fertiliser, heavy tillage and monocultures are thought to increase loss of soil C (Jarecki and Lal, 2003; Bhattacharya et al., 2016), and adoption of measures such as no till, residue incorporation and agroforestry are advocated to reverse this trend (Jarecki and Lal 2003, Lal 2020)

There is great potential for increasing the C content of agricultural soils if effective techniques can be identified and practiced. This is the aim of the “4 per mille” campaign, a drive to increase the world's soil C content by an average of 0.4 % per year to mitigate climate change (Minasny et al. 2017, Arrouays and Horn 2019). Though there is much discussion on the realism of the target, (van Groenigen et al. 2017a, de Vries 2018, King et al. 2018, Poulton et al. 2018), there is a general

consensus that an increase in soil C is a feasible and desirable objective (Van Groenigen et al. 2017b, Poulton et al. 2018, Demenois et al. 2020). Though addition of organic matter, can increase soil C, it can also, paradoxically, cause the loss of pre-existing soil C through a phenomenon known as priming (Kuzyakov et al. 2000, Blagodatskaya and Kuzyakov 2008, van der Wal and de Boer 2017, Bailey et al. 2019).

2.5.2 Priming

Definitions of priming vary and can include any loss or gain of a variety of soil components including C, N, P and S, due to any action on soil, including additions, tillage and irrigation (Kuzyakov et al. 2000), but the term is commonly used to refer to a short term change in C turnover due to addition of organic materials (Zhang et al. 2013, Stockmann et al. 2013, Poeplau and Don 2015). The phenomenon was first described by Lohnis in 1926 who observed increased mineralisation of soil humus after the addition of leguminous green manures (Kuzyakov et al. 2000, Kuzyakov 2010). Priming of C can occur in both directions though, and negative priming refers to the reduction in the loss of C due to an amendment (Kuzyakov et al. 2000).

A meta analyses by Zhang et al. (2013) of 520 observations found that on average, addition of organic C (plant material or low molecular weight material) increased the SOC degradation by 26.5 % of rates without the addition. This included a range of observations including 69 which showed negative priming, 181 with positive priming and 270 with no significant effect. However, where positive priming occurs, the extent can be large enough to result in a net C loss when the additional C which stimulated the priming is taken into account (Fontaine et al. 2011). Though priming rates are calculated for the volume of soil used in the experiments, priming is known to be concentrated in very small “hotspots” of intense microbial activity at the sites of the interface between amendment and soil (Hill et al. 2008, Kuzyakov 2010).

There are many overlapping theories on the mechanisms causing priming, which are explored further in chapter 4, but priming is driven by soil microbial life, and has never been observed in sterile soil (Kuzyakov et al. 2000, Chaves et al. 2005, Rousk et al. 2015). As mechanisms of priming are still poorly understood, the consequences of adding material to soil are difficult to predict (Liang et al. 2017, Mwafurirwa et al. 2017). It is known that priming is affected by the quality of the substrate, with easily available C sources generally causing greater priming than more recalcitrant forms (Blagodatskaya and Kuzyakov 2008). Soil characteristics such as soil pH (Blagodatskaya and Kuzyakov 2008), microbial biomass (Blagodatsky et al. 2010) and soil pore characteristics (Toosi et al. 2017) also affect priming. Increasing temperatures due to climate change are likely to exacerbate priming effects due to higher microbial activity (Kuzyakov 2010), and there is currently an awareness that greater understanding of

the drivers of priming are needed to inform our environmental and agricultural management choices (Bastida et al. 2019, Chenu et al. 2019).

2.5.3 Potential for sequestration through incorporation of green manures

Though increasing soil C by addition of organic matter is not straightforward, the meta-analyses by Lehtinen et al. (2014) and Poeplau and Don (2015), both conclude that there is potential for increasing soil C through incorporation of plant matter. Lehtinen et al., (2014) studied data from 39 long term experiments, concluding that soil organic C increased by an average of 7 % due to crop residue incorporation. Poeplau and Don, (2015) conducted a meta-analysis, looking specifically at the effect of the incorporation of winter cover crops on soil C. Analysis of data from 139 plots at 37 sites in both temperate and tropical zones, with sampling depths up to 30 cm, found that plots which had received inputs of cover crops had a significantly higher organic matter content than reference plots, amounting to 0.32 Mg ha⁻¹ to a soil depth of 22 cm. They estimated the global potential for C sequestration via cover crop incorporation to be 8 % of the direct annual greenhouse gas emissions from agriculture – a rate of 0.12 Pg C yr⁻¹. The addition of inorganic nutrients, however, is also shown to increase soil C by increasing plant productivity and lowering of respiration of soil C (Zang et al. 2016). However, the continued use of inorganic fertiliser without organic additions may lead to impaired functioning of soil biota reducing the soil's capacity for C storage (Bhattacharya et al. 2016).

It is thought that rates of C sequestration by incorporation of organic matter will decrease over time due to soil C saturation (Lehtinen et al. 2014, Chen et al. 2019, Chenu et al. 2019). Poeplau et al. (2017) determined sequestration from residue incorporation in an agricultural soil in Italy, finding that it slowed after 20 years. Poeplau and Don (2015), however found a greater C sequestration in studies of a duration of over 20 years compared to those of under 5 years, and no decline in C sequestration over time up to 54 years. Though the authors acknowledge that data on long term studies were limited, with 8 data sets being for in excess of 20 years, they also note that modelling has shown C sequestration in agricultural lands to be likely over 100 years, and experiments at Rothamstead show C accumulating since 1844 (Poeplau and Don, 2015).

The benefits of soil organic matter accumulation by addition of organic materials need to be balanced with detrimental effects such as greenhouse gas emissions, nutrient leaching and a possible increase in pathogens (Bhattacharya et al. 2016). In considering the possible benefits of C sequestration by addition of leaf material grown ex-situ as PMGMs, it must be noted that the root fraction of green manures is thought to contribute disproportionately to C sequestration compared to leaf material (Poeplau and Don, 2015), and therefore the proposed use of harvested PMGMs, of leaf material only may have a lower C sequestration effect than incorporation of whole green manure plants. A possible

benefit of the proposed use of PMGMs however, is that living roots contribute to C sequestration via root exudates and dieback/turnover (Schmidt et al., 2011) and therefore, the PMGMs may sequester C in the soil in which they are grown.

2.6. Addressing the drawbacks of green manure use

2.6.1 Improving N synchrony by use of Mobile Green Manures

Improving the synchrony of the supply of N via green manures, and crop N uptake is important to reduce leaching of NO_3^- , emission of N_2O and to improve NUE (Crews and Peoples 2005, Hansen et al. 2019). As discussed in section 2.3, the rate at which N within plant matter becomes available to crops is influenced by the characteristics of the material e.g. C:N ratio, as well as environmental factors. Knowledge of this can be used as a tool to predict and influence the speed at which N becomes available to crops (Chaves et al. 2005). However, the incorporation of green manures in situ, before the sowing of a crop, is still a blunt tool, which does not offer the advantages of targeted N supply of mineral fertilisers (Crews and Peoples 2005, Rees et al. 2020).

With the aim of improving synchrony of N supply and demand, researchers have recently studied the effects of cropping green manures and adding the harvested material to cropland to better match with N demand (Crews and Peoples 2005, Moeller and Stinner 2009, Carter et al. 2014). For example, Sorensen and Thorup-Kristensen (2011) trialled 14 plant species as “mobile green manures” to fertilise crops of cauliflower and kale, with varying results, which indicated the importance of C:N ratio to release of N. In a recent 5 year field trial Burgt et al. (2018) used mixes of legumes and grasses which were either processed into pellets or applied to land directly, and concluded that use of “cut and carry” green manures is feasible to fertilise arable crops as the main source of N without deleterious effects. Research has also been conducted into processing harvested green manures in various ways to increase NUE, including by composting, ensiling and anaerobic digestion (Brozyna et al. 2013, Benke et al. 2017, Hansen et al. 2019, Toleikiene et al. 2020). For example, Moeller and Stinner (2009) harvested green manures in autumn, removing 80 to 120 kg N ha⁻¹ which was processed by anaerobic digestion, with digestate added to soil in spring. Carter et al., (2014) removed green manure leaf matter in autumn which was processed by composting with straw or preserved as silage, and in a six year field study Raberg et al. (2019) trialled the re-application of green manure plant matter which had been ensiled or anaerobically digested. The above three experiments found that removal, processing and re-addition can improve the NUE of green manures, though Moeller and Stinner (2009) noted that careful management is required to minimise emissions of N_2O and NH_3 . The processing of material such as pulverising, drying, digesting and composting also changes the physical and chemical characteristics and this can be used as a tool to influence mineralisation rates (Moeller and Stinner 2009, Carter et al. 2014).

2.6.2 Improving land use efficiency by use of perennial green manures

The use of plant N does not always involve growing and incorporating N fixers in situ. Small scale horticulturalists commonly use liquid feeds made from N rich plants such as the common nettle (*Urtica dioica*) and comfrey (*Symphytum officinale*) which are grown in a permanent patch (Govere et al. 2011, Garmendia et al. 2018). As permanent untilled areas, nettle and comfrey patches may also build up C in soil, and act as a biodiversity reserve. Another method of crop fertilisation gaining popularity among more innovative growers in temperate regions is the use of chipped branch-wood, known as “ramial wood”, which is added to soil either directly or composted (Percival et al. 2009, Barthes et al. 2010, Robert et al. 2014). These methods, which use non-leguminous plants, primarily redistribute N from one area to another, rather than add to the total amount of fixed N in the system.

In tropical agroforestry systems however, it is common to add prunings from N fixing trees to soil, therefore increasing the total fixed N within the agroecosystem (Palm 1995 Ajayi et al. 2011, Sileshi et al. 2020). Annual crops are often intercropped with one or more species of tree which are regularly coppiced, and the prunings mulched or incorporated into soil (Rao et al. 1997, Sileshi et al. 2020b). Examples of annual crops and their perennial partners include sorghum intercropped with *Vachellia* (acacia) trees in Kenya, (Sileshi et al. 2020), *Gliricidia sepium* intercropped with maize in Malawi (Makumba et al. 2006) and *Alnus nepalensis*, intercropped by cardamom in the Himalayas (Sharma et al. 2002). Other techniques comprise multi-layered systems of mature trees and shrubs which can be comprised of two or three species, or be more complex such as the home gardens which can include over 100 species (Sileshi et al 2020b). The rates of N fixing by trees in such systems vary, but can be substantial, for example N fixing in *Alnus nepalensis*, can reach 155 kg ha⁻¹ year⁻¹ in 15-year-old stands (Sharma et al. 2002) and *Gliricidia sepium* is estimated to fix over 100 kg ha⁻¹ year⁻¹ (Sileshi et al. 2020a).

As with the use of green manures in temperate systems, there are often low rates of N uptake from the added material. Typically only 10 to 30 % of N added in tree prunings is taken up into the crop, the rest of which may be immobilised, lost from soil or taken up again by the trees (Palm 1995, Snoeck et al. 2000, Sileshi et al. 2020b). As discussed in section 2.3, the availability of N from plant matter is influenced greatly by the chemical composition of the leaf matter, and it is likely that tree prunings, which contain more structural materials will contain a higher amount of secondary metabolites such as lignin, than non woody green manures such as clovers and vetches. Many studies have researched improving the N uptake from tree prunings, for example by manipulating the speed of N release by optimising the timing of addition, residue placement (mulching or incorporating) or combining residues of different properties (Handayanto et al. 1997, Mafongoya and Nair 1997, Cadisch et al.

1998, Hartemink and O'Sullivan 2001, Makumba and Akinnifesi 2008). As a resource to help predict N availability and choose appropriate methods to match with the material, Palm et al. (2001) constructed a database listing the qualities of plant matter of over 300 tropical species including N, lignin and polyphenol contents. The importance of agroforestry systems for soil health and biodiversity is increasingly recognised (Dollinger and Jose 2019) and research continues to work to increase efficacy by maximising the synchrony of N supply with demand (Youkhana and Idol 2009, Hernandez-Mucino et al. 2015, Sileshi et al. 2020b).

2.6.3. A novel solution: Perennial Mobile Green Manures

The use of a combination of the methods described above could improve both NUE and land use efficiency of organic N fed systems in temperate climates. It is envisaged that perennial N fixing plants could be grown in permanent areas from where plant tissue can be harvested and added to cropland to meet N demand. These PMGMs could be grown on lower quality land, such as flood plains and hillsides, therefore reducing the demand for arable land compared to that used by temporary fertility building leys. Considering the urgent need for increasing ecosystem services, the direct replacement of temporary leys with permanent N producing areas, even on high quality agricultural land, would also be of environmental benefit by increasing the amount of unploughed land and therefore C sequestration (Lal 2020), while still providing an N supply to crops. In addition, the areas on which PMGMs are grown, could, as with permanent comfrey and nettle areas also provide habitat for wildlife, therefore contributing to ecosystem services.

The ex-situ cultivation of green manures also opens up possibilities for increasing the range of species grown for use as green manures to include herbaceous perennials, as well as and woody shrubs and trees as used in tropical systems. To use plant species not currently used as green manures, it is necessary to gain an understanding of the performance of the leaf litter in supply of N to a crop, and the effect of the N and C dynamics on the NUE and GHG balance. There are a variety of N fixing perennials, which could be investigated, but three species have been chosen for this study: Alder (*Alnus glutinosa*), Gorse (*Ulex europaeus*); and Gunnera (*Gunnera manicata*). Alder is a deciduous tree, native to the UK, commonly found growing near water and tolerant of flooded ground (McVean 1953, Claessens et al. 2010). It gains fixed N via a symbiotic relationship with *Frankia*, a filamentous actinobacterium (Pawlowski and Demchenko 2012). Gorse is an evergreen shrub, also native to the UK, which often grows on exposed ground with thin soils (Cordero et al. 2016). Like other plants in the *Fabaceae* family, it has a symbiotic relationship with rhizobia bacteria. Though native, gorse can be invasive and therefore operations to remove it are often undertaken on farmland and nature reserves (Atlan et al. 2015, Cordero et al., 2016). *Gunnera manicata* is a non-native, large herbaceous perennial

growing to over 2 meters tall (RHS 2017), which forms a non-obligate symbiotic relationship with *Nostoc* cyanobacteria (Bergman et al. 1992). Gunnera is native to South America, but commonly grown as a garden plant in the UK and has become naturalised in some lowland areas of the UK (NNSS 2011).

In addition to tolerance of wet or exposed conditions, which enables use of lower quality land, the species have been chosen for other potentially beneficial effects. Increased planting of alder, as a native tree, would be beneficial to biodiversity. Alder re-grows after being coppiced to ground level (McVean 1953), so enabling the creation of short rotation coppice with stands of trees at different stages which would provide a mosaic of different habitats. In contrast, gunnera as a potentially invasive non-native must be planted with caution. However, in the case of both gunnera and gorse where plants material is regularly removed for control of the species, an economic use of the plant matter can be a bonus.

Because alder and gorse are woody species, leaf material needs removing from the more carbon rich stems. Gunnera however, as a herbaceous perennial does not present this difficulty, and the lack of a waxy cuticle suggests that gunnera tissue may be lower in decomposition-resistance secondary metabolites. Though it may be unwise to plant large areas of gunnera due to potential invasiveness, gunnera could produce a useful amount of material within small plantings due to its fast growth. Small patches of gunnera patches grown similarly to the present practice of growing comfrey could provide a useful amount of material for the horticultural industry.

Information on N fixation rates of these species is limited, however gorse has been reported to fix 100 to 200 kg N ha⁻¹ year⁻¹ (Magesan et al. 2012). Trees with an actinorhizal symbiosis, as alder has, have been estimated to be capable of fixing 30–50 g N tree⁻¹ season⁻¹, though rates can be lower due to environmental conditions or pest attack (Vessey et al. 2005). N fixing rates of *Gunnera manicata* appear to be unknown, but another member of the genus (*G. arenaria*) has been calculated to fix 72 kg N ha⁻¹ yr⁻¹ (Bergman et al. 1992). Desired N supply rates will differ depending on crop type. For example, N use efficiency for fast growing salads may be best served by regular additions of a green manure with a high mineralisation rate, whereas slower growing crops may benefit from an addition with a delayed release to supply N when access by machinery is problematic.

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Chapter 3: Evaluating PMGMs for efficacy as N fertiliser and climate change impact: A Pot Experiment

Contributions: PWH, DRC and CW conceived and designed the experiment. Experimental work, data processing and statistical analysis were performed by CW. CW wrote the chapter with advice and edits from PWH and DRC.

Abstract

N fixing green manures add N to soil to fertilise crops without the CO₂ emissions which result from industrial N fixing in the production of mineral N fertiliser. The incorporation of green manures to soil, however, can have similar environmentally detrimental effects to adding inorganic N, specifically, the emission of the greenhouse gas N₂O, and the leaching of NO₃⁻. Here, the alternative N-fixing species *Alnus glutinosa* (Alder), *Gunnera manicata* (Gunnera), and *Ulex europaeus* (Gorse), were trialled as PMGMs to investigate their provision of N to crops and potential pollution by N₂O and NO₃⁻, in comparison to the conventional green manure *Trifolium pratense* (red clover). These alternative species are adapted to growing on low quality land, from where leaf tissue can be harvested and applied to agricultural soil, giving the added advantage of increasing land use efficiency. A “crop” of *Lolium perenne* (ryegrass) was pot-grown for one year in soil supplied, prior to sowing, with the equivalent of 200 kg ha⁻¹ N as leaf tissue of alder, gunnera, gorse, and red clover. The experiment also included a range of N-rates of NH₄NO₃ fertiliser, and no addition (control) treatments.

Results indicate that the PMGMs were effective as N fertiliser and caused lower N pollution than clover or NH₄NO₃. The gunnera treatment resulted in delayed ryegrass growth compared to other treatments, but with a significantly higher overall mean biomass (shoot + root) of 90 % higher than the control dry biomass, whereas dry biomass resulting from 200 kg ha⁻¹ N as alder, gorse, clover or NH₄NO₃ were less than 40 % higher than that of the control after one year ($P < 0.001$). In addition, the potential for leaching of NO₃⁻ was found to be lower for PMGMs than from clover or NH₄NO₃ with mean NO₃⁻ concentrations in soil solution after PMGM treatments peaking at below 25 mg N l⁻¹ in comparison to over 130 mg N l⁻¹ after clover and NH₄NO₃ additions. Emissions of N₂O from PMGMs were considerably lower than those from clover and NH₄NO₃ treated soil with applied N lost as N₂O-N from gunnera being 0.34 %, alder 0.61 %, and gorse 1.20 %, compared to 5.3 % from clover, and 2.23-3.58 % for a range of the different NH₄NO₃ rates up to 70 kg ha⁻¹ N ($P < 0.001$). (N₂O emission data are not available for the higher applications of NH₄NO₃). The relative N₂O emissions are likely to have been caused by high concentrations of NO₃⁻ and DOC occurring simultaneously in clover treated soils, whereas the lower NO₃⁻ concentrations in PMGM treated soil, and lower DOC concentrations in NH₄NO₃ treated soils probably limited N₂O production by denitrification. It was concluded that PMGMs offer a promising low impact alternative to traditional green manures and NH₄NO₃ fertiliser and research is recommended in field conditions.

3.1 Introduction

Using traditional N fixing green manures in agriculture, supplies N to crops without the CO₂ emissions caused by industrial N fixing, and can improve soil health by adding organic matter to soil (Cherr et al. 2006, Blanco-Canqui et al. 2015, Carr et al. 2020). These benefits, however, are offset by the increased area of cropland required for N fixation and the limited flexibility in the addition of the green manure N to soil (Crews and Peoples 2005, Smith et al. 2018). This lack of flexibility in timing N additions can lead to poor synchrony of N supply with crop demand resulting in inefficient use of N and pollution by leaching of NO₃⁻ and emission of N₂O (Crews and Peoples 2005, Moller 2018, Hansen et al. 2019). A solution to this may be the use of N fixing perennial plants grown as PMGMs on low quality land, from which plant material is harvested and applied to cropland. With knowledge of the rate at which N from the tissues becomes available to plants, PMGMs can be added to synchronise N release with plant demand.

3.1.1 Crop N supply by green manures

For N within plant matter to become available for crop uptake, most plant organic material must be decomposed to smaller molecules (Bloom 2015). Plants have long been known to uptake N as NO₃⁻ and NH₄⁺, but the role of organic N (e.g. early protein breakdown products such as short peptides and amino acids) in plant nutrition is increasingly recognised in crop species (Hill et al. 2011, Ma et al. 2018, Enggrob et al. 2019). There are potential advantages of crop uptake of organic N, by reducing opportunities for N loss which arise from mineral N, and increasing efficiency by energetic benefits to the plants (Paungfoo-Lonhienne et al. 2012, Franklin et al. 2017).

The rate at which N supplied as green manure becomes available to plants is determined by the chemistry and structure of the green manure material and the soil environment (Whalen 2014, Paul 2016). N generally becomes available to plants faster from materials with a low C:N ratio, and addition of materials with a high C:N ratio can result in initial N immobilisation, with N later becoming available for plant uptake as the C:N ratio of the substrate diminishes with microbial respiration (Whalen 2014, Chen et al. 2014, Hansen et al. 2019). There is broad agreement between many experiments that immobilisation of N occurs when the C:N ratio of the substrate is above 15 to 20:1 (Iritani and Arnold 1960, Chadwick et al. 2000, Chen et al. 2014). Other factors of leaf chemistry such as polyphenol content can also delay N release due to allelopathic effects, or binding of N in protein complexes (Trinsoutrot et al. 2000, Chomel et al. 2016).

3.1.2 Assessing organic amendments for crop N fertilisation.

The N fertilisation effects of organic amendments are commonly quantified as N recovery efficiencies (NREs) and fertiliser equivalents (FEs) (Cassman et al. 2002, Kubota et al. 2018, De Notaris et al. 2018).

Both can be assessed by use of pot or field experiments in which known amounts of N supplied within the organic material are added to soil and the resulting crop biomass analysed for N content (Bhogal et al. 2016, Ovsthus et al. 2017, De Notaris et al. 2018). These metrics can be used in conjunction with the number of cumulative day degrees (CDDs), a measure of the total amount of time above a certain temperature, typically 5 °C, during an experiment, which gives an assessment of the time in which biological activity of decomposition and plant growth was not restricted by excessive cold (Nicholson et al. 2013, Bhogal et al. 2016).

3.1.3 Nitrogen recover efficiency (NRE)

The nitrogen recovery efficiency (NRE), is a measure of the proportion of the N supplied which is taken up by the crop (Cassman et al. 2002, Mosier et al. 2013). It can be calculated by the “difference method” in which the N uptake of control plants is subtracted from the N uptake of plants which have been given the additional N. This value is then divided by the amount of N added, and expressed as a proportion of 1 or converted to a percentage (Cassman et al. 2002, Ovsthus et al. 2017, Salim and Raza 2020). NREs calculated in this way are sometimes referred to as apparent NREs, to acknowledge that the source of N uptake to the plant is not known, and increases in N uptake may be due to changes in the uptake of soil N which was present before the addition (Mosier et al. 2013, Ovsthus et al. 2017, Yan et al. 2020). Isotopic studies using labelled N additions can trace the added N to the crop giving more accurate NREs (Yan et al. 2020). NREs are always higher when the N supplied is below the optimum for crop growth, as the proportion of added N taken up declines as N becomes less of a limiting factor for crop growth (Salim and Raza 2020). Therefore, lower yielding crops which are inadequately provided with N, can have a high NRE, yet have a negative environmental effect due to the need for more land area for production.

3.1.4 Fertiliser Equivalent (FE)

The Fertiliser Equivalent (FE), also known as the fertiliser replacement rate is a measure of the amount of fertiliser N which would be required to give the same increase in biomass or N uptake as an applied organic treatment (Kumar and Goh 1999, Rigby and Smith 2014). Experiments designed for calculation of FEs use a series of fertiliser response plots, which are treated with various levels of mineral N fertiliser, to allow for comparison with the organic treatments. A regression of the biomass or N uptake against the amounts of N added in the response plots is used to calculate the fertiliser equivalent of the organic addition (De Notaris et al. 2018, Sánchez-Rodríguez et al. 2018). In studies to determine the N effect of amendments, it is important to provide the soil with appropriate levels of any other nutrients necessary for crop growth so that N is the only nutrient factor limiting crop growth (Rigby and Smith 2014, De Notaris et al. 2018).

3.1.5 N efficiency of green manures and mineral fertilisers

Due to the very reactive nature of fixed N, there are inefficiencies in the uptake of N into crops in both mineral N and organic N fed systems, and on average, globally around 40 % of N supplied is estimated to be taken up into crops (Conant et al. 2013). Intensive horticultural systems with high N fertilisation rates, can have low N recovery rates, typically being lower than 50 % or even as low as 20 % (Di and Cameron 2002). N recovery rates from green manures or plant residues vary greatly. A review by Crews and Peoples (2005), listed the N recovery from 12 different green manure applications in 9 different studies, ranging from 9 to 49 %. Lower NRE's from green manures than from mineral fertilisers are to be expected due to a higher proportion of the N remaining in organic material in soil (Janzen et al. 2003, Chen et al. 2014, Hu et al. 2015). For example Janzen et al. (2003) found that between 9 and 27 % of green manure N (flatpea/lentil) and between 17 and 44 % of $(\text{NH}_4)_2\text{SO}_4$ N was recovered in a wheat crop after 14 months. Similarly, in an experiment using cabbage residue, the N recovery in grass shoots was 27.5 to 31.4 % from the residue, but 70.2 % from KNO_3 fertiliser (Araya et al. 2010). Low NREs from organic amendments are not necessarily an indication of low N efficiency as organic N remaining in soil can become available for crop growth in future years (Hansen et al. 2019). Therefore, NREs calculated over many years can give a better assessment of N supply from amendments.

3.1.6 Nitrous oxide emissions from green manures

Excess mineral N in soil above that which can be taken up by the crop can result in N_2O emissions, principally by nitrification of NH_4^+ or denitrification of NO_3^- (Butterbach-Bahl et al. 2013, Rees et al. 2013). Therefore, poor synchrony of N supply and demand, which can result in high concentrations of mineral N, is a risk factor for N_2O emissions (Hansen et al. 2019). N_2O emissions have been found to be negatively correlated with the C:N ratio of plant residues, due to faster N mineralisation (Huang et al. 2004). Emissions by denitrification can be increased if concentrations of dissolved organic carbon (DOC) are increased by additions of plant matter due to the requirement for C as an electron donor, (Mitchell et al. 2013, Thangarajan et al. 2013). N_2O production by denitrification is also increased by anaerobic conditions with maximum emissions occurring when WFPS is in the region of 70 % (Bateman and Baggs 2005, Butterbach-Bahl et al. 2013). Additions of organic matter can also play a role in creating anaerobic conditions by increasing soil respiration of added C which reduces oxygen levels (Chen et al. 2013). N_2O emissions from N additions to agricultural soil are commonly expressed as emission factors (EFs), a calculation of the percentage of the N supplied which is emitted as N_2O (De Klein and Harvey 2015). Expressing emissions as EFs allows comparisons between situations where different amounts of N are added.

3.1.7 Aims and experimental design

For PMGMs to be effective for climate change mitigation, they need to supply N to a crop without causing deleterious levels of pollution by emission of N_2O or leaching of NO_3^- . We aimed to assess the N fertilisation value of *Alnus glutinosa* (Alder), *Gunnera manicata* (Gunnera), and *Ulex europaeus* (Gorse), by quantifying the plant N uptake regularly over time. To understand the potential effects of the chosen PMGMs on N loss via NO_3^- leaching and N_2O emissions we aimed to study the C and N dynamics within the soil-plant system, as well as quantify losses of N_2O from it.

To achieve the above aims, we undertook a pot experiment to assess the potential for N fertilisation and pollution from alder, gunnera and gorse as PMGMs. By growing and regularly harvesting ryegrass (*Lolium perenne*) in soil fertilised by the PMGMs, we used the N uptake into grass shoots to assess the different rates of decomposition to plant-available N, as used by Araya et al (2010) and Chadwick et al. (2000). From this the calculation of NREs and Fertiliser Efficiencies enabled comparison with the fertilisation effects of NH_4NO_3 and the tradition green manure, red clover (*Trifolium pratense*). In addition, regular sampling of the soil solution allowed quantification of DOC and various forms of soil N.

To assess the PMGMs in conditions favourable to organic matter decomposition, and N_2O production, we conducted the experiment at temperatures averaging at 18 to 29 °C, and with WFPS at 70% (Davidson et al. 2000, Bateman and Baggs 2005, Giles et al. 2012). This allowed the chemistry and physical characteristics of the substrate to be the main influence on speed of decomposition. These conditions may occur in irrigated polytunnels in temperate regions, or in the field in tropical areas, and give an indication of the maximum N pollution which may result in “worst case scenario” conditions, but data can also be used to estimate the smaller, but real differences which may occur in situations less favourable to N_2O production, for example in drier soil containing anaerobic microsites.

3.2 Methods and materials

3.2.1 Green manures

Clover and alder leaves were harvested from plants growing at Henfaes Research Station in Abergwyngregyn (53°14'N, 4°01'W), gorse from Bangor university campus, North Wales (53°13'N, 4°07'W) and gunnera from pot-grown plants, on 03/10/16. All leaf material was cut to pieces of ≤ 4 cm. Gorse stems of ≥ 4 mm diameter and gunnera petioles were removed. All green manure material was stored in polythene bags at 4 °C for 11 days until the start of the experiment. Samples of the green manures were dried at 80 °C for 48 hours, and the weights of fresh and dried samples were used to determine moisture content of the fresh material. To determine total C and N content, samples were ground to ≤ 1 mm, using a FOSS CT 193 Cyclotec mill (Hilleroed, Denmark) and analysed using a TruSpecR analyser (Leco Corp., St Joseph, MI). Characteristics of the green manures are shown in Table 3.1.

Table 3.1 Green manure properties (means \pm SEM, $n = 3$)

Plant	Dry matter content (%)	Carbon (g kg ⁻¹ dry leaf)	Nitrogen (g kg ⁻¹ dry leaf)	C:N ratio
Clover	21	4.86 \pm 0.010	0.481 \pm 0.004	10.1 \pm 0.10
Alder	15	5.11 \pm 0.013	0.338 \pm 0.001	15.1 \pm 0.01
Gunnera	40	4.67 \pm 0.017	0.228 \pm 0.004	20.5 \pm 0.33
Gorse	37	5.29 \pm 0.012	0.198 \pm 0.002	26.8 \pm 0.24

3.2.2 Soil

Soil, classified as a Eutric Cambisol (FAO), was collected from Henfaes Research Station, Abergwyngregyn, North Wales (grid ref: 53°14'N, 4°01'W) from a depth of 2 to 12 cm and sieved to ≤ 10 mm. Course sieving was chosen to reduce the disruption of the soil structure, and resulting effects on microbial activity (Jones and Willett 2006). Soil was stored at 4 °C for 18 days until the experiment began. Subsamples were taken to determine water content by drying at 105 °C for 24 hours. Dry soil was analysed for C and N content as described for leaf tissue. Extracts of fresh soil were prepared using a 6 g subsample in 25 ml 0.5 M K₂SO₄, shaken for 60 minutes at 200 rev min⁻¹, and centrifuged for 10 min at 2,000 *g*. The supernatant was filtered and stored at -20 °C until analysis. To determine microbial biomass a second 6 g subsample was fumigated in CHCl₃ vapour using the method described by Vance et al. (1987). Both fumigated and unfumigated extracts were analysed for DOC and total dissolved nitrogen (TN) using a Multi N/C 2100S analyser (AnalytikJena, Jena, Germany). Microbial

Table 3.2. Background Soil characteristics. (means \pm SEM, $n = 5$, except pH $n = 3$). Where SEMs are not stated, the values are a single measure obtained from NRM laboratories.

Soil Property	
pH	5.65 \pm 0.018
Microbial biomass (mg g ⁻¹)	0.33 \pm 0.06
Total soil C (g C kg ⁻¹)	21.9 \pm 0.86
Total soil N (g N kg ⁻¹)	2.21 \pm 0.10
Soil C:N ratio	9.91 \pm 0.48
DOC (mg C L ⁻¹)	14.35 \pm 3.35
TN (mg N L ⁻¹)	23.71 \pm 1.99
DON (mg N L ⁻¹)	3.55 \pm 0.92
NO ₃ ⁻ (mg N L ⁻¹)	18.97 \pm 2.01
NH ₄ ⁺ (mg N L ⁻¹)	0.04 \pm 0.03
Amino Acids (μ N L L ⁻¹)	12.71 \pm 3.53
Non-AA organic N (mg L ⁻¹)	2.84 \pm 1.12
Available P (mg L ⁻¹)	35.4
Available K (mg L ⁻¹)	103
Available Mg (mg L ⁻¹)	54

biomass C was calculated by subtraction of unfumigated soil C from fumigated soil C, using a kec conversion factor of 0.45 (Joergensen, 1996).

Unfumigated extracts were also analysed for NO₃⁻ and NH₄⁺ using a PowerWave XS BioTek spectrophotometer (Winooski, VT, US) using Gen 5 software, following the methods described in Miranda et al. (2001) for NO₃⁻ and Mulvaney (1996) for NH₄⁺. Amino Acids were analysed using a Varian Cary Eclipse Fluorescence Spectrophotometer (Palo Alto, California) according to the method described by Jones et al. (2002). Amino acid readings were adjusted for interference by fluorescence from NH₄⁺ as follows. The fluorescence of NH₄⁺ standards, was measured and used to calculate the level of fluorescence caused by the NH₄⁺ content of each sample (known from previous analyses), which was then subtracted from the amino acid reading as in Jones et al (2002). Dissolved organic N (DON) was calculated by subtracting NH₄⁺ and NO₃⁻ concentrations from the TN concentration. Soil was analysed for pH in water at a ratio of fresh soil:deionised water of 1:2.5. The original field soil had previously been analysed for P, K and Mg by NRM laboratories, Bracknell, Berkshire UK (reported 10th March 2016), and had had no additions since that date. Soil characteristics are shown in Table 3.2.

3.2.3. Experimental set up

The experiment consisted of 5 replicates of each treatment supplied to pots of soil sown with *Lolium perenne* and run for 364 days. The treatments were: no addition (control), 200 kg ha⁻¹ N equivalent of the green manures: clover, alder, gunnera and gorse, and a range of NH₄NO₃ levels at the equivalent of 35, 70, 140 and 200 kg N ha⁻¹ (referred to as: AN35, AN70, AN140 and AN200). The experiment was set up as follows: 40 plastic “lock and lock” (HPL819) pots (Seoul, Korea) of 272 x 113 x 800 mm (internal) each weighing 184 g were filled with 1216 g of fresh soil to give a total weight of 1400 g at a bulk density of 1.06 g cm⁻³. The weight of fresh leaf tissue containing the equivalent of 200 kg ha⁻¹ N, was calculated using the N content, H₂O content and the pot surface area. Green manures were thoroughly mixed with soil prior to pot filling, except for 20 mm of soil without green manure in the uppermost layer to allow for even and uninhibited seed germination.

To ensure that nutrients other than N did not limit grass growth, S, K and P were supplied as recommended for grass establishment by UK fertiliser guidelines (Defra 2010), based on the levels shown in Table 3.2. This amounted to a rate equivalent to 16 kg ha⁻¹ S, 66.4 kg ha⁻¹ K and 13.2 kg ha⁻¹ P, supplied as a mixture of K₂SO₄, K₂PO₄ and P₂O₅. NH₄NO₃ treatments equivalent to 35, 70, 140* and 200* kg N ha⁻¹ were added in solution, and the same weight of distilled water was added to all other treatments. Distilled water was added by weight as necessary to a water content of c. 400 g kg⁻¹, dry soil which gave a water filled pore space (WFPS) of c. 70 %. 1 g seed of *Lolium perenne* was sown evenly on the soil surface of each pot and covered with 10 g of soil (sieved to 2 mm). The pots were arranged in a random block design in a heated greenhouse with the thermostat set to a minimum of 5 °C and maximum 10 °C at night, and a day min of 15 °C and max of 20 °C, and lit for 12 hours daily. A temperature logger was placed between the plant pots. The mean temperatures for each period of grass growth ranged from 17.5 to 28.8 °C and are shown in Table 9 appendix 1. WFPS was maintained, by regular weighing of pots and addition of distilled water, to 70 ± 10 % throughout the experiment, and 70 ± 3 % at the time of gas and soil solution sampling.

*The treatments of AN140 and AN200 were assessed in an addition to the main experiment, which was conducted at a later date. This additional experiment included the control and AN70 treatments alongside the two higher fertiliser treatments and followed the same methods as the main experiment. The soil of the additional experiment had the same characteristics as that of the main experiment, with the exception of pH (6.1) and potassium (146 ± 4 mg L⁻¹). Microbial biomass was not measured. It was considered valid to use the data collected from the AN140 and AN200 treatments, alongside the data from the main experiment for a parameter when the results of the control and AN70 treatments did not differ between the additional and main experiment either in the actual amounts, or the proportional difference between the 70 kg N ha⁻¹ treatment and the control. Differences for each parameter were determined by a t-test. Differences between the two experiments are likely to be due to unavoidable differences in temperature.

3.2.4 Biomass sampling and analyses

Biomass production was measured by cutting all grass in each pot at a height of 1 cm above the soil surface level on days 31, 59, 94, 129, 164, 199, 234, 269, 304, and 340. Any self-seeded dicotyledonous plants were removed before reaching 20 mm height by cutting at ground level, and their biomass processed with the harvested grass. The total dicotyledonous (weed) biomass was estimated at < 0.5 % of the grass biomass. Plant material was dried at 80 °C for 24 hours, weighed and ground using a Retch mixer mill MM 200 (Haan, Germany) to ≤ 1 mm, and analysed for C and N as described above.

On day 364 all vegetation above the soil surface was removed and processed as for the previous biomass samples. The soil of each pot, which was consolidated due to root growth, was cut evenly into two blocks. The roots within one block were washed from the soil, dried at 80°C for 48 hours, ground and analysed for C and N as for leaf samples. Soil taken from the remaining block was retained in polythene bags at 4 °C until analyses.

3.2.5 Gas sampling and analyses

Gas was sampled from the headspace of each pot after 0.08, 1, 3, 5, 13, 18, 39, 53, 67, 95, 151, 206, 263, 318 and 347 days as follows. The pots were sealed with air-tight lids, each which had been fitted with a suba-seal™. 20 ml gas samples were taken by syringe through the suba-seal™ at timepoints 0, 30 and 60 minutes after lids were fitted. Samples were stored in pre-evacuated 20 ml glass vials prior to analysis. Gas was analysed using a Perkin Elmer 580 Gas Chromatograph fitted with an ⁶³Ni Electron Capture Detector (ECD) (Beverly CT, USA), and N₂O concentration was calculated against a series of certified N₂O standards.

It was calculated that each pot had a headspace of 1.15 L above the soil surface from which the samples were taken. The molecular volume was corrected for temperature and pressure, and fluxes calculated by linear regression following the method set out in Scheer et al. (2014). Negative fluxes were not removed. Cumulative N₂O was determined by calculating the area under the curve of N₂O flux over time by use of the trapezoidal rule (Li et al. 2016). The emission factors (EFs) were calculated by subtracting the cumulative N₂O emissions for the control treatment from cumulative emissions of the treatment replicates (paired by block) and dividing by the amount of N added as described in De Klein and Harvey (2015).

3.2.6 Soil sampling and analyses

Soil solution was sampled on days 1, 3, 5, 9, 13, 18, 25, 32, 39, 53, 67, 82, 95, 124, 151, 180, 206, 263 and 318 using 50 mm Rhizons™ (male Luer 19.21.22, Rhizosphere research products, Wageningen, Netherlands). These were inserted at a c. 45 ° angle, reaching a depth of c. 70 mm below the soil

surface. Soil solution was drawn out via a needle fitted to a 9 ml vacuum tube. Soil retained after plant harvesting was extracted in 0.5 M K₂SO₄ (6 g fresh soil and 25 ml 0.5 M K₂SO₄) and extractions were analysed for NO₃⁻, NH₄⁺, amino acids, DOC and TN as described above. Amino acid data are not available for AN140 and AN200 as interference from high NH₄⁺ content made measurements impractical. Soil solution was also analysed for total phenolics using a PowerWave XS BioTek spectrophotometer using Gen 5 software, at wavelength 725 nm using Folin–Ciocalteu reagent (Swain and Hillis 1959).

3.2.7 Calculations and data analyses

Biomass dry weights were not used directly to analyse differences between treatments, as the dry weights of the control and AN70 treatments differed between the main and the additional experiments. However, the increase in the dry biomass of the AN70 treatments over the control were the same ($P < 0.05$) between the two parts of the experiment, therefore the increase in biomass over the control for each treatment can be compared together. Results are presented as the % increase in biomass over the control, calculated by subtracting the mean control dry weight from that of each replicate and converting to a % of the control dry weight.

The shoot and whole plant fertiliser equivalents (FEs) were calculated by use of a linear regression of N offtake against N added for the fertiliser treatments, as in Sánchez-Rodríguez et al. (2018). The shoot FEs were also calculated at various stages of the experiment according to the cumulative day degree (CDD). The CDD is a measure of the total of the mean daily temperatures above 5 °C and was calculated as in Bhogal et al. (2016). This allows comparison of the N uptake with that of other experiments conducted at different temperatures. It was not possible to calculate FEs of biomass, as the biomass production of gunnera treated grass was higher than that from the highest level of the fertiliser treatments. Apparent nitrogen NREs were calculated as in Ovsthus et al. (2017) by subtracting the N uptake of the control replicate from the N uptake of each treated replicate (paired by block), dividing by the amount of N added and converting to a percentage.

All statistical analyses were performed using SPSS v25 (IBM Corp., Armonk, NY). Data were tested for homogeneity of variance using Levene's test. One way analyses of variance (ANOVA), and the Tukey's honest significant difference test were conducted to determine significant differences between treatments. Relationship between data were identified using linear regression, multiple regression and Pearsons correlation as appropriate.

3.3 Results

3.3.1 Above ground biomass

The biomass data are presented here as the dry weight relative to the control, so that the biomass production of the higher fertiliser levels (AN140 and AN200) can be considered together with those of the other treatments (see methods section 3.2.3). Clover, alder and gorse treated soil produced the same amount of shoot biomass over the course of the experiment as that from AN200, whereas the gunnera treatment resulted in a higher total cumulative shoot biomass than all other treatments at 68 ± 5 % additional biomass over that of the control ($P < 0.001$). Fig. 3.1.

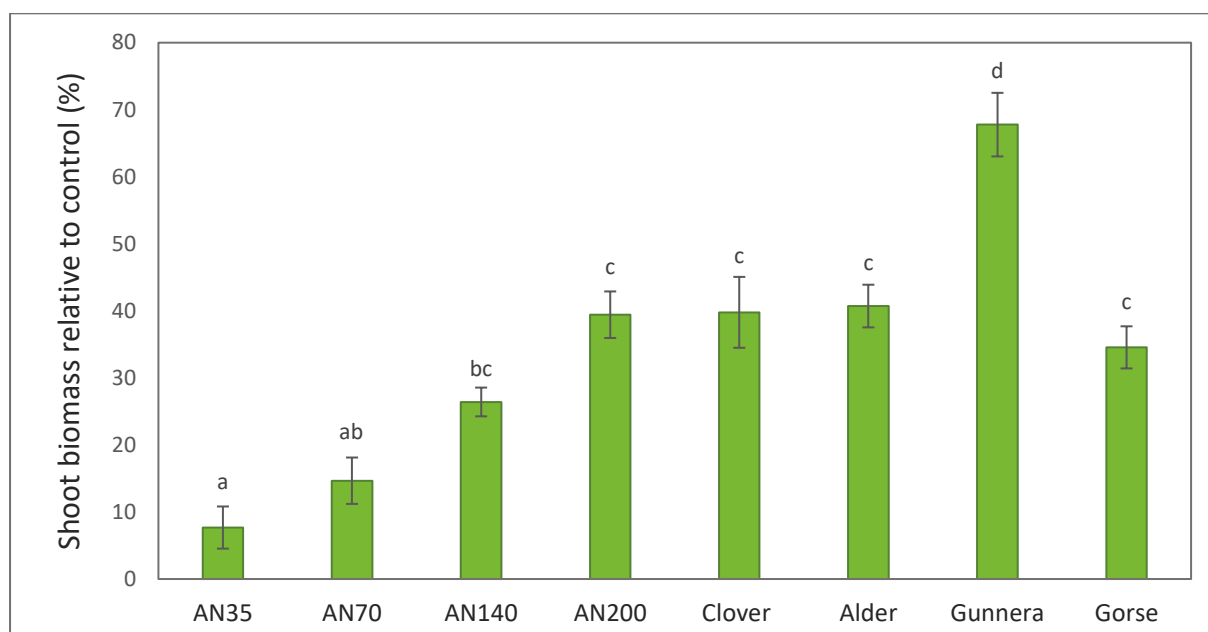


Fig. 3.1. Shoot biomass total dry weight produced over the experiment (% increase over the control biomass). Means \pm SEM, ($n = 5$). Letters denote significant differences at $P < 0.05$.

Biomass production from the AN140 and AN200 treatments was highest between days 31 and 59 (Fig. 3.2). The AN200 treatment produced an additional 294 % more biomass than the control during this period. The growth response from green manures was later than that from fertiliser treatments. The highest growth rates from clover, alder and gorse occurred between 59 and 94 days. The gunnera treatment produced a more delayed response. Gunnera treated shoot biomass produced in the first 31 days was the same as that of the control, but between 94 and 164 days, the biomass growth rate from gunnera was much higher than all other treatments with a 287 % increase over the control between days 94 and 129 ($P < 0.001$).

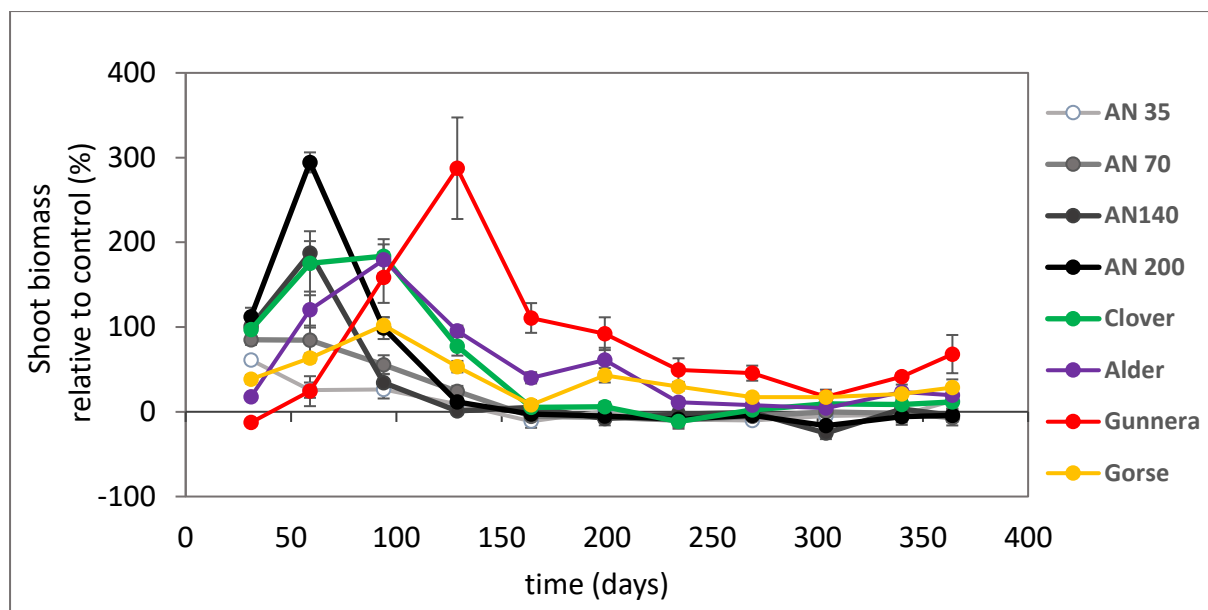


Fig. 3.2. Shoot biomass dry weight. % increase over control. Means \pm SEM ($n = 5$)

3.3.2 Below ground

Gunnera treated soil resulted in a much higher root biomass with an additional 171 ± 31 % more than the control root biomass and was different to all other treatments ($P < 0.02$, Fig. 3.3). The whole plant biomass (root and shoot) from the gunnera treatment was 90 ± 9 % more than the control whole plant biomass (Fig. 3.4). The root to shoot ratio of grass grown in gunnera-treated soil was also higher than that of all other treatments except AN35 and gorse ($P < 0.05$, Table 3.3).

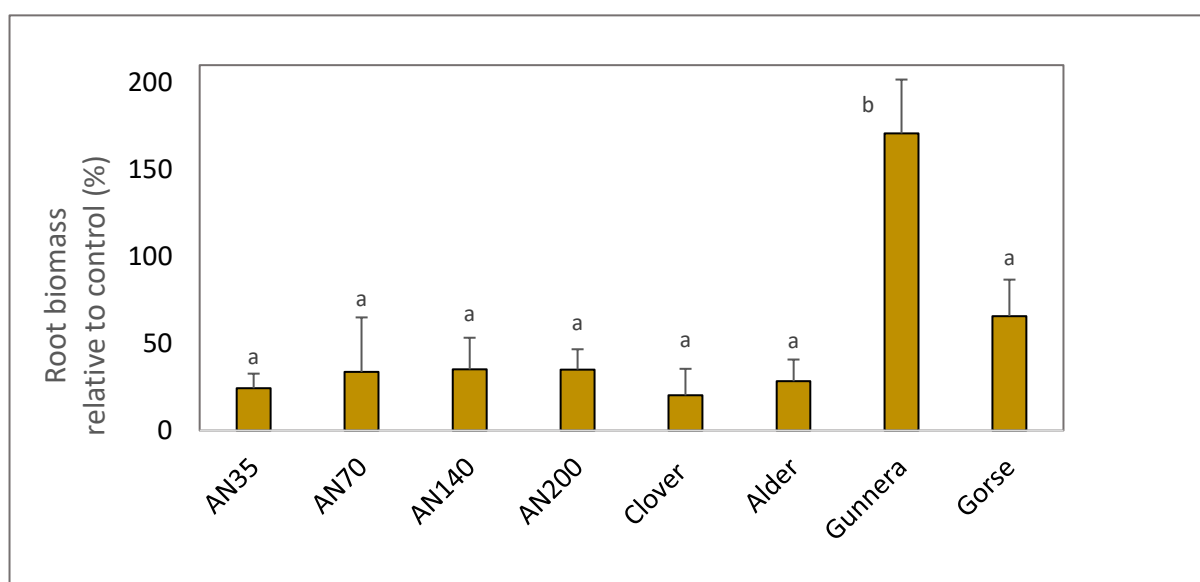


Fig. 3.3. Root biomass dry weight. % increase over control after 364 days of growth. Means \pm SEM ($n = 5$). Letters denote significant differences between treatment at $P < 0.05$)

Table 3.3 Ratios of root:shoot dry weight. Means \pm SEM ($n = 5$) Letters denote significant differences between treatment at $P < 0.05$)

Treatment	Mean \pm SEM
Control	0.27 \pm 0.02 a
AN 35	0.31 \pm 0.02 ab
AN 70	0.27 \pm 0.04 a
AN 140	0.27 \pm 0.04 a
AN 200	0.25 \pm 0.02 a
Clover	0.23 \pm 0.03 a
Alder	0.25 \pm 0.02 a
Gunnera	0.43 \pm 0.05 b
Gorse	0.33 \pm 0.04 ab

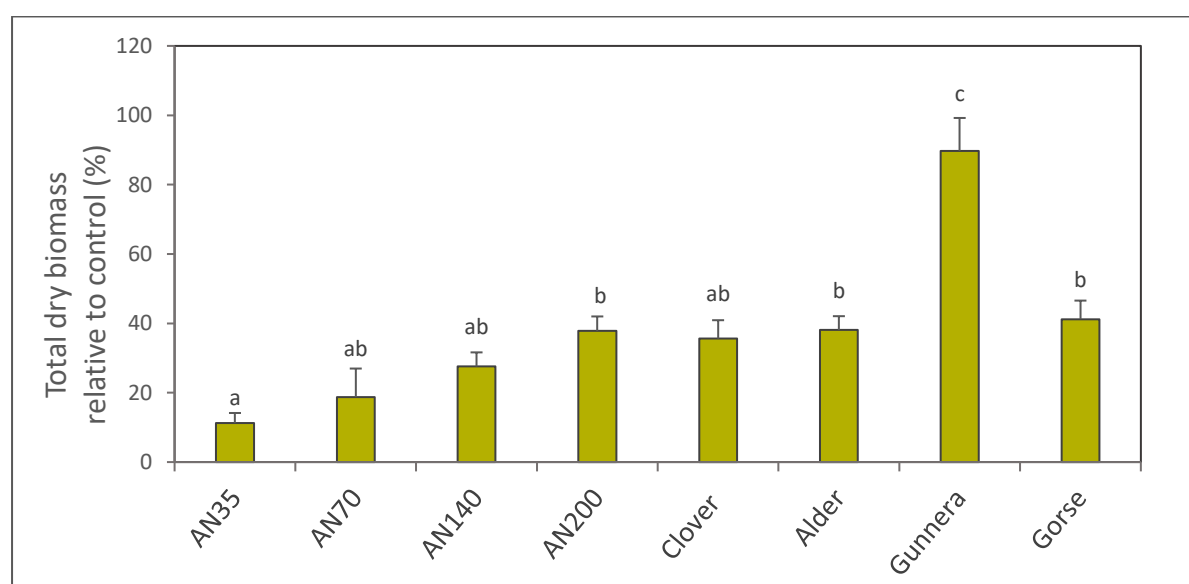


Fig. 3.4. Total biomass dry weight (shoot and root). % increase over the control. Means \pm SEM ($n = 5$). Letters denote significant differences between treatment at $P < 0.05$)

3.3.3 Biomass N content

The shoot N content per cut and cumulative shoot N content are shown in Figs. 3.5 and 3.6. There were large differences in the % N of the shoot tissue between treatments in cut 1 (31 days) and cut 2 (59 days) (Table 1 appendix 1), and therefore large differences in the amount of N in shoots. After the first 31 days, alder and gunnera treated grass shoots only contained the same amount of N as the control grass shoots. In the same time period, the N in the clover treated grass was over three times that of gunnera treated grass, and the shoot N content from AN140 and AN200 treated grass was higher than all other treatments ($P < 0.001$). A regression between the C:N ratios of the green manures

and shoot N content of cut 1 showed a negative relationship (Fig. 3.7, $P = 0.06$). However, gorse treated grass had a higher N content of $3.9 \pm 0.1 \text{ g m}^{-2}$ than is to be expected by its C:N ratio of 26.8 according to the regression.

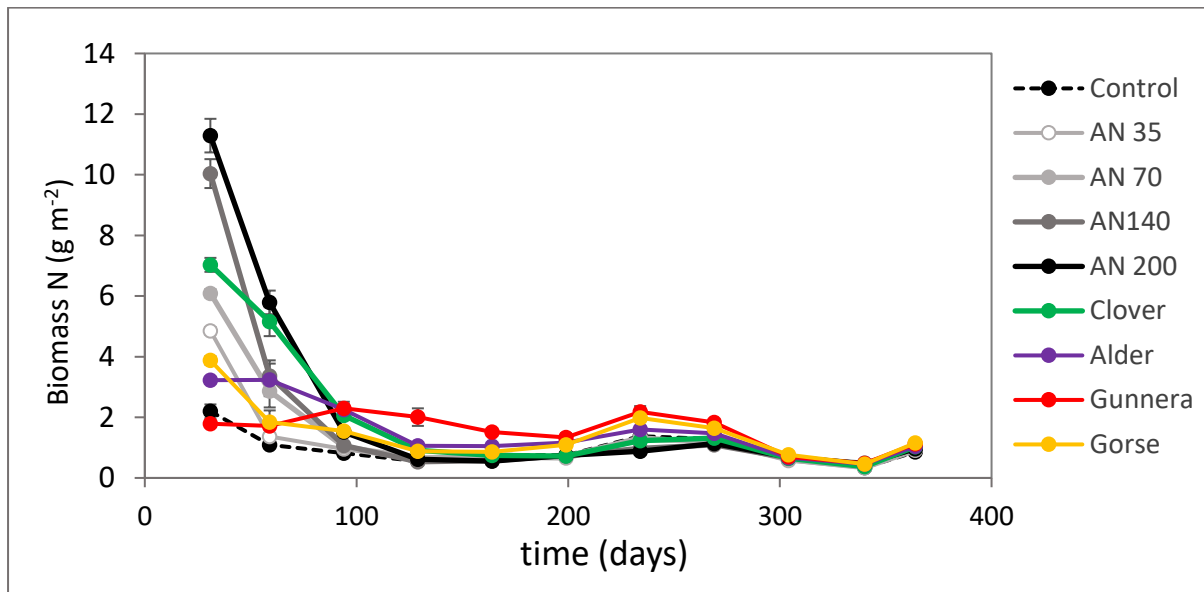


Fig 3.5. Shoot N offtake. Means \pm SEM ($n = 5$).

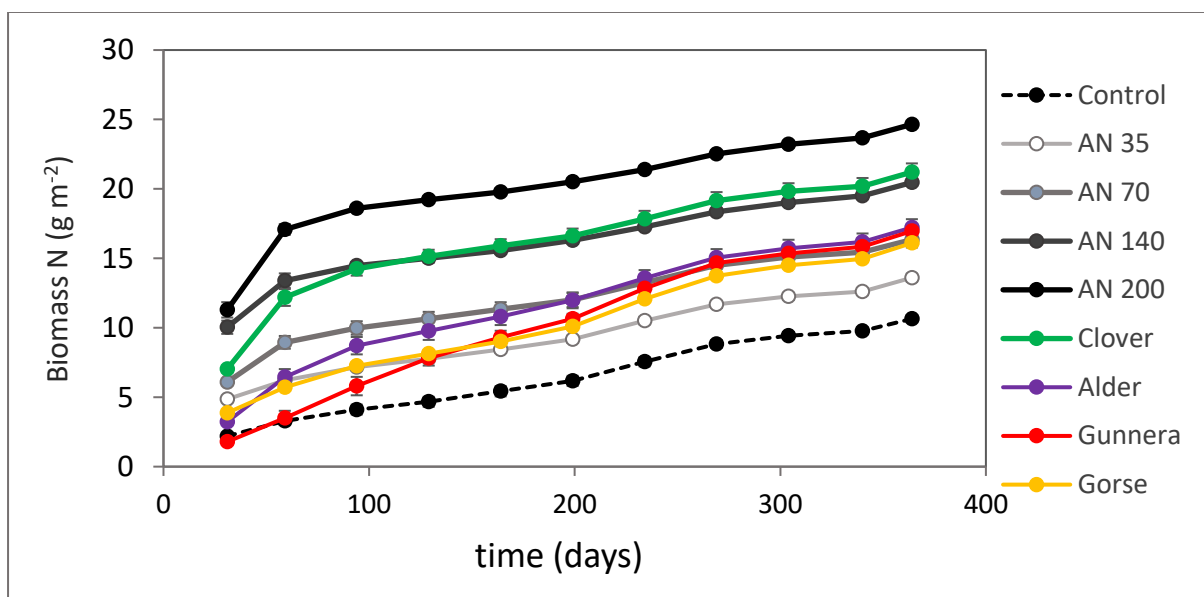


Fig 3.6. Cumulative Shoot N offtake. Means \pm SEM ($n = 5$).

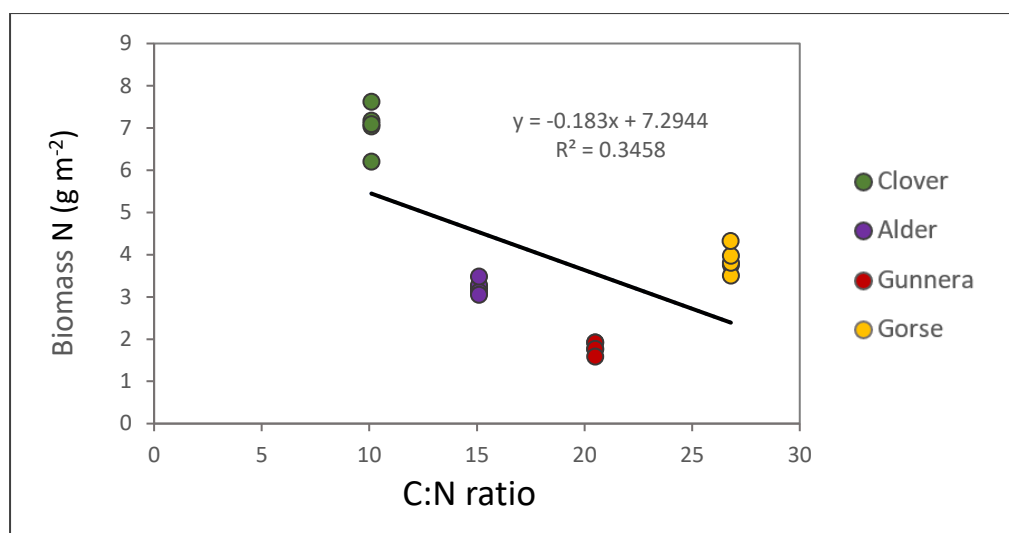


Fig 3.7. Relationship between the C:N ratio of the green manures, and the N content of the grass shoots cut on day 31. Data points are individual replicates.

Fig. 3.8 shows the root N offtake and Fig. 3.9 the total whole plant offtake (shoot plus root). There was no difference in the percentage N of root tissue between the treatments (Table 1 appendix 1), but the high root biomass from the gunnera treatment made a large contribution to the total plant N content. This resulted in a FE of the whole plant (root plus shoot) of gunnera-treated grass which was the same as that from the clover treatment, and higher than that from alder and gorse ($P < 0.04$, Fig 3.10). The FEs of shoot uptake calculated at various stages of the experiment in relation to CDDs and are shown in Table 3, Appendix 1, and used in Chapter 5 in comparison with N uptake of the field experiment.

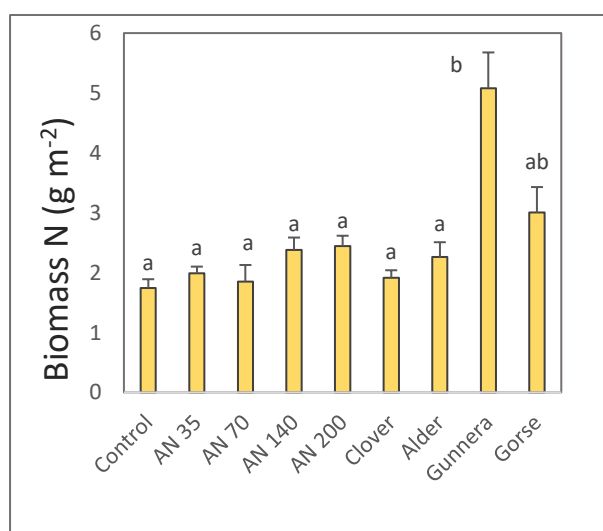


Fig 3.8. Root Biomass N content after 364 days of growth. Means \pm SEM ($n = 5$). Letters denote significant differences between treatment at $P < 0.05$)

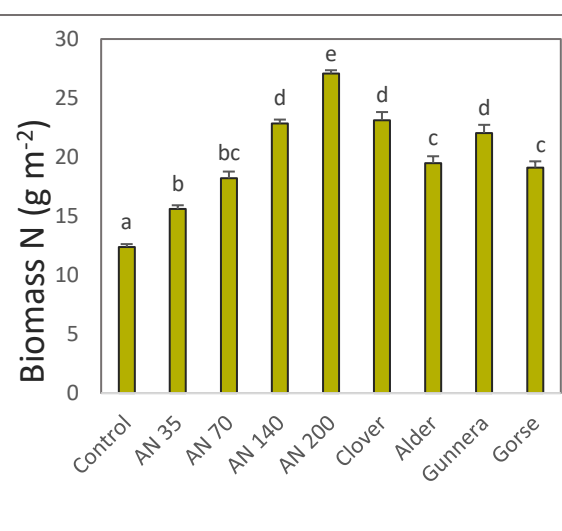


Fig. 3.9. Whole plant Biomass N content after 364 days of growth. Means \pm SEM ($n = 5$). Letters denote significant differences between treatment at $P < 0.05$)

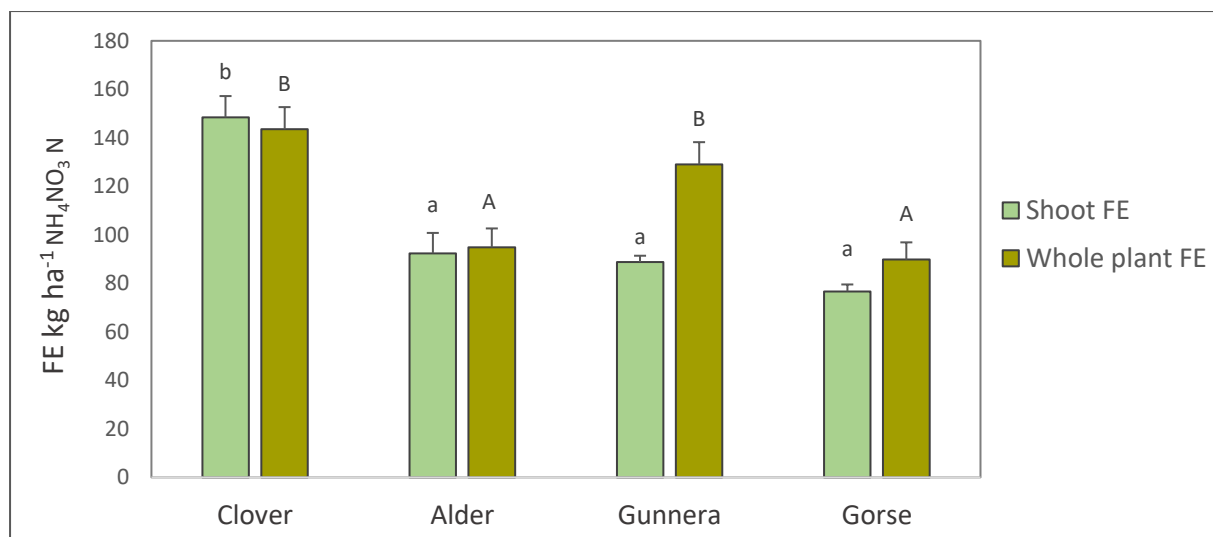


Fig. 3.10. Fertiliser equivalent values. Means \pm SEM ($n = 5$). Letters denote significant differences between treatment at $P < 0.05$

NREs are a measure of the proportion of the N added which was taken up by the plant. The PMGMs had lower NREs than fertiliser treatments ($P < 0.04$, Fig. 3.11). In the case of the gunnera treatment, the root N contributed over a third of the whole plant N recovery.

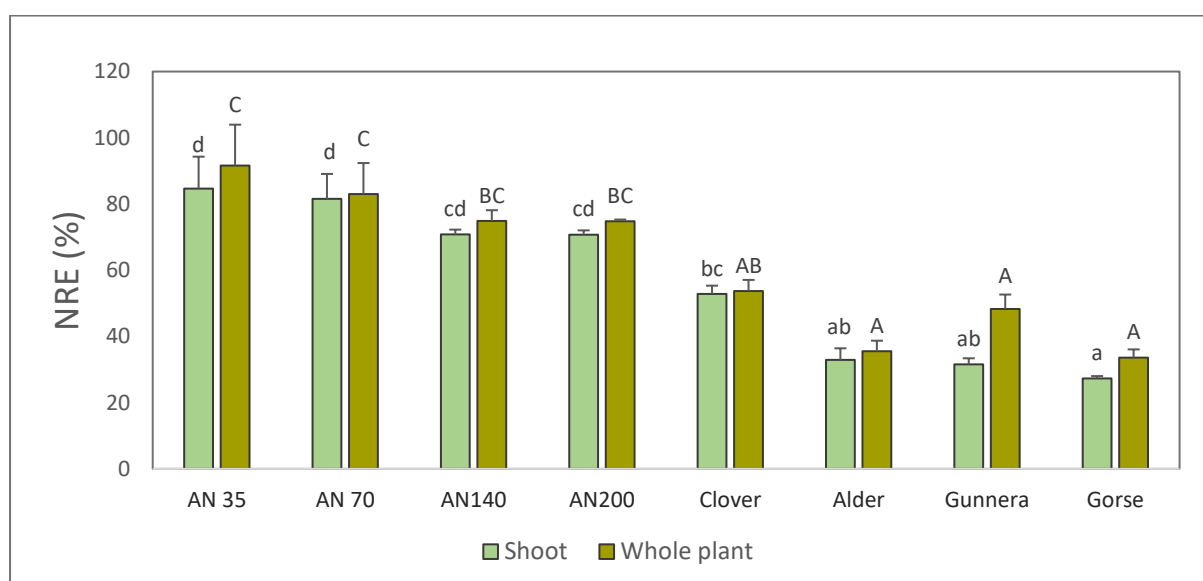


Fig 3.11. NREs after 364 days of growth. Means \pm SEM ($n = 5$). Letters denote differences between treatments at $P < 0.05$

3.3.4 Soil solution

Some measurements of N in solution for the control and AN70 differed between the main and additional experiments, and therefore data for soil solution N is only shown here for treatments of the main experiment. Data for the additional experiment are shown in appendix 1, tables 4 to 7. Total

N (TN) and NO_3^- in soil solution are shown in Figs. 3.12 and 3.13. Fertiliser N resulted in high NO_3^- and TN levels from day one, which reduced after day 18. Clover-treated soil solution TN was slower to reach its maximum concentration of over 140 mg L^{-1} on day 32. In contrast, the TN in solution from the control or PMGMs remained below 35 mg N L^{-1} , with the highest concentrations occurring within the first 9 days. From day 67 onwards, levels of TN from all treatments were below 20 mg N L^{-1} . During the rest of the experiment gorse was the only treatment which had different levels of TN in soil solution. Though the TN concentrations were low, they were consistently higher than most treatments, and higher than all other treatments on days 95 and 151 ($P < 0.001$). Organic N in soil solution is shown in Fig. 3.14. Treatments AN35 and AN70 as well as clover had the highest levels at the start of the experiment.

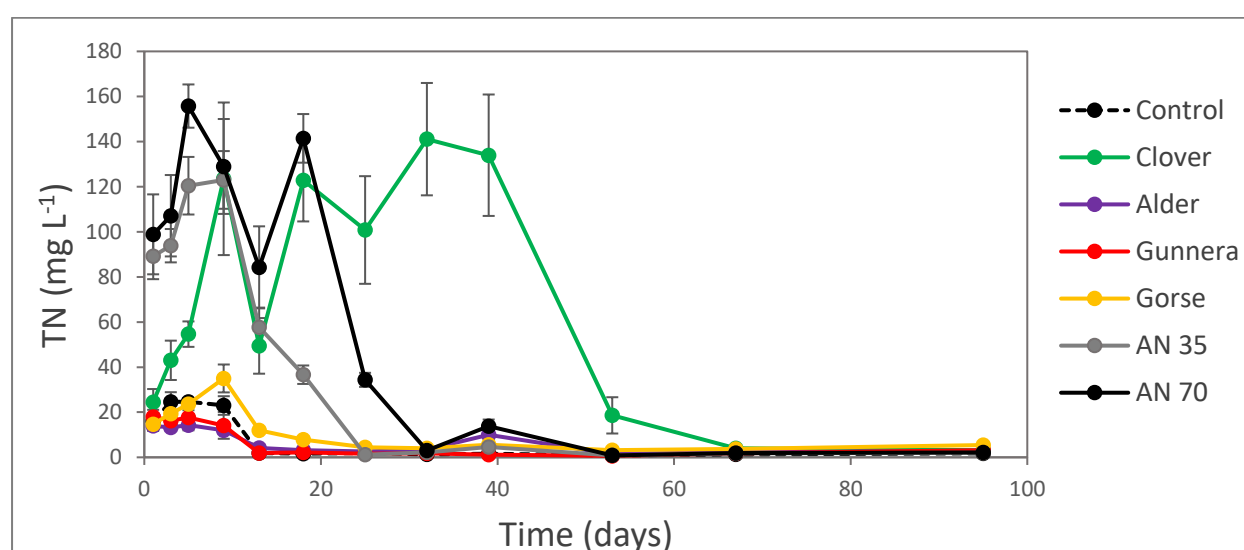


Fig. 3.12. Total N in soil solution. Means \pm SEM ($n = 5$).

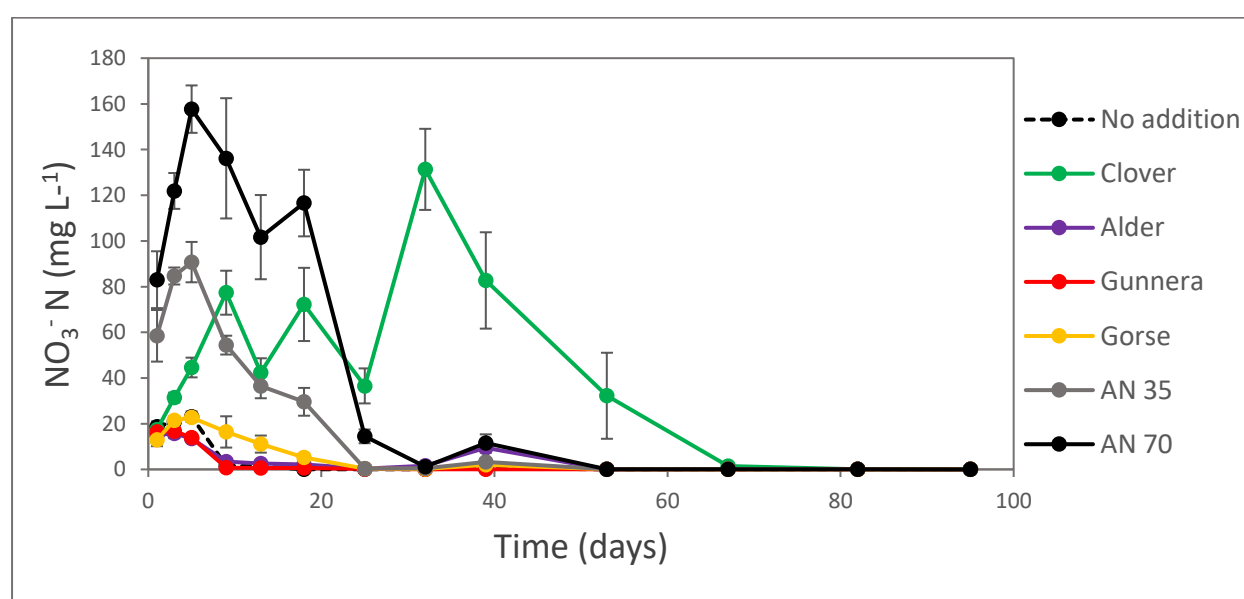


Fig. 3.13. Total nitrate in soil solution. Means \pm SEM ($n = 5$).

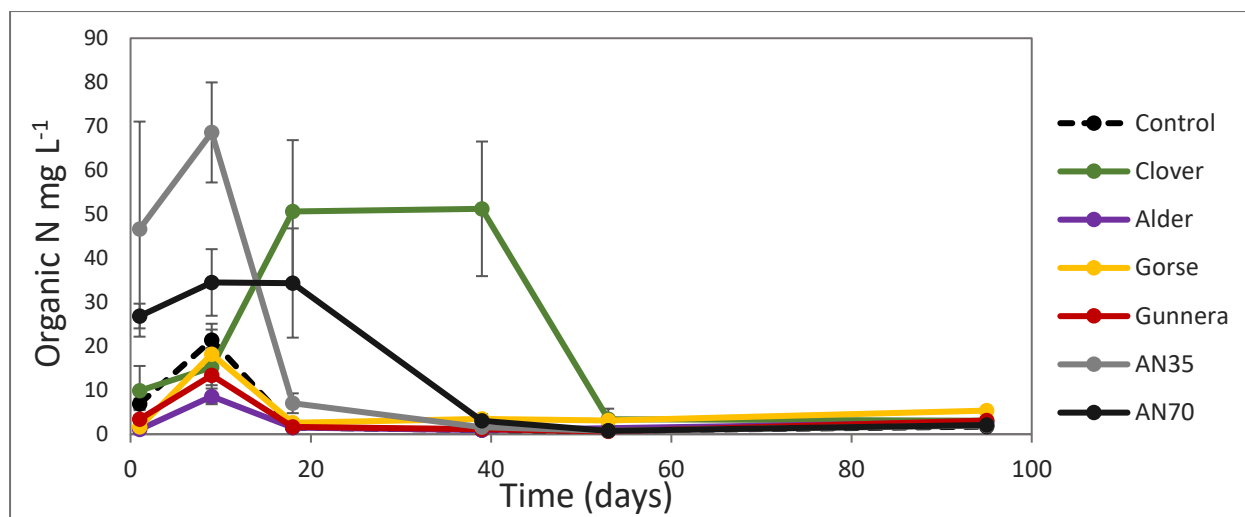


Fig 3.14 Organic N in soil solution. Means \pm SEM ($n = 5$).

Regressions of the shoot N content of grass cut on day 31 against average TN in solution per day (weighted mean of TN from day 1 to 31) showed that N content increased by 0.13 g m^{-2} for each 1 mg L^{-1} in the soil solution of PMGM treated soil ($P = 0.015$), but at the higher TN levels produced by clover and AN35 and AN70 treatments, the increase was only $0.03 \text{ g m}^{-2} \text{ N}$ for each 1 mg L^{-1} of TN ($P = 0.01$; Fig. 3.15).

NH_4^+ in soil solution reached no higher than $6.39 \pm 0.76 \text{ mg L}^{-1} \text{ NH}_4^+ \text{-N}$ for treatments other than AN140 and AN200 at any point. AN200 treated soil reached $213.32 \pm 63.49 \text{ mg L}^{-1} \text{ NH}_4^+ \text{-N}$ on day 1 but dropped to below $15 \text{ mg L}^{-1} \text{ NH}_4^+ \text{-N}$ from any treatment in the additional experiment from day 25. (Table 6, appendix 1) AN70 and clover treatments resulted in initially high amino acid N contents of 535.7 ± 137.4 and $211.2 \pm 124.9 \mu\text{g L}^{-1}$, respectively, on day 1, which reduced and stay below $20 \mu\text{g L}^{-1}$ from day 13. (Table 7, appendix 1).

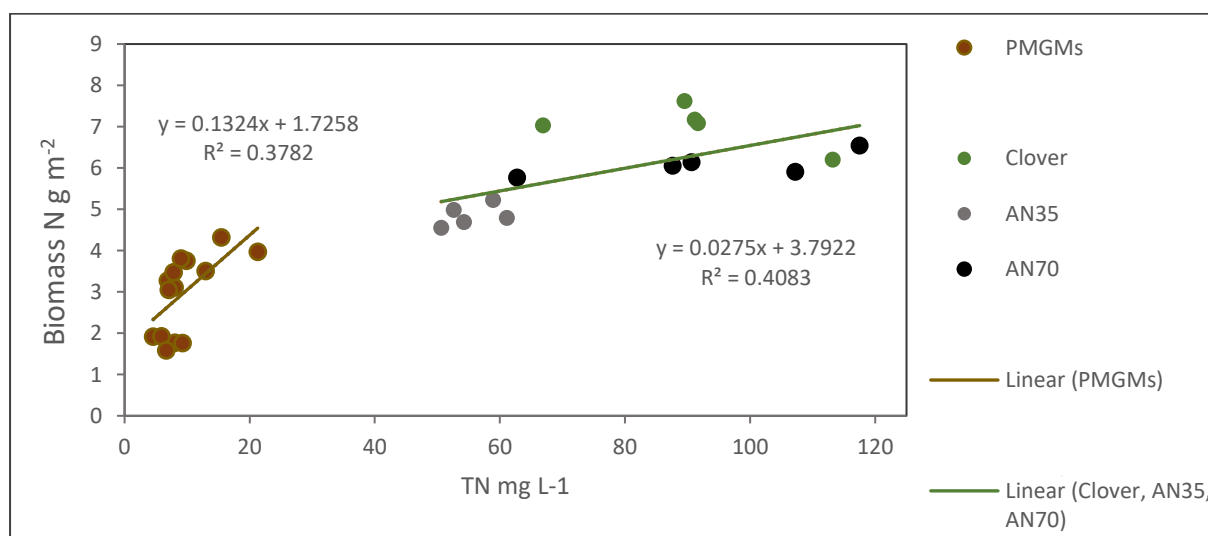


Fig. 3.15. Relationship between TN in solution (weighted mean average per day, d1-d31) against shoot N content.

Concentrations of phenols in soil solution varied little between treatments, except for the gorse treated soil (Fig. 3.16). Though gorse treated soil had a low level of phenols at the start, over the course of the experiment, it had a higher level than the control and AN70 treatments ($P < 0.05$), and a higher level than all other treatments on day 263 ($P < 0.001$). Gorse treated soil had the highest level of DOC in soil solution from day 24 onward ($P < 0.05$). There were no differences between levels of microbial biomass C between treatments at the end of the experiment (Table 8, appendix 1).

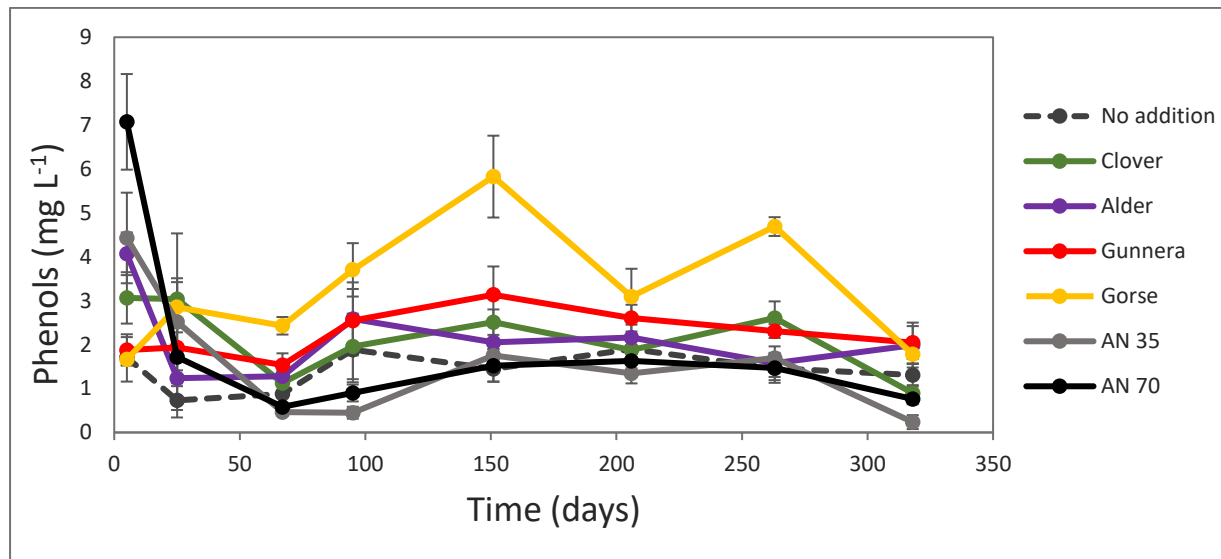


Fig 3.16. Phenolics in soil solution. Means \pm SEM ($n = 5$).

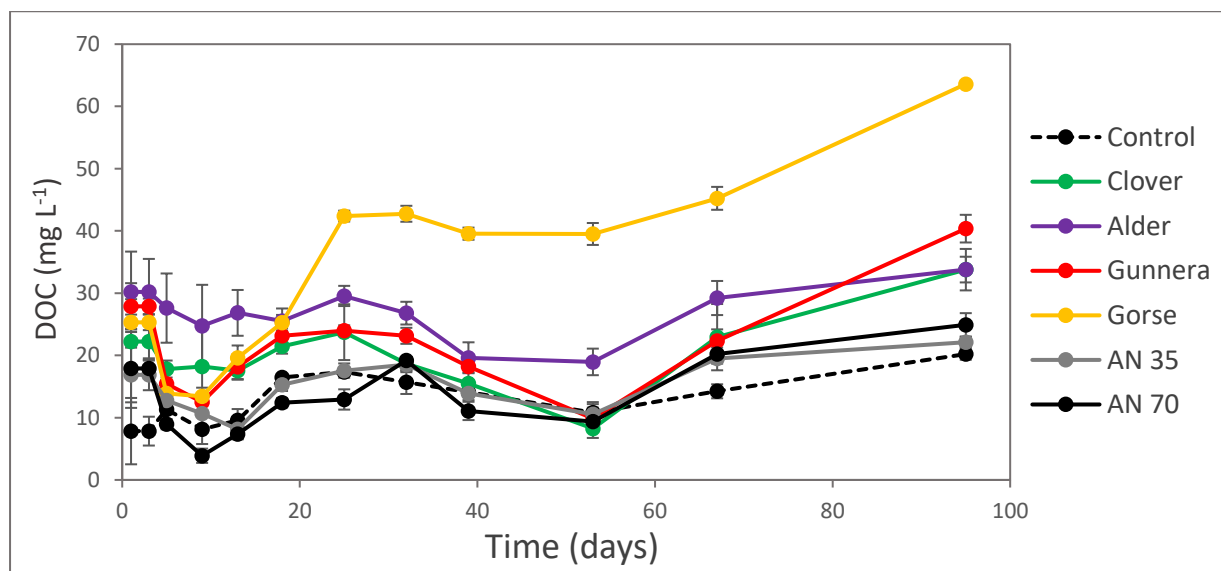


Fig 3.17 DOC in soil solution. Means \pm SEM ($n = 5$).

3.35 Nitrous oxide

N₂O flux rates varied greatly between treatments, until day 53, when all fluxes reduced to below 100 $\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ (Fig. 3.18). Flux rates from clover treated soil were higher than all other treatments between days 13 and 39, and the clover treatment produced a cumulative emission per unit area which was over three times higher than that from any other treatment (Fig. 3.19a). Clover treated soils had a higher EF than the PMGMs with 5.3 % of clover-N added being lost as N₂O-N, compared to only 0.6, 0.3 and 1.2% of N supplied as tissue of alder, gunnera and gorse and respectively (Fig. 3.19c). The AN35 and AN70 treatments had lower N₂O emissions than clover on a unit area or biomass basis (Figs 3.19 a and b). However, EFs for AN35 and AN70 were as high as the clover EF due to the lower amount of N added in these fertiliser treatments (Fig 3.19c). All the PMGM treatments resulted in lower EFs than both clover and AN70, but only gunnera treated soil had lower N₂O emissions per unit of grass biomass dry weight than the AN 70 treatment (Fig 3.19b).

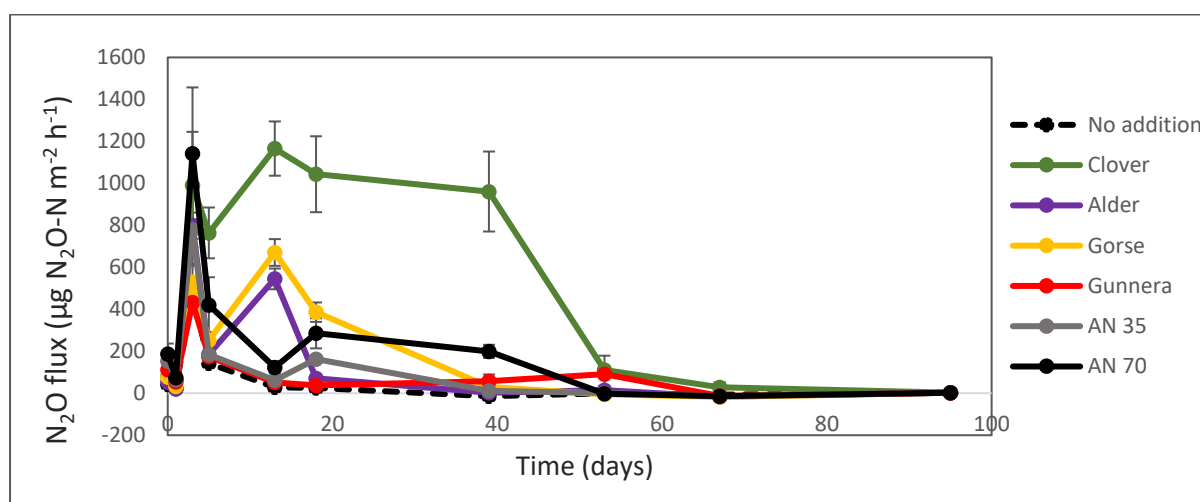


Fig 3.18. N₂O Fluxes. Means \pm SEM ($n = 5$).

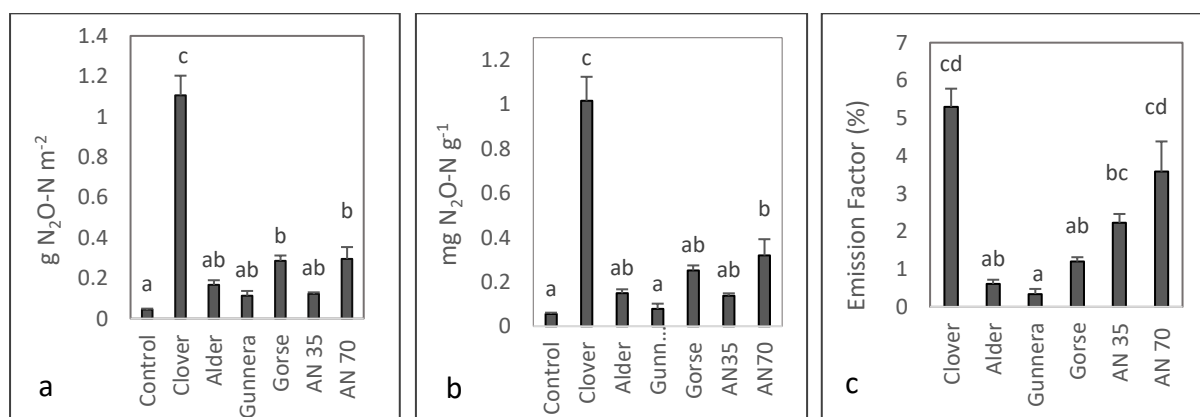


Fig 3.19 Total cumulative N₂O emissions by a) on an area basis, b) by dry weight of biomass, and c) by Emission Factor (EF). Means \pm SEM ($n = 5$). Letters denote significant differences between treatment at $P < 0.05$

N₂O emissions were negatively correlated with the C:N ratio of the green manure ($P = 0.002$, $r^2 = 0.41$, Fig. 1 Appendix 1). A regression of N₂O against NO₃⁻ for days 1 to 53 shows that N₂O fluxes increased on average by 3.27 ug N₂O-N m⁻² h⁻¹ for each 1 mg L⁻¹ increase in NO₃⁻ in soil solution (Fig 3.20, $P < 0.001$, $r^2 = 0.117$). The level of NO₃⁻, however, only accounts for 11.7 % of the variation in N₂O production across the experiment. Multivariate regressions for each timepoint of N₂O fluxes against DOC and NO₃⁻ in soil solution show that the relationship varies greatly over time (Table 3.4). On some days there was no significant relationship between N₂O and NO₃⁻, but a strong relationship on other days of up to an increase of 7.13 ug m⁻² h⁻¹ for each 1 mg L⁻¹ increase in NO₃⁻ ($P < 0.001$) on day 39. DOC shows a positive relationship with the N₂O fluxes on day 13 only.

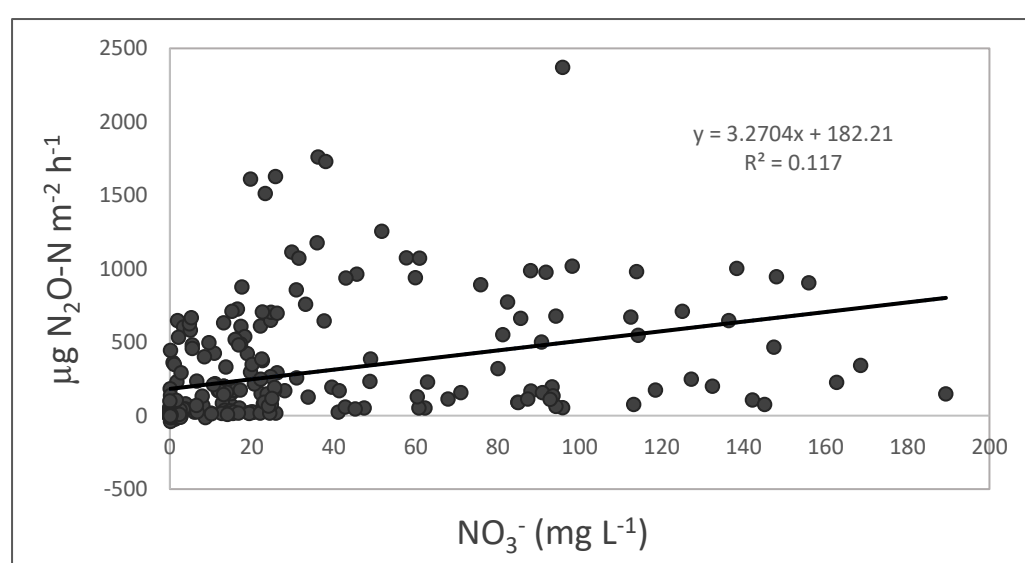


Fig 3.20 Regression of N₂O flux against NO₃⁻ in soil solution. Data points are individual replicates for time points 1 to 53 days across all treatments.

Table 3.4. Multivariate regressions with N₂O as the dependent variable and NO₃⁻ and DOC as the independent variables. Unstandardised coefficients represent the change in ug N₂O-N m⁻² h⁻¹ for each additional mg L⁻¹ NO₃⁻ /DOC

Day	r ²	Nitrate Unstandardised Coefficient	DOC Unstandardised Coefficient
1	0.154	0.525*	-0.378
3	0.163	3.50	-10.78
5	0.051	1.19	2.21
13	0.193	1.395	22.669*
18	0.233	4.40**	21.04
39	0.441	7.13**	-5.08
53	0.77	1.10	-.615

*Significant at $P < 0.05$ **Significant at $P < 0.001$

Data suggest that there were interactions between the effects of NO_3^- and DOC in soil solution on N_2O fluxes. Fig. 3.21 shows scatterplots for selected treatments on days 5, 13 and 18 when N_2O fluxes from clover treated soil were particularly high. For example, on day 13 the N_2O fluxes from the clover treatment were over 9 times those from AN70 (Fig. 3.18), despite clover treated soil NO_3^- levels being less than half that from AN70 treated soil. Gunnera treated soil with high DOC, but low NO_3^- emitted a very low amount of N_2O (Fig 3.21b). It is the clover treatment, with high of levels of both $\text{DOC} > 7$ mg L^{-1} and $\text{NO}_3^- > 25$ mg L^{-1} which emitted the highest levels of N_2O .

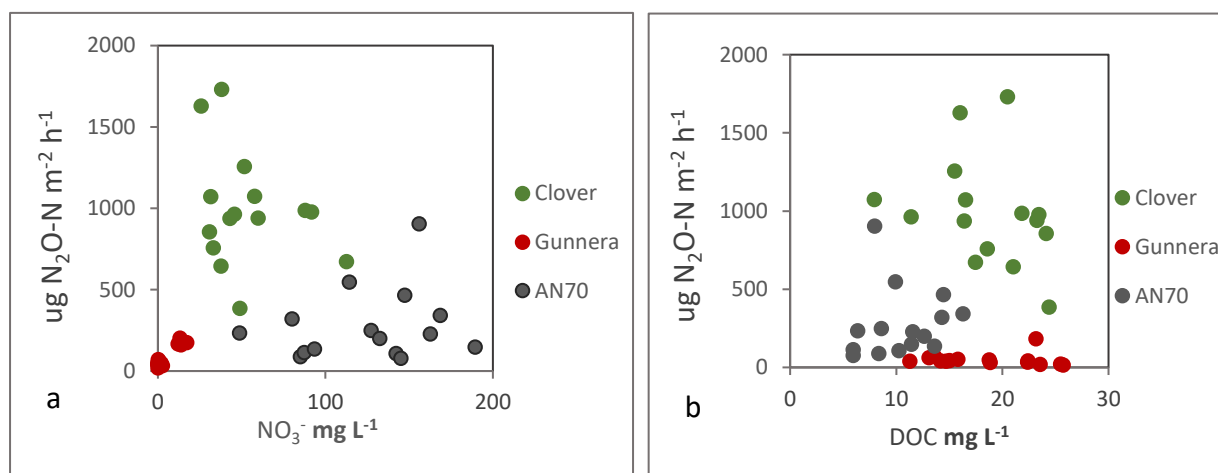


Figure 3.21. Relationship between N_2O fluxes and a) NO_3^- and b) DOC in solution on days 5, 13 and 18 for clover, gunnera and AN70 treatments.

3.4 Discussion

3.4.1 Effectiveness of PMGMs as fertiliser

The biomass production shows that PMGMs were effective as N fertiliser. Notably, the gunnera treatment resulted in a much higher production of grass biomass than the same amount of N supplied as either traditional clover or as NH_4NO_3 . The NREs are at the high end of a widely varying range previously reported for green manures and fertiliser (Crews and Peoples 2005, Araya et al 2010). The FEs and NREs demonstrate a lower overall N uptake from PMGM N than from N supplied as NH_4NO_3 . This is not necessarily indicative of less effective fertilisation by the PMGMs. Crop uptake of N higher than that needed for plant growth (luxury uptake) is not always beneficial, and N which is retained in soil, can yet be decomposed to plant available forms to supply future crops (Hansen et al. 2019). In addition, if C sequestration is to occur, retention of some N is necessary as a component of organic compounds (Knicker 2011, Poulton et al. 2018).

The whole plant FEs and NREs showed that gunnera supplied the same amount of N to the crop as the clover treatment, but without the high N_2O emissions or high NO_3^- in soil solution which increases the risk of N leaching. Gorse and alder treatments also met a considerable amount of the N demand. The FEs demonstrate that the addition of 200 kg N ha^{-1} within gorse and alder tissue provided the equivalent N as adding 89.8, and 94.8 kg ha^{-1} , respectively of $\text{NH}_4\text{NO}_3\text{-N}$. FEs need to be considered alongside the potential for N pollution, the effect on soil organic matter, and the possibility of green manure N becoming available for plant uptake in future years. Thus, it is important not to dismiss an amendment solely on the basis of having a low FE. Although the highest NH_4NO_3 treatment of 200 kg ha^{-1} had an NRE of 70.5 %, this experiment did not allow leaching of NO_3^- . In a farm situation, leaching could be considerable at the end of the cropping season due to the high NO_3^- levels in soil solution, whereas less leaching would be possible from the lower concentrations of NO_3^- in PMGM treated soils (Campiglia et al. 2011, Congreves and Eerd 2015). Therefore, conditions of high rainfall in a farm situation could result in the fertiliser and clover treatments having a lower NRE than shown in this experiment due to NO_3^- leaching leading to lower plant uptake. A potential inaccuracy of the NRE is that soil amendments may stimulate mineralisation of existing soil N (priming) which can give an artificially high apparent NRE (Kuzyakov et al. 2000, Shahbaz et al. 2018, Liang et al. 2017).

3.4.2 Synchrony of N supply with demand

Maximum synchrony of N supply and demand is key to increasing the ratio of crop production to N pollution. The high initial shoot N uptake from fertiliser treatments is expected due to the direct supply of NO_3^- and NH_4^+ , but this also occurred in clover-treated soil indicating very fast decomposition of

clover. There were high concentrations of N in both inorganic and organic forms in clover treated soil with NO_3^- reaching over 130 mg L^{-1} and DON over 50 mg L^{-1} . It is likely that there was a rapid turnover of organic N. The half-life of peptides in soil solution due to microbial consumption has been measured at < 1 minute, and fast microbial uptake of the amino acids results in half-lives measured at < 0.5 minute (Hill et al. 2012). Therefore, it is likely that there were very high rates of both production and consumption of organic N. The grass may also have taken up organic N directly. *L. perenne* has been shown to take up amino acids when grown hydroponically (Thornton and Robinson 2005) and many other temperate plant species have been shown to take up organic N from soil, for example uptake of glutamine by *Arabidopsis thaliana* (Ganeteg et al. 2017), of glycine by wheat (Ma et al. 2018), and of L-alanine and L-tri-alanine by a variety of grassland plants (Wilkinson et al. 2015).

High amino acid concentrations from the AN70 treatment were unexpected and may be due to fast microbial growth and death and decomposition or microbial exudates (Bottomley et al. 2012). During the first 31 days, the high N content in clover and AN70/140/200 treated grass shoots of $> 5 \%$ and total N in soil solution of $> 50 \text{ mg L}^{-1}$, indicates that plant available N exceeded that which the grass could take up and therefore was not a limiting factor to plant growth. The regression of cut 1 grass shoot N content to TN in soil solution shows that additional TN in fertiliser and clover treated soil solution when concentrations were over 50 mg L^{-1} made little difference to shoot N uptake. Growth may instead have been limited by temperature (averaging 17.5°C during the first 31 days) and/or light levels, with excess N being retained in soil or lost as gases. Optimum temperature for *Lolium perenne* growth is considered to be between 20 to 25°C (Hannaway et al. 1999). The availability of non-N nutrients is not likely to have limited biomass production as adequate levels were provided to the seedbed.

Excess N which was taken up into the crop over that needed for growth and functioning, though safe from denitrification, was taken out of the system by harvesting and therefore could not contribute to further biomass production. This reduced the efficiency of biomass production by clover and AN200 treated grass. A high N content is advantageous to some crops, such as fodder for animal feed (AHDB 2014), or grain content of wheat (Jones and Olson-Rutz 2012). However, for other crops high N may not be desirable, and high biomass is more advantageous. Some vegetable crops have been found to contain high levels of NO_3^- , which has been associated with health risks, for example, methaemoglobinemia and gastric cancer (Chen et al. 2004, Hord et al. 2009). Although the average diet is unlikely to exceed the safe limit of $222 \text{ mg NO}_3^- \text{ day}^{-1}$ for an adult, it can be a risk for people consuming large quantities of leafy vegetables (Brkić et al., 2017). In situations where high N is not necessary for crop quality, preservation of N in the soil, is advantageous. Therefore, for many crops, a high biomass FE value is more desirable than a high N FE value.

The negative correlation of green manure C:N ratio with the shoot N uptake in the first 31 days is in agreement with N release shown in previous studies (Mary et al. 1996, Trinsoutrot et al. 2000, Nicolardot et al. 2001). Here the green manure C:N ratio only accounted for 35 % of the variation in shoot N content. This is to be expected as many factors other than the C:N ratio affect N mineralisation rates such as phenolic content, and the lability of the C and N compounds (Crews and Peoples 2005, Chen et al. 2014, Whalen 2014). The fast uptake of N into the grass shoots from the clover treatment indicates that the clover tissue had both a high lability and that immobilisation of N by microbes did not prevent uptake by the grass. Where uptake of N into grass was slow for alder and gunnera treatments, it is unknown whether the green manures were slow to decompose or whether low molecular weight N had been immobilised in microbial biomass. It must be noted that we have assessed the timing of N uptake to shoots by assuming that most N taken into roots is quickly transported to shoot tissue. The gunnera treated grass allocated over 20 % of the N uptake to roots, for which the timing of uptake of growth and N allocation is unknown.

That fertilisation by gunnera or alder did not result in elevated N content in the grass tissue or in soil solution, shows that plant-available N was not in excess of the demand by the grass. The gunnera treated grass had a lower initial N uptake than the clover or AN200 treated grass but resulted in higher crop growth between days 95 and 234, making more efficient use of N. Low levels of mineral N in soil do not necessarily indicate that N is a limiting factor for plant growth as both organic matter mineralisation and plant uptake of mineral N can be rapid and may be in balance (Jackson et al. 2008). In addition, the grass may have taken up some organic N, so bypassing the need for N mineralisation. The NH_4^+ concentration of soil extracts is not an accurate indication of plant available NH_4^+ as the ions adsorb strongly to negatively charged clay particles or soil colloids, reducing the NH_4^+ dissolved in solution (Nommik and Vahtras 1982). However, lower % N in shoot tissue and slower growth rates combined with low N in solution indicate that plant available N was likely to have been a limiting factor in grass growth for treatments other than AN70 and clover, and for all treatments after day 31.

After each grass cut, plant uptake of N is likely to be reduced due to smaller grass plants taking up less N. In addition, it has been shown that N used for grass re-growth during the first week after cutting is predominantly remobilised from roots and stubble rather than being taken up from soil (de Boer et al. 2016). It is likely that N was mineralised after dieback of root tissue resulting in the increased NO_3^- and TN in solution on day 32. A smaller rise of NO_3^- and TN also occurred on day 39 in some treatments.

3.4.3 Possible allelopathic effect of gorse treatment

The shoot growth of gorse treated grass, cut after 31 days, had an N offtake of $3.9 \pm 0.1 \text{ g m}^{-2}$ which is higher than is to be expected by its C:N ratio of 26.8. This may be due to heterogeneity of the gorse amendment, which included thin stems with what appeared to be a woody interiors. It is possible that N-rich leaves and stem exteriors with a low C:N ratio decomposed before a more recalcitrant C-rich interior. N uptake by gorse treated grass was low in relation to N in soil solution, compared to other treatments. For example, between days 94 and 199, shoot N uptake from the gorse treatment was lower or equivalent to shoot N uptake from all other treatments, despite a higher availability of TN throughout this period in gorse treated soil. Though these differences are small, taken together with the high N_2O emissions which were equivalent to those from the AN70 treatment per unit area, this suggests that N was not being taken up by grass at as high a rate as the N availability would allow.

This low N uptake may be explained by an allelopathic effect of compounds within gorse tissue. Gorse has been found to emit volatile aromatic compounds such as isoprene (Boissard et al. 2001), and emissions from gorse branches have been shown to inhibit root growth in seedlings (Pardo-Muras et al. 2018). The content of specific volatile organic compounds in gorse tissue is not well known (Pardo-Muras et al. 2018). However, the higher levels of phenolic acids found in the gorse-treated soil solution is in accordance with a possible phenolic-mediated allelopathic effect. The allelopathy of some phenolic compounds in decomposing leaf litter, for example caffeic acid, can suppress the growth of young roots (Batish et al., 2008; Chomel et al., 2016). In addition, higher DOC and TN levels in gorse treated soil suggest a possible restriction on organic matter breakdown, which could be due to limitation of microbial action by phenolic compounds (Batish et al. 2008, Chomel et al. 2016). Another possible cause is the binding of phenolic compounds in protein complexes which limits substrate availability preventing breakdown of organic materials (Halvorson et al. 2009, Gabriel et al. 2016, Garcia-Palacios et al. 2016). This could result in retention of organic molecules and reduced respiration of DOC.

3.4.4 Unravelling the effect of gunnera

The gunnera treatment produced an unforeseen response with delayed shoot growth, and higher shoot and root biomass, root:shoot ratio and total N offtake. Higher root:shoot ratios are known to occur in low nutrient environments (Hermans et al. 2006, Cambui et al. 2011, Andrews et al. 2013). However, the gunnera treated soil did not have lower soil N levels than the control soil. There are some possible explanations for increased root biomass in soil treated with organic amendments. Root proliferation can be a response to an uneven distribution of decomposing organic matter, (Robinson 1994, Hodge et al. 1999). A high density of roots may be a response to availability of NH_4^+ , which is

produced before NO_3^- in the mineralisation process, a higher root mass, being better able to take up the less mobile NH_4^+ and compete more effectively with microbial uptake (Jackson et al. 2008, Kuzyakov and Xu 2013). There is some evidence that a high level of organic N in solution, and a higher proportion of uptake of organic N, stimulates an increase in root:shoot ratio, but at present this is mainly limited to laboratory studies in non-soil environments (Cambui et al. 2011, Lonhienne et al. 2014, Franklin et al. 2017).

None of the above offers an explanation on why gunnera-fertilised grass would have a different root:shoot ratio to the other organic amendments. The leaf tissue was distributed similarly within soil for all green manure treatments and none of the green manures had high levels of NH_4^+ in soil solution. Another explanation is that chemicals other than N affected the root:shoot ratio, for example, a stimulation effect from a chemical released from gunnera tissue, or the gunnera immobilising a necessary nutrient, the lack of which stimulated root proliferation. Although the above offers some explanations, the cause of the dramatically higher root mass in gunnera-treated grass is not known.

The higher shoot biomass from gunnera-treated soil may simply be due to a delayed but continuous release of plant available N, which being well synchronised with demand enabled low N losses and resulted in the high shoot biomass production which occurred from day 129 to day 269. It is possible that the chemistry of the gunnera tissue, other than the C:N ratio, influenced the decomposition speed to give a gradual, but long-term release of plant available N. Another explanation is that the increased root surface area of gunnera-treated grass may have enabled increased N uptake from soil, resulting in more efficient use of N than alder or gorse treated grass. Franklin et al (2017) researched the effect of increased root:shoot ratio alongside other factors which can result from organic N uptake. Using previous lab based experiments, they constructed a model which indicated that the direct uptake of organic N by plants has the potential to increase NUE, both by the lower C costs of assimilation of organic N molecules, as well as by the increased capacity for plant uptake of N due to higher root mass. They conclude that agricultural crop yields may benefit from a higher supply of organic N. A greater root mass can also increase N mineralisation, as root exudates and other materials such as mucilage and damaged cell contents provide energy for increased microbial activity (Clarholm 1985, Jackson et al. 2008, Zhu et al. 2014). This could have caused increased decomposition of gunnera tissue and/or other soil organic matter (priming).

3.4.5 N_2O emissions

Though the N_2O EF of 5.5 % from the clover treatment is high, others of a similar magnitude have been recorded from crop residues. For example, Velthof et al. (2002) recorded an EF of 6% from addition of broccoli residue with a C:N ratio of 13.7, also in a pot experiment. Most EFs from residues or cover

crops, however, are much lower. A global meta analyses of field experiments by Charles et al. (2017) reported an average EF of only 0.19 ± 0.08 from residues, though the average amount of N added was only 83 kg ha^{-1} . The EF used by the IPCC for residues in wet soil is 0.6 % with an uncertainty range of 0.1 to 1.1 % (Hergoualc'h et al. 2019). The 3.58 % EF for the AN70 treatment was also very high compared to previous recorded fertiliser EFs, and it is likely that the EF of an AN200 treatment would be higher still. For example Cowan et al. (2020) found an EF for NH_4NO_3 in the UK averaging at 0.6 % when applied to arable crops and 1.3 % when applied to grasslands. Chadwick et al. (2016) similarly found an EF from NH_4NO_3 of 0.52 % when added to arable land and 1.12 % for grassland. The UK country specific EF used at present for NH_4NO_3 to arable land is 0.791 %, and to grassland is 1.3 % (Wakeling et al. 2017) and the EF used by the IPCC for mineral N in wet soil is 1.6 % with an uncertainty range of 1.3 – 1.9 % (Hergoualc'h et al. 2019). However, emissions from NH_4NO_3 can also be highly variable, and Charles et al. (2017) report EFs varying between -0.99 to 12.8% of that added.

The high emissions from the AN70 and clover treatments are not entirely unexpected due to the warm temperatures (monthly means of 17.5 to 28.8 °C) and 70 % WFPS which favour N_2O production. In light of this, the very low emissions from the PMGMs, especially the EFs of 0.61 and 0.34 from alder and gunnera respectively are promising. Emissions also need to be considered in relation to crop yield. A low EF can be expected if the crop is under-supplied with N, and this is not an environmental gain if crop production is inefficient. Here, the gunnera treatment had lower emissions per unit of dry biomass than those from the AN70 treatment, whereas the emissions from the clover treatment are higher than all other treatments. The high variation in fluxes between sampling points shows the importance of short term high N_2O fluxes to cumulative emissions (Henault et al. 2012). Variability in temperature is likely to have influenced N_2O production, and regressions on each sampling point allows analyses without the temperature effect.

From day 39, some negative fluxes were recorded in samples from all treatments except clover, though no single flux was lower than $-40 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$. N_2O consumption can occur in anaerobic conditions, due to the activation of the oxygen intolerant enzyme N_2O reductase which promotes the denitrification of N_2O to N_2 (Chen et al. 2013). As well as N_2O consumption by complete denitrification, N_2O consumption by nitrifier nitrification has also been reported (Chapuis-Lardy et al. 2007). Whether N_2O consumption exists in soil to any significant extent has been a controversial topic (Chapuis-Lardy et al. 2007, Cowan et al. 2014). A review by Chapuis-Lardy et al. (2007) found that negative fluxes are not uncommon and concluded that they should be considered a real phenomenon. More recently however, an investigation by Cowan et al. (2014) using high precision analyses with a detection limit of $4 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ concluded that most reported negative N_2O fluxes

are likely to be due to instrument noise. Here, although it is feasible that N_2O consumption did occur in the soil within anaerobic microsites, the negative fluxes were small enough that they could also be due to inaccuracies in the measurement, and not large enough to impact on the overall scale of the N_2O produced.

3.4.6 Interactions between C and N as drivers of N_2O production

The negative correlation between total N_2O emissions and the C:N ratio of the green manure is in line with other studies (Chen et al. 2013, Shan and Yan 2013, Charles et al. 2017), and accounts for 41 % of variation in N_2O . A higher C:N ratio can reduce N_2O production as C-rich materials promote microbial growth which can immobilise N, reducing the NH_4^+ and NO_3^- available for nitrification and denitrification (Garcia-Ruiz and Baggs 2007, Li et al. 2019). Here, higher C:N materials may have also had slower mineralisation of N due to lower lability.

The positive correlation between N_2O emissions and soil NO_3^- is also expected, due to the provision of a substrate for denitrification. Here though, the variation in NO_3^- only accounts for 11.7 % of the variation in N_2O production. The concentrations of DOC are likely to have interacted with NO_3^- levels in providing the necessary factors for N_2O production by denitrification, with DOC functioning as an energy source by electron donation (Baggs et al. 2002, Thangarajan et al. 2013, Hansen et al. 2019). The high N_2O fluxes occurred in clover treated soils when there were elevated levels of both NO_3^- and DOC on days 5, 13 and 18. Neither gunnera-treated soil with DOC levels as high as clover treated soil but low NO_3^- levels, or the AN 70 treatment with very high NO_3^- levels but lower DOC levels, produced high N_2O emissions. The decomposition of PMGMs was not fast enough for high NO_3^- levels to build up, reducing the substrate for denitrification, even when DOC was in plentiful supply.

Both the clover and gorse treatments had higher N_2O emissions than would be predicted by their C:N ratios as compared to alder and gunnera, as is the case with shoot N uptake. Higher N_2O emissions are in accordance with the higher mineral N availability from these treatments. In the case of clover, this also could be an effect of high lability of C. Low C:N ratio residues have been found to have higher levels of water soluble organic C, many forms of which are bio-available (Boyer and Groffman 1996, Hadas et al. 2004, Huang et al. 2004). In addition to increasing DOC, higher availability of C can also result in increasing anaerobic microsites due to high respiration by heterotrophic microbes. These anaerobic microsites can increase incomplete denitrification to N_2O (Butterbach-Bahl et al. 2013, Hansen et al. 2019). Chen et al (2013) emphasise the importance of anaerobic microsites as a challenge to the dominant paradigm that it is N substrate provision and DOC as energy which are the main influences on N_2O emissions from residues. The possible allelopathy of chemicals within gorse do not appear to have any effect on N_2O producing microbes.

It cannot be assumed that similar N_2O emissions would occur in an open field. Chen et al. (2013) notes that laboratory or pot studies on average report higher emissions than field studies, and that, as here, lab studies are often conducted in higher temperatures than those of field studies. Other varying environmental factors will also affect emissions. For example, in a free draining field, soil NO_3^- levels may be reduced due to leaching after fertilisation by NH_4NO_3 or clover. This transfers the problem to one of eutrophication of other areas or waterways by NO_3^- and/or indirect N_2O emissions from the leached NO_3^- . As a large proportion of N_2O is produced during short term events, the accuracy of the N_2O emission results could have been improved by more gas sampling points during the first 60 days. This highlights the importance of improving methods of data collection such as the use of automated chambers which allow more frequent measurements (Henault et al. 2012). Even with very frequent measurements, short-lived high emissions can be missed and sampling may not adequately represent diurnal fluctuations (Charles et al. 2017), therefore ongoing development of accurate measuring is crucial.

3.4.7 Other potential losses of N

Over 85 % of the N added in the AN70 treatment was accounted for by the N in the plant tissues and that lost as N_2O , although it is also possible that substitution with primed soil N occurred, and priming by PMGMs is investigated in chapter 4 and further discussed in chapter 7. The amount of N accounted for as N_2O and in grass tissue from the green manures is much lower, ranging from 35 % of gorse N to 59 % of clover N. The proportions of N which may have been lost by ammonia volatilisation, denitrification to N_2 or NO , or remaining in soil are unknown. Ammonia volatilisation relies on a high NH_4^+ to H^+ ratio, and is greater in dry soils, and where NH_4^+ has more contact with air (Liu et al. 2007, Niraula et al. 2018). Here, the low soil solution NH_4^+ levels, wet soil and low pH suggest that a significant amount of ammonification is unlikely (Smil 1999, Moeller and Stinner 2009). In a field situation, especially where green manures are mulched on the soil surface, losses to ammonia volatilisation should be considered. The amount of complete denitrification to N_2 in agricultural soils is poorly understood, but it is estimated that 6 to 12 % of fixed N added to agricultural soils is lost as N_2 (Galloway et al. 2003). For example it has been found that between 11 and 14% of fertiliser and manure N applied to arable soil was lost to denitrification via all N gasses, including N_2 (Smil 1999). Therefore, it is likely that most of the green manure N unaccounted for from the green manure treatments remained in the soil as organic matter, including microbial biomass.

3.4.8 Conclusions

These results show that PMGMs have the potential to fertilise crops while resulting in lower N pollution than mineral fertiliser or clover. The highly contrasting biomass production, N offtake and

N₂O fluxes between treatments demonstrates the importance of green manure management to agricultural efficiency and GHG emissions, and the potential for strategic use of green manures to contribute to climate change mitigation. Gunnera and alder have the potential to be highly effective at providing N to crops while incurring little N loss or pollution. Gorse appears to be the least suitable due to the possible allelopathic response and higher N₂O emissions than the other PMGMs.

The matching of the rate of release of mineral N to demand by the crop is all the more crucial if an organic source of N is used, which by default adds C to the soil. If the soil is not already high in available C, the amendment may provide the missing factor for N₂O production by denitrification. The PMGMs tested, did not release N to plant available forms fast enough to warrant successive applications during the growing season, as proposed. Instead, the slow release of plant-available N from PMGMs makes alder and gunnera suitable as slow release fertilisers incorporated in one application to the seed bed. The lower N₂O emissions from PMGMs than from clover or NH₄NO₃ demonstrate their potential to contribute to climate change mitigation, and gunnera notably had lower N₂O emissions per unit of crop produced, than either AN70 or clover. The emissions measured here in moist, warm conditions may be relevant to current horticultural practice in protected growing in temperate climates. For example, if cover crops are grown and dug into irrigated soil in poly tunnels. This should be noted by researchers and practitioners of organic growing, and by policy makers if advocating an increase in organic production by protected cropping.

3.4.9 Further research:

This study gives an indication of the N pathways and resulting crop growth and N pollution from three PMGMs in controlled conditions. These data can be used as a guide to predict the, rates of decomposition, resulting N availability, crop demand and N₂O emissions which may occur in a field situation. Research now needs to be conducted in field cropping to assess how N pathways differ from a pot experiment to those in the field, to enable assessment of the optimum timings, PMGM species and mode of application in which maximum climate change mitigation and soil health can be achieved. Use of dried green manures would be more practical than fresh material for applying in large quantities at field scale and allow flexibility of application time, especially for alder and gunnera which are dormant in winter. Processing of the tissue such as drying or shredding, can affect mineralisation and N₂O emissions and requires investigation (Chen et al. 2013, Charles et al. 2017). Application of PMGMs as a mulch would allow reduced tillage, with the associated benefits to soil health, but may risk N loss through ammonia volatilisation, which is greater when N is added at the soil surface (Cameron et al 2013). Priming of soil C may also occur due to new inputs (Kuzaykov 2010, Schmatz et al. 2017, Bailey et al. 2019, Kan et al. 2020). Therefore, research is needed at field

scale, with practical means of application and quantification of losses by leaching and ammonia volatilisation as well as those monitored here. To complete the picture for GHG mitigation data are needed on the effect on C priming and sequestration by use of the PMGMs.

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Chapter 4: Effect of PMGMs on soil organic carbon: An incubation experiment

Contributions: PWH conceived the experiment. PWH and CW designed the experiment. Experimental work, data processing and statistical analysis was performed by CW. CW wrote the chapter with advice and editing by PWH.

Abstract

Use of PMGMs to fertilise crops can increase NUE, and reduce N pollution, including the greenhouse gas N_2O , compared to use of traditional green manures. Organic amendments can also have a beneficial C sequestering effect, however, it is also possible for pre-existing soil C to be lost due to a stimulation effect of the additions (priming). For PMGMs to be useful in climate change mitigation they would ideally increase soil C stocks, and at the least should not have a detrimental effect which outweighs the positive impact on NUE.

To assess the priming effects of PMGMs we conducted three sets of incubations of agricultural soil with treatments of clover, alder, gunnera and gorse, NH_4NO_3 and no amendment for 42 days. The three sets allowed for assessment of C emissions from different sources. In one set, respired CO_2 was trapped from soil organic carbon (SOC) which had been labelled with ^{14}C , and in a second set where the green manures were labelled with ^{14}C . A third set measured the total CO_2 emissions. The most labile green manures resulted in more priming of SOC during the incubations, but predictions of the soil C stocks after one year suggest that use of more recalcitrant green manures could result in the most loss of C. These predictions, however, do not take into account additional factors of real agricultural conditions such as crop roots, meso and macro fauna and weather. We consider how priming may be affected by field conditions and various strategies for agriculture which could increase C stocks from green manure use.

4.1 Introduction

Increasing the C content of soil as a climate change mitigation strategy is promoted by the “4 per 1000” initiative, launched at the 2015 Paris Climate Summit (Rumpel et al. 2018, Arrouays and Horn 2019). One method of achieving this is through the addition of organic material to agricultural soil. A drawback of this strategy identified by Poulton et al. (2018), is the limited availability of organic material to farmers. The use of PMGMs, which typically have a higher C:N ratio than short lived green manures, could alleviate this problem, by adding larger quantities of C. Another technique to increase soil C sequestration is the conversion of agricultural land to forest, but again, Poulton et al. (2018) noted the drawbacks of this in the implications for food security of reducing the area of productive land. However, on organic farms, where a proportion of land is devoted to short lived N fixing green manures, N fixing by PMGMs instead would reduce the cropping area required (Williams et al. 2006, Powlson et al. 2011, Smith et al. 2018). This would free up land which could be converted to woodland, either purely for C sequestration and biodiversity value, or also for N fixing by PMGMs within short rotation coppice areas. Therefore, use of PMGMs could increase C sequestration both by enabling conversion of some arable land to unploughed C sequestering areas, and by addition of C within the PMGM tissues.

The addition of organic material to agricultural soil, however, does not always lead to a net C gain. Addition of organic materials can cause the loss of previously stable SOC, a phenomenon commonly referred to as the priming effect (van der Wal and de Boer 2017). In some cases, the quantity of C primed from existing SOC can be greater than the C added (Kuzyakov et al. 2000). In other cases, it may reduce the soil C gain. Guenet et al. (2018) found that when the effects of priming were added into a model of global C sequestration, the gain of C was reduced by 51 %.

Loss of some green manure C is unavoidable. If crops are to take up green manure N, decomposition of the added organic matter must occur at least to the level of peptides, necessitating some respiration of the C within (Hill et al. 2011, Ganeteg et al. 2017, Ma et al. 2018). In addition it is the turnover of soil organic matter which is beneficial to soil functioning (Powlson et al. 2011, Whalen 2014, Poulton et al. 2018). For C sequestration to occur, some of the green manure C must remain in, or be converted to stable forms. It is important therefore to study the C dynamics, and interactions between the pre-existing SOC and the added green manure C, to enable development of agricultural methods which result in effective soil functioning, provision of nutrients to the crop as well as a gain of net soil C.

4.1.1 Priming as a result of green manure addition

The effect on SOC of adding plant material to soil varies widely, but positive priming effects are common e.g. Liang et al. (2017), and Lerch et al. (2019) found increases in soil C loss of 31.5 % and 21 % respectively after addition of plant material. A meta analyses by Zhang et al. (2013) of 520 observations found that on average, addition of organic C (plant material or low molecular weight material) increased the SOC degradation by 26.5 % compared to unamended soil. This included a range of observations, 69 of which showed negative priming, 181 showed positive priming and 270 with no significant effect.

The magnitude and direction of priming has been shown to depend on many factors including the characteristics of the plant material e.g. C:N ratio and lability (Chen et al. 2014, Wang et al. 2015, van der Wal and de Boer 2017), and of the soil, including its C:N ratio, texture and moisture content (Zhang et al. 2013, Toosi et al. 2017, Bastida et al. 2019) and environmental factors including temperature and precipitation (Bailey et al. 2019, Gross and Harrison 2019). A distinction has been made between “apparent” priming, the loss of SOC from microbial biomass, and “real” priming, the loss of SOC from non-living soil organic C (Kuzyakov 2010, Bastida et al. 2019).

4.1.2 Mechanisms

There has been much research into the causes of priming, but mechanisms remain difficult to identify (Blagodatskaya and Kuzyakov 2008, Shahbaz et al. 2017, van der Wal and de Boer 2017, Liu et al. 2017). The most prominent theories on the mechanisms of priming are listed below:

1. Labile material provides energy for enzyme production. The co-metabolism theory states that addition of highly labile fresh organic matter stimulates the decomposition of SOC by provision of C as an energy source for enzyme production (Kuzyakov et al. 2000, Wang et al. 2015, van der Wal and de Boer 2017, Fang et al. 2018).

The following theories (2, 3 and 4) are all due to changes in the relative dominance of various microbial groups:

2. *K* selected microbes increase due to accumulating necromass of *r* selected microbes. This theory states that provision of labile material promotes the growth of *r* selected microbes. The proliferation and death of the *r* selected microbes increases abundance of *K* selected microbes which feed on the structural polymers of the microbial necromass. The enzymes produced by *K* selected microbes to degrade the structural polymers of the necromass are also capable of decomposing the more recalcitrant soil organic matter, and this occurs when the amount of necromass declines, leaving the

K selected microbes and the enzymes produced with extra capacity for degradation (van der Wal and de Boer 2017, Bailey et al. 2019).

3. The stoichiometry theory states that priming is highest when the substrate's C:N ratio matches with that required for microbial growth. (Chen et al. 2014, Di Lonardo et al. 2018). For example, Di Lonardo et al. (2019) found that both the decomposition rate of the substrate and priming were higher when the C:N ratio of the substrate was well matched to microbial demand, and hypothesised that the well matched C:N ratio increased microbial activity, which resulted in more priming. This optimal C:N ratio has been calculated to be around 16 to 20 (Kuzakov et al. 2000, Wang et al. 2015).

4. Proliferation of K strategists due to recalcitrant substrate (or “like primes like” theory): A theory proposed by Fontaine et al (2003) states that priming of SOC can occur when an amendment is similar to the SOM in its chemistry (other than C:N ratio), for example phenolic content (Fontaine and Barot 2005). This promotes the growth of *K* strategists which produce enzymes capable of decomposing both the amendment and SOC (van der Wal and de Boer 2017), whereas a dissimilar substrate chemistry will increase a microbial population which has little effect on existing SOM. Research by Di Lonardo et al. (2017) found that the chemical make up of added substrates had a larger effect on priming than the energy content. They compared amendments with differing levels of similarity to the SOM and found that the amendment with the most similar chemical structure to the SOM, vanillic acid, had the highest priming effect and stimulated an increase in microbes which decompose old SOC. Liang et al. (2017) also concluded that the residue chemistry e.g. polyphenol content may be influencing decomposition and therefore priming effect. A variation proposed by Fontaine et al (2003) is that *K* strategists increase their populations by decomposing the remaining less labile material of an amendment, after the most labile material has been consumed by *r* strategists. The enzymes remaining available after the polymers from the amendment are degraded then break down SOC (Fontaine et al. 2003).

5. The N mining theory states that when C is in excess of N in comparison to that required for microbial growth, microbes decompose existing soil organic matter to acquire the necessary N (Craine et al. 2007, Fontaine et al. 2011, Chen, Liu, et al. 2014, Wang et al. 2015).

4.1.3 Common methods to determine priming and limitations

Priming of soil C is commonly studied by addition of a ^{13}C or ^{14}C labelled substrate to soil and the labelled C traced to respired CO_2 , DOC or microbial biomass (Kuzakov 2010). The substrate is often glucose or other simple organic compounds (e.g. Shen and Bartha 1996, Jagadamma et al. 2014, Liu et al. 2017, Bastida et al. 2019). Labelled plant material is also used to study the effects of crop residues

or green manures (e.g. Guenet et al. 2010, Liang et al. 2017, Di Lonardo et al. 2017, Di Lonardo et al. 2018), or both labelled substrate and labelled plant material (e.g. Shahbaz et al. 2018). The reverse method of adding an unlabelled substrate to labelled SOM is also increasingly used (e.g. Rousk et al. 2015). Kuzyakov (2010) advised that experiments should be designed to identify more than one C source so that both soil and substrate C can be traced. It has been suggested that results taken as evidence of priming have on occasion been due to uneven labelling of leaf tissue which can lead to an over-estimation of C derived from soil, and a false conclusion of the occurrence of priming (Shen and Bartha 1996). It has also been questioned whether the loss of SOC measured as a result of addition of glucose is merely an experimental artefact which would not occur in real soil conditions, though this theory was rejected in a review by Kuzyakov (2010).

4.1.4 Objectives and experimental design

We aimed to assess the C impacts of the use of PMGMs by separately quantifying the C loss from stable SOC and from added green manures over a 42 day incubation. The PMGMs were compared with the traditional green manure red clover, as well as NH_4NO_3 fertiliser and no addition (control). We then used the data from the incubations to predict the C budget of the different treatments over a full year. An additional aim was to elucidate whether SOM could have been an important contributory source of N to plant uptake in the pot experiment (chapter 3).

In agriculture, it is common to delay sowing a crop for several weeks after green manure incorporation. This experiment simulated this initial time period in which green manures are incorporated into soil without crops. We used soil previously labelled with ^{14}C and ^{14}C labelled green manure in separate incubations to allow the determination of their pathways to respired CO_2 and microbial biomass. Regular trapping and analyses of ^{14}C efflux was used to monitor green manure and SOC-derived respiration rates. Measurement of the total respiration rate of a third set of unlabelled samples was used in comparison with data of respired ^{14}C captured to assess the accuracy of the data, and provide an overall estimation of C loss.

4.2 Materials and methods

Three sets soil with the same treatments, were incubated for 42 days to separately monitor the CO₂ efflux derived from different sources: mineralisation of C from added leaf material, mineralisation of stable SOC, and total C mineralisation from leaf and soil.

4.2.1 Soil

Soil, classified as a Eutric Cambisol (FAO), was collected from a depth of 2 to 12 cm, from a sheep-grazed *Lolium perenne* dominated grassland at Abergwyngregyn, Gwynedd, UK (53°140N, 4°10W). One set of soil was dug from an area which 8 years previously had been isotopically labelled by addition of a diluted ¹⁴C glucose solution as described in Rousk et al. (2015). Unlabelled soil was dug from another area approximately 6 meters away, to obtain soil which had characteristics as similar as possible to the labelled soil.

4.2.2 Analyses of soil

Soil moisture was determined by weighing samples before and after drying at for 24 hours at 105 °C. Dried soil (labelled and non-labelled) was analysed for total C and N using a TruSpecR analyser (Leco Corp., St Joseph, MI, USA). Fresh soil was analysed for pH, and electrical conductivity (EC) in deionised water. ¹⁴C-activity of labelled dry soil was measured (in triple replicate) by combustion of dried samples in an OX400 biological oxidiser (Harvey Instruments Corp., Hillsdale, NJ, USA). CO₂ from each sample was trapped in 15 ml Oxysolve C-400 scintillant (Zinsser analytica, Frankfurt, Germany), and counted in a Wallac 1409 liquid scintillation counter (Wallac EG&G, UK) to determine the ¹⁴C activity.

Soil extracts were prepared using a 5 g subsample of fresh soil in 25 ml 0.5 M K₂SO₄, shaken for 30 minutes at 200 rev min⁻¹, and centrifuged for 10 min at 2880 g. The supernatant was filtered through Watman no 1 filter paper and stored at -20 °C until analysis. A second 5 g subsample was fumigated in CHCl₃ vapour using the method described by Vance et al. (1987) and both fumigated and unfumigated extracts were analysed for dissolved organic carbon (DOC) and total dissolved nitrogen (TN) using a Multi N/C 2100S analyser (AnalytikJena, Jena, Germany). Microbial biomass C was calculated by subtraction of unfumigated soil C from fumigated soil C, and using a kec conversion factor of 0.45 (Joergensen 1996). Extracts were analysed for ¹⁴C activity as described below for NaOH solutions, and the % of ¹⁴C within soil microbial biomass was calculated from the ¹⁴C contents of the fumigated minus the unfumigated extractions, as for total microbial biomass C.

Characteristics of the soils are shown in table 4.1. Due to natural heterogeneity, there were some differences between labelled and unlabelled soils, but there was no difference between the CN ratios of the two soils. Soil was sieved to 2 mm and stored at 4 °C.

Table 4.1 Background Soil characteristics. (Means \pm SEM, $n = 3$)

Soil Property	Labelled	Unlabelled
Activity (2.2 Bq g ⁻¹)	70.1 \pm 2.2	-
H ₂ O (g g ⁻¹)	0.60 \pm 0.004	0.78 \pm 0.005
pH 1 : 2.5 (in H ₂ O)	5.2 \pm 0.02	5.2 \pm 0.003
EC 1 : 2.5 (in H ₂ O) (μ S cm ⁻¹)	74.0 \pm 2.3	94.7 \pm 0.3
Microbial biomass (g C kg ⁻¹)	1.53 \pm 0.01	1.48 \pm 0.04
¹⁴ C within microbial biomass C (%)	3.46 \pm 0.38	-
Total soil C (g kg ⁻¹)	28.97 \pm 0.84	36.00 \pm 0.26
Total soil N (g kg ⁻¹)	3.41 \pm 0.05	4.15 \pm 0.04
Soil C:N ratio	8.5 \pm 0.005	8.7 \pm 0.1
DOC (g C kg ⁻¹)	0.19 \pm 0.01	0.22 \pm 0.02
TN (g N kg ⁻¹)	0.04 \pm 0.003	0.05 \pm 0.003

4.2.3 Green manures

Labelled and unlabelled green manure material of the traditional green manure red clover, and the PMGMs alder, gunnera and gorse was harvested from pot grown plants, while leaves were photosynthesising. A set of plants of each species had been previously labelled with ¹⁴C on three successive days as follows. Potted plants were placed outside in plastic trays and covered with bags of transparent flexible plastic sheeting which were sealed to the trays to provide air-tight chambers. 0.5 ml of ¹⁴C NaHCO₃ solution containing 2.05 MBq, was pipetted through a slit in the plastic sheeting, into a plastic container (11 mm deep by 35 mm diameter), which contained 3 ml of 2 M HCl. The slit was immediately taped up to provide an air-tight chamber. Plants took in the ¹⁴CO₂ evolved from the reaction between ¹⁴C NaHCO₃ and HCl, which was incorporated into plant tissue via photosynthesis. After a period of time (one hour in sunny conditions and up to two hours in cloudy conditions), the plastic coverings were removed. Following the third labelling, leaves were cut from the plants and dried in an oven at 80 °C. In the case of gorse, this also included stems of < 2 mm diameter. Dry material was crumbled to pieces of less than 4 mm. Though there were no discernible physical differences between the labelled and unlabelled plants, there were some differences in leaf tissue due to natural heterogeneity, resulting in a small difference in C content between labelled and unlabelled alder leaves (Table 4.2). Dried green manure samples were analysed for ¹⁴C activity as described above for soil. Labelled and non-labelled green manures were analysed for total C and N after grinding in a

Retsch mixer mill MM 200 (Retsch, Haan, Germany) to ≤ 1 mm, by a TruSpecR analyser (Leco Corp., St Joseph, MI, USA).

4.2.4 Experimental set up

Three sets of soil incubations, with added treatment or no treatment (control) were set up as described below. NH_4NO_3 was added dissolved in water at a rate of 10 % water to weight of fresh soil, and replicates not receiving NH_4NO_3 had the same volume of water added as the AN treated replicates within their respective sets of incubations. This resulted in the water contents shown in table 4.1. Due to unforeseen differences in the initial water content, rates of addition to labelled soils were 9.7 % less per unit dry soil than to the unlabelled soil of the “leaf” and “total” incubations. All replicates in all incubations were weighed (including containers) prior to the start of the experiment, and then every 14 days to check for water loss (none found).

Table 4.2 Treatments (Means \pm SEM, $n = 3$). ^{14}C labelled leaf was used in incubations to measure efflux of leaf-derived C. Non labelled leaf was used in incubations to measure soil derived ^{14}C and total C respired.

Treatment (g kg ⁻¹ fresh soil)		¹⁴ C labelled leaf				Non labelled Leaf		
		C added (g kg ⁻¹ fresh soil)	N added (g kg ⁻¹ fresh soil)	C/N ratio	Specific activity (kBq g ⁻¹ dry leaf)	C added g kg ⁻¹ fresh soil	N added g kg ⁻¹ fresh soil	C/N ratio
Clover	10	4.43 \pm 0.04	0.30 \pm 0.011	14.6 \pm 0.5	306.1 \pm 10.5	4.33	0.31	14.09
Alder	10	4.86 \pm 0.03*	0.24 \pm 0.004	20.0 \pm 0.2**	123.4 \pm 9.0	4.75 \pm 0.02*	0.25 \pm 0.0002	19.2 \pm 0.09**
Gunnera	10	4.42 \pm 0.02	0.18 \pm 0.005	24.6 \pm 0.6**	41.7 \pm 5.3	4.49 \pm 0.2	0.16 \pm 0.008	28.3 \pm 0.3**
Gorse	10	4.65 \pm 0.10	0.18 \pm 0.013	26.8 \pm 2.5	107.0 \pm 2.3	4.84 \pm 0.01	0.16 \pm 0.002	27.0 \pm 0.3
NH ₄ NO ₃	0.857	-	-	-	-	-	0.30	-

* Alder C contents differed between labelled and unlabelled leaf ($P = 0.048$). ** CN ratios differed between labelled and unlabelled leaf.

4.2.5 Incubations for measurement of leaf and soil C mineralisation by ^{14}C capture

Four replicates of 200 g ^{14}C labelled fresh soil (70.1 ± 2.2 Bq g⁻¹) mixed with unlabelled treatments as in table 4.2, plus control were made up in 840 ml plastic tubs for measurement of $^{14}\text{CO}_2$ evolution from soil organic matter. Four replicates of 10 g unlabelled soil and with labelled green manure treatments as in table 4.2, for measurement of CO_2 evolution from green manure leaf were made up in 50 ml centrifuge tubes. All incubations were in the dark and at a temperature of 18.5 ± 3 °C. The larger size of the containers used for trapping of ^{14}C from SOC was to enable the capture of a larger quantity of CO_2 , with sufficient isotope activity as activity was lower in labelled soil than in the labelled leaves.

$^{14}\text{CO}_2$ evolved from incubations was captured in 1 M NaOH traps for reading by a scintillation counter as follows. 5 ml vials containing 1 ml NaOH were added to the centrifuge tubes containing labelled

leaf. 20 ml vials containing 3 ml NaOH were placed inside the containers with the labelled soil only and labelled soil with NH_4NO_3 treatments. 20 ml vials containing 18 ml NaOH were placed inside the containers which contained labelled soil and the green manure treatments. This larger amount of NaOH was necessary to prevent saturation by the high levels of CO_2 evolved from the green manure treated soil (derived from both leaf and soil C). Soil not treated with leaf matter and therefore producing less CO_2 required a smaller NaOH trap so that the lower amount of labelled C evolved was at a high enough concentration within the NaOH to be read by the scint counter.

Traps were changed after 1, 2, 3, 5, 7, 10, and 14 days, for labelled soil incubations and on days 1, 3, 7, and 14 days for labelled leaf incubations, and then weekly until the end of the incubation on day 42. Optiphase HiSafe 3 (Fisher Scientific, UK) scintillant was added at a ratio of 1:4 (scintillant to NaOH), well shaken, and left to stand for over 2 hours. The resulting mixture was analysed in a Wallac 1409 scintillation counter (Wallac EG&G, Milton Keynes, UK). After 42 days of incubation, extracts of 5 g fresh soil were prepared as above and analysed for TN and DOC, and microbial biomass. K_2SO_4 extracts were also analysed for ^{14}C activity as for NaOH samples.

4.2.6 Incubations for measurement of total C mineralisation

Three replicates of 50 g fresh soil, mixed evenly with unlabelled treatments as in table 4.2, plus control (no treatment) were added to 300 ml glass jars and connected to a Micro-Oxymax multichannel respirometer fitted with a CO_2 sensor (Columbus Instruments, Ohio, USA). The jars were covered in aluminium foil to exclude light, randomly assigned to the channels, and CO_2 evolution was automatically measured every six minutes. The treatments were in replicates of 3, which was the maximum allowed by the number of channel connections to the machine.

4.2.7 Calculations

Leaf and soil derived carbon

The weight of leaf and soil derived C respired as CO_2 was calculated by dividing the activity of captured ^{14}C , in NaOH traps by the activity of leaf or soil ^{14}C added at the incubation starting point and multiplying this by the weight of the leaf or soil starting point C. We assumed that the accuracy with which the ^{14}C leaf respiration data represented the accuracy of the ^{14}C leaf respiration data increased with time after the most labile material has been mineralised. More labile plant material is likely to have accumulated a disproportionately high amount of ^{14}C , due to a higher incorporation of C into biologically active cells, compared to less labile, more structural material. This more labile material, containing disproportionately high ^{14}C activity is likely to be respired sooner, leaving less labile, more representative material behind. On this assumption, we plotted the cumulative leaf and soil ^{14}C loss during weeks 3 to 6 and undertook a regression to assess the effect of leaf C mineralisation on soil C

mineralisation. The leaf-derived C contained within soil DOC at the end of the incubation was calculated as above using the activity of captured ^{14}C in soil K_2SO_4 extractions.

Estimation of carbon pools after one year

To predict the amount of respiration of leaf, soil and total C which would occur over one year, data for each replicate were fitted with disintegration curves, using the “dynamic fit” function of SigmaPlot version 13 (Systat Software, San Jose, CA, USA). The total respiration, respiration of leaf C and respiration of soil C for each replicate were modelled by fitting exponential decay, double, 4 parameter curves (equation 1), yielding R^2 values of > 0.995 . P values for curve fits were < 0.0003 for total respiration, < 0.001 for the respiration of leaf C and 0.014 to 0.0001 for the respiration of soil C.

$$f = a \cdot \exp(-b \cdot x) + c \cdot \exp(-d \cdot x) \quad [1]$$

where f is the size of the remaining carbon pool, a and c are carbon pools with differing labilities, b and d are decay constants and x is time.

The control and AN treatments for ^{14}C soil respiration could not be fitted with exponential decay, double, 4 parameter curves, and instead modified single, 3 parameter curves (equation 2) were fitted with an R^2 of > 0.9994 and $P < 0.001$.

$$f = a \cdot \exp(b/(x+c)) \quad [2]$$

where f is the size of the remaining carbon pool, a is the carbon pool subject to mineralisation, b and c are constants and x is time. The formulas were used to calculate remaining pools after one year.

Calculation of old soil carbon fraction

Previously published data from a similar soil were used to estimate the size of the C pool represented by the labelled fraction of the soil organic matter. Heim and Schmidt (2007) calculated the proportion of SOC of > 10 years old in a Eutric Cambisol under ryegrass, in similar climatic conditions, as 66% of SOC. Making the assumption that the pool of SOC represented by the ^{14}C label in the soil here (labelled 8 years previously) was similar to the SOC over 10 years in age in Heim and Schmidt (2007), the labelled fraction of the SOM was estimated to represent 66 % of the SOC. This was used to calculate a cautious estimate of C > 8 years old remaining after one year of priming as predicted by the decay curves. It should, however, be noted that although 8 year old SOM is likely to be quasi-stable, all SOC > 8 years old may not behave the same (von Luetzow et al. 2008). Consequently, estimates of loss as CO_2 may overestimate true values.

Microbial biomass

Total microbial biomass C was calculated as described above for the original soil. Leaf and soil-derived microbial biomass C was calculated by use of measured ^{14}C activity in the extractions using a k_{ec} value based on calculations by Glanville et al. (2016) for an incubation of 42 days at 20 °C. They calculated the k_{ec} values from incubations with additions of ^{14}C labelled alanine and glucose by comparison with experimentally derived k_{ec} values using extractions from known mineralizable C pools. Soil and leaf derived microbial biomass was calculated from the leaf and soil derived C in fumigated and unfumigated DOC using a k_{ec} conversion factor of 0.25. In the case of SOC derived microbial biomass, the ^{14}C was assumed to represent 66 % of the total C, as described above.

Data analyses

Data analyses was performed in SPSS version 25 (IBM, New York, USA). Data were first tested for homogeneity using Levene's test, before using one way ANOVA. Significant differences between treatments were identified using Tukey's HSD test. Pearson's correlations were performed to assess relationships between C:N ratio and ^{14}C efflux and regressions on ^{14}C soil efflux against ^{14}C leaf efflux.

4.3 Results

4.3.1 Total CO₂ emissions

After seven days all green manure treatments had resulted in over 4 times the total CO₂ emissions (leaf + soil) of the control, with clover-treated soil emitting over 10 times more (Fig 4.1). The rates of emissions declined over time. Clover-treated soil had the steepest decline so that in week 6 rates were less than double that of the control and lower than all the PMGMs (Table 4.3). The relative rates of loss between treatments changed over time, so that in the last week, gunnera and alder treated soil had higher emissions than the control, AN and clover treated soils.

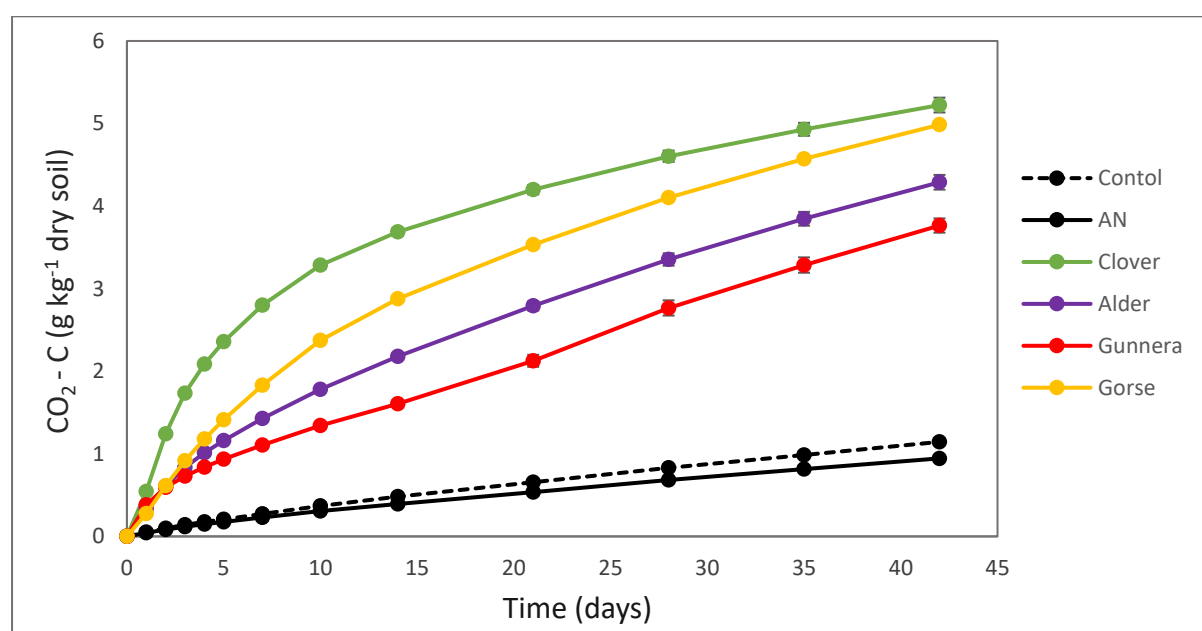


Fig. 4.1. Total cumulative C respired from incubations of unlabelled soil and leaf. Data are means \pm SEM ($n = 3$)

Table 4.3. Total weekly C loss as CO₂ from incubations of unlabelled leaf and soil (g C kg⁻¹ dry soil per week). Data are means \pm SEM ($n = 3$)

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
	Days 0 to 7	Days 8 to 14	Days 15 to 21	Days 22 to 28	Days 29 to 35	Days 36 to 42
Control	0.27 \pm 0.012 ^a	0.21 \pm 0.003 ^a	0.18 \pm 0.003 ^a	0.17 \pm 0.002 ^a	0.16 \pm 0.007 ^a	0.16 \pm 0.012 ^a
AN	0.23 \pm 0.003 ^a	0.17 \pm 0.004 ^a	0.14 \pm 0.004 ^a	0.15 \pm 0.003 ^a	0.13 \pm 0.003 ^a	0.13 \pm 0.003 ^a
Clover	2.80 \pm 0.046 ^e	0.89 \pm 0.010 ^d	0.51 \pm 0.012 ^b	0.41 \pm 0.012 ^b	0.33 \pm 0.010 ^b	0.29 \pm 0.012 ^b
Alder	1.43 \pm 0.017 ^c	0.75 \pm 0.015 ^c	0.61 \pm 0.026 ^c	0.56 \pm 0.019 ^c	0.49 \pm 0.009 ^{cd}	0.44 \pm 0.014 ^{cd}
Gunnera	1.11 \pm 0.021 ^b	0.50 \pm 0.023 ^b	0.52 \pm 0.029 ^b	0.64 \pm 0.020 ^d	0.52 \pm 0.005 ^d	0.48 \pm 0.007 ^d
Gorse	1.83 \pm 0.012 ^d	1.04 \pm 0.017 ^e	0.66 \pm 0.011 ^c	0.57 \pm 0.011 ^c	0.47 \pm 0.009 ^c	0.41 \pm 0.006 ^c

Letters denote significant differences between treatments at $P < 0.05$

4.3.2 Green manure derived C emissions

Cumulative emissions of green manure-derived CO₂ as percent of ¹⁴C are shown in Fig. 4.2. Fig. 4.3 shows the estimated total C respired from leaf material calculated from the trapped ¹⁴C and the % of the leaf C which was labelled. Emissions steeply declined over time, during the first 14 days. Although there were no differences between cumulative emissions at the end of the incubation, alder-treated soil had the highest emissions per week during weeks three to six ($P < 0.005$, Table 4.4).

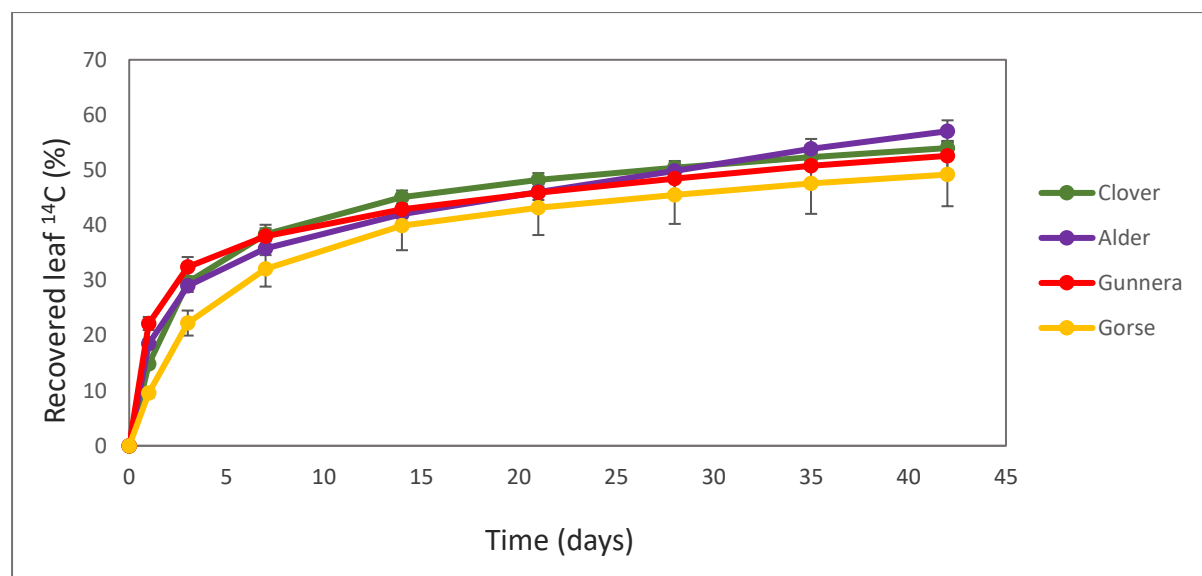


Fig. 4.2 Percentage of ¹⁴C added trapped recovered as CO₂. Means \pm SEM ($n = 4$).

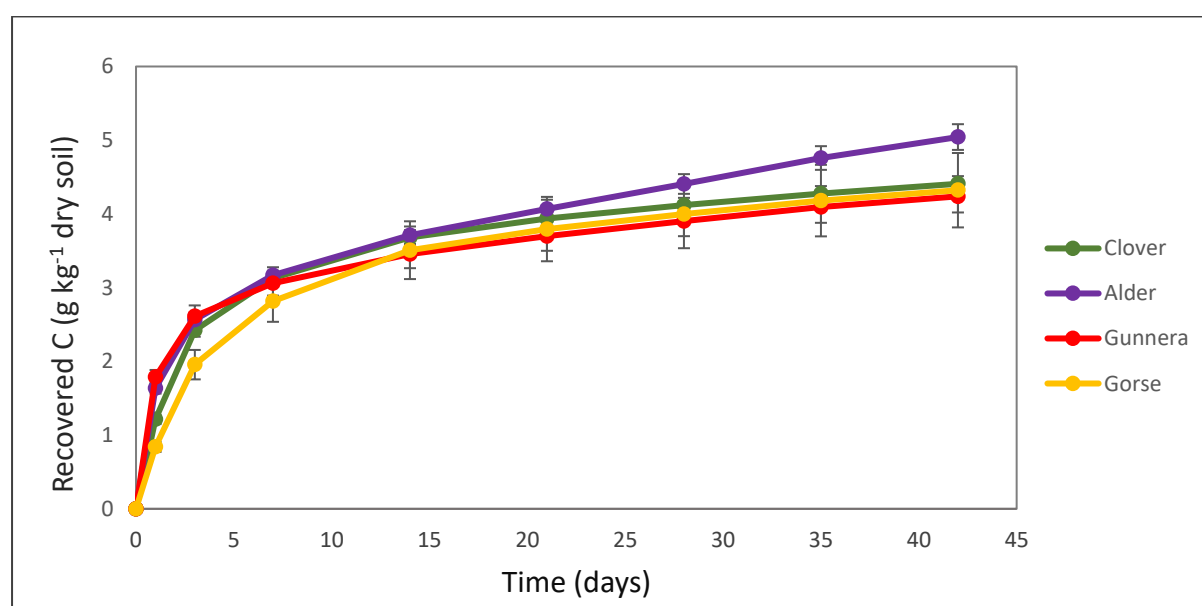


Fig. 4.3 Estimated respired leaf C calculated from trapped ¹⁴C. Means \pm SEM ($n = 4$).

Table 4.4 Leaf-derived C loss as g CO₂-C kg⁻¹ dry soil per week, and percent of added ¹⁴C in brackets. Means ± SEM (*n* = 4)

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
	Days 0 to 7	Days 8 to 14	Days 15 to 21	Days 22 to 28	Days 29 to 35	Days 36 to 42
Clover	3.13 ± 0.09 * (38.3 ± 1.1)	0.56 ± 0.03 ^a (6.7 ± 0.3)	0.26 ± 0.03 ^a (3.1 ± 0.2)	0.18 ± 0.004 ^a (2.2 ± 0.04)	0.16 ± 0.003 ^a (1.9 ± 0.04)	0.14 ± 0.003 ^a (1.6 ± 0.04)
Alder	3.17 ± 0.11 * (35.8 ± 1.2)	0.56 ± 0.01 ^a (6.2 ± 0.2)	0.37 ± 0.01 ^a (4.0 ± 0.2)	0.35 ± 0.02 ^b (3.8 ± 0.2)	0.36 ± 0.04 ^b (4.0 ± 0.3)	0.29 ± 0.03 ^b (3.2 ± 0.3)
Gunnera	3.06 ± 0.17 * (38.0 ± 2.1)	0.37 ± 0.03 ^a (4.9 ± 0.4)	0.24 ± 0.01 ^a (3.0 ± 0.1)	0.20 ± 0.01 ^a (2.5 ± 0.1)	0.19 ± 0.01 ^a (2.3 ± 0.1)	0.14 ± 0.01 ^a (1.8 ± 0.1)
Gorse	2.82 ± 0.28 * (32.1 ± 3.2)	0.71 ± 0.16 ^a (7.9 ± 1.3)	0.29 ± 0.06 ^a (3.3 ± 0.5)	0.20 ± 0.04 ^a (2.3 ± 0.3)	0.18 ± 0.03 ^a (2.1 ± 0.3)	0.14 ± 0.03 ^a (1.6 ± 0.3)

Letters denote significance between treatments at *P* < 0.05. * Data for days 0 to 7 showed leaf-derived C loss as higher than total C loss (table 4.3), and therefore are not treated as valid - see main text.

The data show that the C losses from leaf tissue (calculated from ¹⁴CO₂ production) shown in table 4.4 were higher than the total CO₂ losses during the first week shown in table 4.3, (*P* < 0.05). This inaccuracy in the data, is likely to be due to uneven labelling of leaf tissue causing an over-estimation of leaf C respired in the first time points. It has been suggested that a cause of inaccurate data in experiments on litter degradation is the non-uniform ¹⁴C labelling of leaves (Kuznyakov et al. 2000, Blagodatskaya and Kuznyakov 2008). The green manure tissues are likely to have had a higher proportion of ¹⁴C incorporated into more labile materials such as sugars, than more stable materials e.g. structural materials in petioles and veins. Capture of this labile ¹⁴C if not representative of the total leaf C would result in an over-estimation of leaf C evolved. Morphological variations between the plant species would account for differences in irregularity of labelling between treatments. Unfortunately, fully homogenous labelling of the green manures would have required labelling over a longer time period than was available e.g. some of the gorse tissue was over a year old, so would potentially have required regular labelling over a period of more than one year. As the incubations proceed however, the less labile material is likely to make up a higher proportion of the C evolved, making respired ¹⁴C more representative of total leaf C. Consequently, estimates of total respired leaf C must be treated with caution, but in conjunction with total and soil respiration rates give an indication of rates of C respiration between green manure species and over time.

4.3.3 Soil derived ¹⁴C emissions

The percentage of ¹⁴C evolved from labelled soil did not show the steep decline in C respiration rates over time that was shown by the total CO₂ or leaf derived CO₂ (Fig. 4.4, Table 4.5). In contrast to the

total CO₂ where there was no difference between AN and control, the AN treated soil had lost 12.6 % more ¹⁴C than the control by the end of the incubation, indicating a positive priming effect ($P < 0.001$). Total ¹⁴C evolution from all the green manures was higher than the control and AN treatments ($P < 0.001$). Rates of ¹⁴C recovery from alder-treated soil increased over time in relation to those from other green manure treatments. At the end of the incubation alder-treated soil had lost 68.3 % more ¹⁴C than the control, and more than any other treatment ($P < 0.001$). Alder treatment also had the highest soil ¹⁴C weekly emission rates during weeks three to six ($P < 0.001$), as was the case for leaf-derived CO₂ (Table 3). Gorse had the lowest soil ¹⁴C loss of all the green manures ($P < 0.09$), but higher than the control or AN ($P < 0.001$).

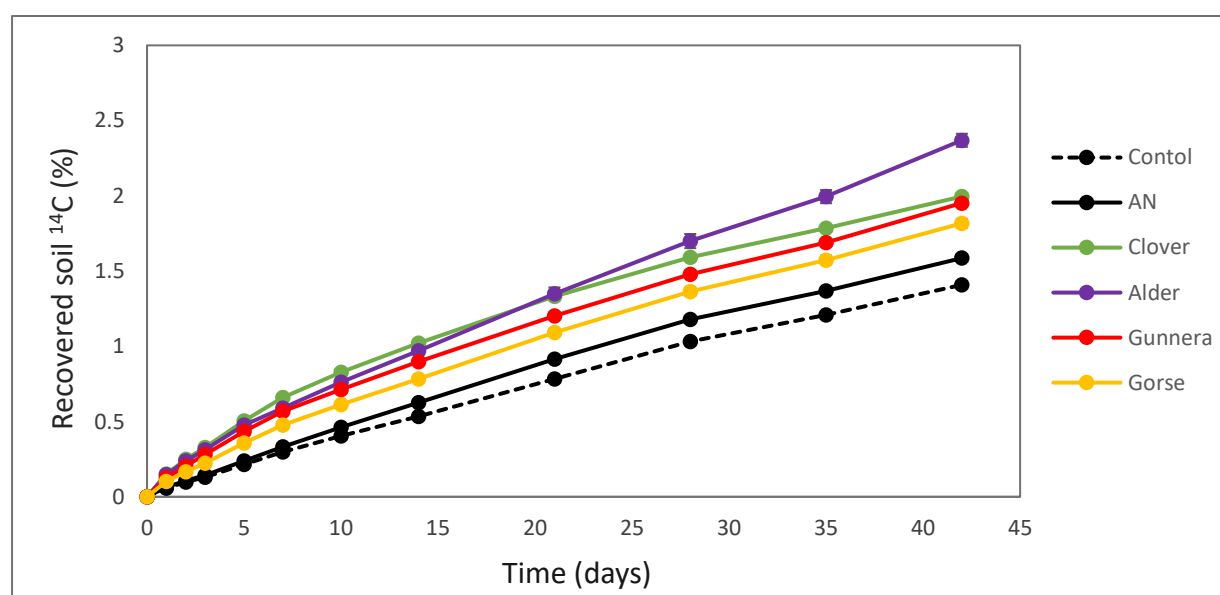


Fig 4.4. Percentage of soil ¹⁴C recovered as CO₂ from incubations of labelled soil with unlabelled leaf. Means \pm SEM ($n = 4$).

Table 4.5. % ¹⁴C evolved from labelled soil per week. Means \pm SEM ($n = 4$).

Time (days)	0 to 7	8 to 14	15 to 21	22 to 28	29 to 35	36 to 42
Control	0.30 \pm 0.003 ^a	0.24 \pm 0.002 ^a	0.25 \pm 0.005 ^a	0.25 \pm 0.003 ^a	0.18 \pm 0.004 ^a	0.20 \pm 0.005 ^a
AN	0.33 \pm 0.002 ^a	0.29 \pm 0.002 ^b	0.29 \pm 0.004 ^b	0.26 \pm 0.001 ^{ab}	0.19 \pm 0.003 ^{ab}	0.22 \pm 0.003 ^{ab}
Clover	0.66 \pm 0.017 ^c	0.36 \pm 0.007 ^d	0.31 \pm 0.011 ^b	0.26 \pm 0.005 ^{ab}	0.19 \pm 0.004 ^{ab}	0.21 \pm 0.002 ^b
Alder	0.59 \pm 0.047 ^c	0.38 \pm 0.009 ^d	0.38 \pm 0.009 ^c	0.35 \pm 0.005 ^c	0.30 \pm 0.005 ^c	0.37 \pm 0.004 ^e
Gunnera	0.57 \pm 0.013 ^{bc}	0.33 \pm 0.009 ^c	0.30 \pm 0.003 ^b	0.28 \pm 0.008 ^b	0.21 \pm 0.012 ^b	0.26 \pm 0.002 ^d
Gorse	0.48 \pm 0.014 ^b	0.31 \pm 0.004 ^{bc}	0.31 \pm 0.009 ^b	0.27 \pm 0.004 ^b	0.21 \pm 0.009 ^b	0.24 \pm 0.003 ^c

Letters denote significant differences between treatments at $P < 0.05$

There was a negative correlation between the C:N ratio of the green manures (unlabelled) and the ¹⁴C SOC loss after seven days ($P = 0.005$), but no correlation in subsequent weeks. Fig. 4.5 shows a

regression of soil ^{14}C loss against leaf ^{14}C loss during weeks 3 to 6 ($P < 0.001$). The CO_2 losses from the earlier part of the incubation were excluded to remove the effects of uneven leaf labelling as far as possible.

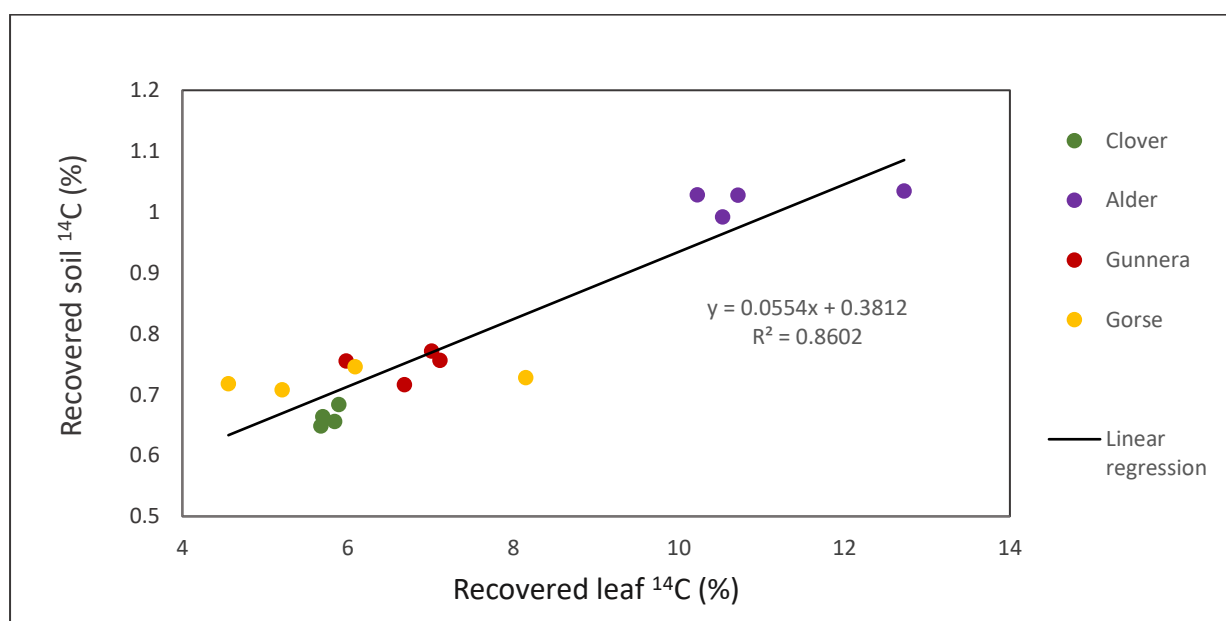


Fig 4.5 Regression of percentage of soil ^{14}C against the percentage of leaf ^{14}C captured in NaOH traps during weeks 3 to 6 of the incubation.

4.3.4 Predicted soil carbon pools.

Pools of C which are estimated to be remaining after one year are shown in table 4.6. All incubations had a predicted decline in the total C stocks (leaf and soil) and the estimated SOC > 8 years. Over 92 % of all green manure C was predicted be lost over one year, and alder and gunnera treatments are predicted to retain the lowest total C stocks. All green manure treatments predict a loss of old soil C greater than the control or AN treatments, and alder is predicted to cause the most loss of soil C > 8 years old ($P < 0.05$), with a loss of 16.7 %. Paradoxically, the AN and control treatments in which no organic matter was added were predicted to have higher total C stocks after one year than the green manure treated soils.

4.3.5 Microbial biomass

Alder, gunnera and clover-treated soil had higher total levels of microbial biomass C than AN treated soil ($P = 0.006$, Table 4.7). The proportion of microbial biomass C which was calculated to be derived from C of > 8 years, ranges from 5 % (gorse treatment) to 22 % (alder treatment). The microbial biomass C which was calculated to be derived from the green manures shows inaccuracies, as the amounts derived from clover and alder are above the total microbial biomass measured. This is likely to be due to uneven labelling of leaf tissue.

Table 4.6 Predicted C pools remaining in soil after one year (g C kg⁻¹ dry weight soil, means \pm SEM). In brackets are the percentages of the ¹⁴C predicted to be in the remaining pools. Total C *n* = 3, Leaf C and SOC C > 8 years *n* = 4. Letters denote significant differences between treatments *P* < 0.05.

	Total C	Leaf C	SOC > 8 years
Control	31.6 \pm 0.2 ^c		22.6 \pm 0.05 ^d (95.2 \pm 0.2)
AN	32.2 \pm 0.1 ^c		22.6 \pm 0.02 ^d (95.3 \pm 0.1)
Clover	29.5 \pm 0.3 ^b	0.29 \pm 0.05 ^a (3.69 \pm 0.6)	21.2 \pm 0.04 ^c (89.1 \pm 0.1)
Alder	27.3 \pm 0.4 ^a	0.08 \pm 0.02 ^a (0.93 \pm 0.2)	19.8 \pm 0.05 ^a (83.3 \pm 0.2)
Gunnera	26.8 \pm 0.3 ^a	0.26 \pm 0.08 ^a (3.28 \pm 1.0)	20.8 \pm 0.06 ^b (87.4 \pm 0.3)
Gorse	28.6 \pm 0.3 ^b	0.64 \pm 0.25 ^a (7.66 \pm 3.0)	21.0 \pm 0.08 ^{bc} (88.3 \pm 0.3)

Table 4.7 Microbial biomass (g C kg⁻¹ dry soil), by C source after 42 days incubation. In brackets are the percentages of ¹⁴C which were released as DOC by fumigation and recovered in the extract (% ¹⁴C of unfumigated DOC - % ¹⁴C of fumigated DOC). Means \pm SEM (*n* = 4). Letters denote significance *P* < 0.05

	Total	Leaf derived	Soil derived > 8 years
Control	1.01 \pm 0.11 abc		0.42 \pm 0.17 a (0.55 \pm 0.22)
AN	0.82 \pm 0.05 a		0.32 \pm 0.21 a (0.42 \pm 0.27)
Clover	1.30 \pm 0.04 c	1.62 \pm 0.07 bc (0.79 \pm 0.03)	0.56 \pm 0.189 a (0.73 \pm 0.13)
Alder	1.27 \pm 0.10 bc	1.99 \pm 0.12 c (0.90 \pm 0.06)	0.56 \pm 0.10 a (0.73 \pm 0.13)
Gunnera	1.29 \pm 0.05 bc	1.26 \pm 0.12 b (0.62 \pm 0.06)	0.45 \pm 0.21 a (0.58 \pm 0.28)
Gorse	0.97 \pm 0.07 ab	0.62 \pm 0.15 a (0.28 \pm 0.07)	0.10 \pm 0.06 a (0.13 \pm 0.07)

Other soil characteristics measured at the end of the incubations are shown in table 4.8. Clover and alder treated soil contained more leaf-derived DOC than gunnera treated soil, and clover treated soil had a higher total DOC than control soil. Concentrations of TN in AN treated soil were the highest at over four times that of the control. Alder and gunnera treated soil had TN concentrations which were lower than the control. All three PMGMs increased the soil pH, though clover had no effect.

Table 4.8 Soil characteristics at end of incubation. Means \pm SEM, $n = 4$ Letters denote significance at $P < 0.05$

	DOC (mg kg ⁻¹ dry soil)	Leaf-derived DOC (mg kg ⁻¹ dry soil)	TN (mg kg ⁻¹ dry soil)	pH 1 : 2.5 (in H ₂ O)	EC (μ S cm ⁻¹)
Control	160.4 \pm 27.1 a	-	79.9 \pm 2.9 b	4.7 \pm 0.03 a	151.3 \pm 2.0 c
AN	196.4 \pm 10.9 ab	-	368.4 \pm 12.7 d	4.6 \pm 0.00 a	518.0 \pm 9.4 f
Clover	223.4 \pm 5.7 b	6.2 \pm 0.3 b	175.9 \pm 4.9 c	4.7 \pm 0.05 a	440.3 \pm 10.1 e
Alder	199.4 \pm 7.8 ab	6.2 \pm 0.5 b	35.7 \pm 1.2 a	5.4 \pm 0.05 d	58.5 \pm 3.2 a
Gunnera	210.4 \pm 3.9 ab	0.8 \pm 0.7 a	25.4 \pm 0.5 a	5.2 \pm 0.04 c	117.5 \pm 3.9 b
Gorse	207.8 \pm 3.9 ab	3.7 \pm 1.4 ab	79.6 \pm 4.5 b	4.9 \pm 0.02 b	198.5 \pm 5.0 d

4.4 Discussion

4.4.1 Soil C losses

Loss of ^{14}C -labelled SOC after addition of green manures during the incubation are expected, as positive priming of pre-existing SOC is a known phenomenon after addition of organic materials (e.g. Guenet et al. 2010, Schmatz et al. 2017, Shahbaz et al. 2018, Lerch et al. 2019). That we were able to measure $^{14}\text{CO}_2$ efflux from respiration of 8 year old ^{14}C labelled SOC shows that the C efflux originated from SOC and is not a false conclusion derived from uneven labelling of the amendments, as has reportedly been the case in other experiments (Kuzyakov et al. 2000). Here the higher amounts of ^{14}C derived from SOC in green-manure treated soil than in the untreated control clearly shows loss of soil derived C in response to the amendments.

That the rates of soil ^{14}C loss did not show a steep decline over the 42 days, contrasts with many previous studies (e.g. Guenet et al. 2010, Schmatz et al. 2017, Fang et al. 2018). A meta-analysis by Zhang et al. (2013) found that priming of soil C generally declined after 15 days. Priming, however has been shown to continue over a long time period e. g. of over 80 days (Perele and Munch 2005), and over 360 days (Schmatz et al. 2017). Priming over a longer period from more recalcitrant materials has also been found by Wang et al. (2015) who found that rates of priming induced by more recalcitrant maize stalks were slower to peak than those from more labile maize leaves, and that the stalk-induced priming was still occurring over 100 days after the beginning of the incubation. That soil ^{14}C loss was correlated with mineralisation of leaf tissue is to be expected to some extent as priming cannot be caused by green manure while it is in-tact and having no interaction with microbes. It follows then that materials of lower lability would have a later priming effect.

The higher levels of total microbial biomass C in clover, alder and gunnera treated soils than in the AN-treated soil are to be expected, as unlike the AN treatment, the green manures provided C enabling increased microbial respiration and growth. Although there was a strong correlation between the effluxes of soil ^{14}C and leaf ^{14}C , there were variations in ^{14}C loss which did not correlate with the rates of C mineralisation from leaves. Therefore other factors of the chemistry of the green manures such as allelopathy, or the similarity of compounds to the existing organic matter were likely to be having an influence, as discussed below.

4.4.2 Implications for N dynamics

The priming of SOM will also have promoted the conversion of soil N into plant available forms and demonstrates that N from SOM is likely to have contributed to the very high N uptake rates in the pot experiment (chapter 3). In addition, the lower TN content of the gunnera treated soil than the control

after the 42 days ($P < 0.05$) confirms an N immobilisation effect of the gunnera treatment, which in the pot experiment showed as a trend, but not was not significant. Implications of the combined data on N dynamics will be discussed in chapter 7.

4.4.3 Loss of dead or living SOC?

It is not possible to ascertain whether the SOC respired was from the living or dead components of SOM. For all treatments the sum of the percentages of the soil ^{14}C captured in NaOH and the soil ^{14}C remaining in microbial biomass of labelled soil was less than the percentage of ^{14}C measured in the microbial biomass of the labelled soil at the start of the experiment. For example, the soil derived ^{14}C , of $3.10 \pm 0.17 \%$ ($2.37 \pm 0.04 \%$ in NaOH, plus $0.73 \pm 0.13 \%$ in microbial biomass), recovered from the alder treated soil, which had the highest amount of ^{14}C recovery, was, in total not more than the $3.46 \pm 0.38 \%$ ^{14}C in the initial microbial biomass. Therefore, we cannot assume that the priming is “real” from dead SOC, and it is possible that all ^{14}C loss was from living C in microbial biomass.

The timing of soil ^{14}C loss over the incubation, however, does not fit with research on apparent priming, which has been found to typically occur within the first few days of substrate addition, with priming of dead SOC taking longer (Bastida et al. 2019). That we do not see a steep decline in soil ^{14}C loss during the incubation suggests that dead organic C is being respired. Errors may have occurred in the measured amounts of initial microbial ^{14}C . Although the procedures used were identical it is possible that the thoroughness of the fumigation used to release microbial C was inconsistent, which could have caused inaccuracies in the relative levels of microbial biomass recorded at the start and end of the incubations. It is also possible that there was a decline in microbial biomass during the incubations which was not due to the treatments. That the ^{14}C activity calculated to be in the microbial biomass of the control soil at the end of the incubation of $1.10 \pm 0.43 \%$ suggests that one of the above explanations is the case, as it is much lower than the ^{14}C in microbial biomass ^{14}C measured in the starting soil of $3.46 \pm 0.38 \%$.

4.4.4 Validity of the predicted one-year carbon stocks

The predictions of C stocks after one year give an indication of the long term effects of the ammendments if a period of fallow was to be maintained. Though this would not occur on the farm, it can be cautiously used to assess the relative effects of the treatments which may occur over a longer time period. The use of the assumption that the labelled component of the SOC represents 66% of the SOC for calculating actual quantities of C from the percentages of SOC ^{14}C captured as $^{14}\text{CO}_2$, must be also treated with caution. However, the calculations result in quantities of primed SOC > 8 years old, which are plausible in that they do not exceed the total CO_2 efflux measured but are high enough to

account for a large proportion of the respiration in the control and AN treatments where no C has been added. For example, the primed SOC > 8 years lost after gorse treatment is 15 % of the total C predicted to be lost after one year, and that of the AN treatment 71 %. The effects of other treatments fall within this range.

4.4.5 Effect of green manures on total C stocks

At the end of the incubations the higher total C (added plant C plus existing soil C) remaining in gunnera and alder-treated soil, than in clover and gorse treated soil suggests that gunnera and alder would have the least detrimental effect on soil C of the green manures. However, the rates of C loss from clover and gorse treatments declined more steeply during the incubation than those from alder and gunnera treatments so that by the final week alder and gunnera treated soils had both the highest soil $^{14}\text{CO}_2$ loss rates per week of all treatments and higher total CO_2 loss rates than all treatments except gorse. This is reflected in the predictions after one year, in which the alder and gunnera treated soil were predicted to retain the lowest total C stocks and alder treatment to have resulted in the largest loss of $^{14}\text{CO}_2$ from soil. For gunnera treated soils, the predicted remaining pool of SOC > 8 years old is a higher proportion of the total predicted SOC pool (78 %), than the alder treated SOC > 8 years old is of the total SOC pool (73 %). As the predictions show no difference between the retained leaf C pools of alder and gunnera, this suggests that although gunnera treated soil is predicted to retain more SOC > 8 years old, it may prime a greater quantity of SOC of under 8 years old.

The gorse treated soil had lower soil ^{14}C efflux than the other green manures during the incubation and lower leaf-derived microbial biomass. This is despite C and N being available for microbial growth, as shown by the DOC and TN measured at the end of the experiment. Therefore, factors other than the C and N content of the gorse tissue may be affecting biological activity, possibly an allelopathic effect of the tissue on microbial action. The pot experiment indicated that gorse treatment suppressed the growth of *Lolium perenne*, (section 3.4.3, chapter 3) and recent research has concluded that microbial action can be inhibited by volatile compounds emitted by gorse tissue (Pardo-Muras et al. 2018).

4.4.6 Loss of C from AN treated soil

The AN treated soil had a higher efflux of soil derived ^{14}C than the control in weeks 2 and 3, but no difference in overall CO_2 loss. Addition of mineral nitrogen has previously been found to have both positive and negative priming effects (Kuzyakov et al. 2000). One explanation of positive priming is that the N supplied allows an increase in activity by N limited microbes (Mahal et al. 2019). Although here the soil has an overall C:N ratio of 8.5, which is higher than the level of N in comparison to C

required for microbial growth, N may be a limiting factor within some specific pools of organic matter. It is possible that negative and positive priming are occurring within different C pools simultaneously, which would also explain the differing results between the total C loss and soil ^{14}C loss from AN treatment. AN treated soil had lower levels of total microbial biomass than alder, gunnera and clover, in keeping with its small priming effect.

4.4.7 Possible mechanisms

4.4.7.1 Priming due to the co-metabolism theory

The positive regression of the capture of soil ^{14}C against the capture of leaf ^{14}C shows that priming of soil C of > 8 years increased with increased respiration of the leaf C, during weeks 3 to 6. This concurs with the co-metabolism theory, with the leaf C providing energy for increased microbial activity. Previous studies have also found that the rate of priming is correlated with the speed of mineralisation of the substrate (Di Lonardo et al. 2017, Liang et al. 2017, Mason-Jones et al. 2018). The correlation shows that respiration of leaf C accounts for 86 % of the variation in respiration of ^{14}C leaf C. The remaining variation may be explained by differences in the energy requirements for microbial enzyme production as lower lability materials have a higher C cost of processing (Manzoni et al. 2012, Cotrufo et al. 2013). This energy cost of processing reduces the amount of C available for microbial priming. A regression of the capture of soil ^{14}C against the capture of leaf ^{14}C was not considered valid due to the likely uneven labelling of leaf tissue, but it can be assumed that all materials would provide some easily available C in the form of sugars in the early stages.

Though both the soil ^{14}C loss and leaf ^{14}C loss were higher from alder-treated soil, than other treatments during weeks 3 to 6, the total respiration from the alder treatment, was not higher than from gunnera or gorse. This could be due to alder disproportionately priming older soil C over newer soil C. However, although labelling procedures were the same, it also could be due to uneven labelling of the leaf tissues giving a falsely high leaf respiration from alder, or falsely low for other gunnera and gorse. The one-year predicted total C loss from alder-treated soil, which was equal to that from gunnera and higher than all other treatments, suggests that the total C loss rates were becoming relatively higher for alder compared to other treatments as the incubation progressed. This adds weight to the co-metabolism mechanism being either the sole cause or a contributory cause of priming.

4.4.7.2 Priming due to substrate-induced changes in the microbial population

If the priming was due to changes in the species composition of the microbial biomass (theories 2 to 4), a time lag would be expected between the green manure additions and the respiration of soil ^{14}C ,

while the species composition of the microbial biomass changed (Bray et al. 2012). Therefore, these mechanisms do not offer a full explanation, though they could have been contributory factors. Some priming due to an increase in *K* strategists feeding on *r* the necromass of strategists (theory 2) could have contributed to the priming of soil C, after the first few weeks. Faster leaf mineralisation and higher levels of priming from the alder treatment may have also been partly due to higher microbial growth enabled by the addition of a material with a C:N ratio (19.2 ± 0.09) close to the ratio of 16 to 20 required by microbes as in theory 3, the stoichiometry theory (Kuzayakov et al. 2000, Chen, Senbayram, et al. 2014, Wang et al. 2015). C:N ratios of organic matter typically decrease as C is respired (Trinsoutrot et al. 2000, Whalen 2014), so the C:N of the remaining gunnera and gorse tissues, which were initially higher than that required for microbial growth would have been likely to become a more suitable substrate over time.

Differing levels of priming can also be explained by theory 4 (the “like primes like” theory), in that the chemistry of the amendments (other than C and N) may be influencing the capability of the microbial community. The high levels of priming in the alder treatment during the last three weeks fit with the theory from Fontaine et al. (2003) that the *K* strategists are increasing by utilising a more recalcitrant portion of the substrate after the more easily available material within the alder tissue has been used up.

It is also worth noting that the soil in this experiment was taken from a plot adjacent to some *Alnus rubra* trees. Therefore, there may be a “home field advantage” effect, if *A. rubra*-derived SOM was of a similar chemical composition to *A. glutinosa* (alder) leaf material (Di Lonardo et al. 2018). If material containing the same compounds has previously been decomposed in the soil, there may be a latent population of microbes capable of decomposing the new addition which can increase when a similar substrate is added (van der Wal and de Boer 2017). Although the leaf fall from the *A. rubra* will have been senescent, leaves may still have had a similar chemistry to fresh *A. glutinosa* leaves. This could contribute to faster priming in alder treated soil. Clover was also present in the grass sward, but due to grazing it is unlikely that a large amount of clover leaf tissue was decomposed into the soil.

4.4.7.3 Priming due to the N Mining theory

Priming occurred which could not be explained by a need for microbes to mine N (theory 5). This is shown in the loss of ^{14}C soil C in the AN treated soil, which was higher than in the control during weeks 2 and 3. In addition, the negative correlation between the C:N ratio of the green manures and ^{14}C SOC loss after seven days is the reverse of what would be expected due to the N mining theory. As noted above the ideal C:N ratio of substrates to provide for microbial growth is considered to be in the region of 16 by Kuzayakov et al. (2000), and 20 by Wang et al. (2015) and microbes require addition N

when substrates have a C:N ratio above this. This is based on an average C:N ratio of microbial biomass of 8 and 10 respectively, and an equal quantity of C required for respiration. According to the N mining theory, priming by the clover residue with a C:N of 14.6 would not be expected to cause SOC degradation. N mining is not considered to be as important a driver of soil C loss as previously thought and much recent research has not supported the N mining hypothesis (e.g. Liang et al. 2017, Mason-Jones et al. 2018, di Lonardo et al. 2019).

4.4.8 Further research

A repeat of this experiment using a longer incubation time may result in a percentage of respired ^{14}C which is greater than that in the original microbial biomass, which would confirm the priming of C from dead organic matter. To further explore the likely mechanisms, analyses of microbial groups to quantify changes in K and r selected microbes could elucidate whether a change in the make up of the microbial communities are driving priming. Experiments using substrates of differing C:N ratios alongside the analyses of the starting and resulting C:N ratio of microbial biomass may shed light on whether microbial biomass is increasing due to substrates which provides for the C and N requirements of microbes.

4.4.9 Application to agricultural practice

Loss of C is to be expected in a laboratory incubation due to respiration without photosynthetic inputs, as shown by loss of C from the control soil. The predicted rates of C loss of treated soil in relation to the control give an indication of priming caused by the amendments. However, in a field situation, the C dynamics will be affected by many inter-related factors including crop roots and exudates, root-associated microbial activity, soil meso and macro fauna, soil disturbance, death and decomposition of crop roots and plant nutrient uptake, temperature and precipitation (Kuzaykov et al. 2007, Zang et al. 2016, Bailey et al. 2019). The data presented above can be used to identify methods of green manure application which need further research to assess their effects on soil C stocks.

4.4.9.1 Plant roots

Plants add C to soil in root exudates and root tissue. Gross and Harrison (2019) report that about 45 to 50 % of photosynthetic C is allocated below ground. The importance of root biomass as a C input is shown by the pot experiment in which high variation in root mass occurred under different treatments. This extra input of crop roots, especially the higher root mass produced in response to gunnera treatment could alter the C balance to be positive after one year of crop growth. The combined effect of roots and decomposing amendments on priming have yet to be unravelled (Mwafulirwa et al. 2017), but the presence of roots has been found to affect priming of soil C in diverse

ways from a stimulation of priming due to increased microbial growth (Cheng et al. 2003), to a repression of priming (Barel et al. 2019). Mwafulirwa (2017) found that the genotype of the plant affects the soil C loss, and therefore it may be worth considering the effects of specific combinations of crop genotypes and green manures.

4.4.9.2 Tillage

In this experiment, the green manures were mixed with soil to simulate tillage of the surface horizons. Tillage is known to destabilise aggregates and tilled soils have been recorded to lose more C compared to no or low-till soils (Bailey et al. 2019). The proposed technique of supplying nutrients via PMGMs also allows mulching of the green manures onto the surface as part of a no-till system, and therefore could reduce C loss, compared to traditional green manures which are incorporated by ploughing. Mulching also reduces the contact of the amendment with the soil. Kan et al. (2020) found that the priming effect was higher when residues were incorporated by tillage, than when left on an untilled soil surface. This needs further investigation to assess whether mulching may increase soil C stocks over incorporation.

4.4.9.3 Soil C:N ratio

There is greater potential for C sequestration and priming is lower in soils which contain low C stocks (Kuzyakov et al. 2000, Zhang et al. 2013, Bailey et al. 2019). Therefore, adjustment of residue addition depending of the soil C balance could increase C stocks. The C:N ratios of the amendment and soil affect priming in ways which are yet to be unravelled. Some research has found that addition of mineral N alongside C inputs reduces priming of SOC (Mahal et al. 2019), however, others e. g. Guenet et al. (2010) did not find that priming reduced when N was added along with plant residues. With greater understanding, the supply of N to crops could be adjusted for maximum C retention alongside the considerations of the nutrition of the crop and possible leaching of nitrate and denitrification to N₂O.

4.4.9.4 Type and amount of amendment

There is conflicting evidence on whether priming increases linearly with increasing amounts of amendment. Kuzyakov et al. (2000) concluded that priming increases in proportion with the amount of substrate, but Guenet et al. (2010) and Zhang et al. (2013) did not find a linear relationship. Bailey et al (2019) concluded that priming is in part determined by the relative quantities of amendment to soil biomass, with the priming decreasing when the substrate is over 50% of the microbial biomass C. If priming does not increase linearly with amendment then the C balance may be maximised by adding large amounts of amendments infrequently, instead of more frequent, smaller amounts. The slow

release of N from alder and gunnera tissues, as found in the pot experiment would allow for addition of a large quantity of PMGM while not over-supplying the crop with N. Another strategy which deserves research is choosing amendments which are chemically different to the existing SOC, so reducing the likelihood that the enzymes produced by substrate decomposition would be effective on SOC.

The decomposition of recalcitrant amendments is driven by K selected microbes. Saprophytic fungi are one group of K selected microbes which may increase populations in response to more recalcitrant organic matter (Liang et al. 2017). This highlights the importance of considering the combined effects of agricultural practices, as saprophytic fungi are implicated in increasing N₂O emissions due to incomplete denitrification (Mothapo et al. 2013). However, fungi are not the only K selected microbes to decompose recalcitrant material, and some gram negative bacteria may also act as K selected microbes (Nottingham et al. 2009, Kuzyakov 2010).

4.4.10 Conclusions

Large losses of C from both soil and green manures, resulted in predicted total C stocks after one year from all green manures that were lower than the control C stocks, despite the additional C of the green manures. It is not certain from the soil ¹⁴C efflux that loss of soil C was from dead organic matter, and could instead have been loss of C from microbial biomass. The varying effects of the green manures demonstrate that it is not only the C and N content affecting priming, but also other factors of leaf chemistry. Identifying mechanisms behind the priming is challenging and requires more research, but the pattern of soil C efflux fits with the co-metabolism theory.

C dynamics in field cropping will be influenced by many other factors and there are a wide variety of agricultural strategies which deserve research to improve C retention. Targeted research is needed to enable the development methods of green manure use which increase soil C stocks. The effects on soil C also must be considered alongside other environmental consequences of soil management such as CO₂ emissions from fertiliser production and nitrogen pollution from green manure or fertiliser use.

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Chapter 5: Evaluating PMGMs for efficacy as N fertiliser and climate change impact: A field experiment

Contributions: CW conceived the experiment. PWH, DRC and CW designed the experiment. Experimental work, data processing and statistical analysis were performed by CW. CW wrote the chapter with advice and edits from PWH and DRC.

Abstract

The pot experiment (Chapter 3) demonstrated the viability of incorporated PMGMs to supply N to a crop, while also resulting in very low N₂O emissions compared to N supplied as red clover or NH₄NO₃. However, PMGMs may have different effects when used in field cropping. It may be advantageous in field cropping to add PMGMs as dried material to allow storage and flexibility in timing of addition. Also, mulching instead of incorporation allows the use of no or low till agriculture which is advocated for C sequestration. In this experiment we tested the PMGMs (alder, gorse and gunnera) for their effects on DM yield, plant N uptake and N₂O emissions in field cropping, with two successional crops over a total of 105 days. In addition, alder leaves were tested in four different combinations of fresh and dried leaves, and application by mulching and incorporation. For comparison we tested the effects of a range of NH₄NO₃ application rates, and calculated FEs and NREs as in the pot experiment.

There were no differences between the total DM yield and total N uptake of the PMGM treated plants to those of the control in the total of the two crops, and no differences between the various methods of addition, showing no adverse effects of drying or mulching PMGMs. However, the N uptake in plots with PMGMs increased over time relative to that from NH₄NO₃ N. In the first crop the N uptake of the PMGM treated plants was less than that of the plants supplied with 200 kg ha⁻¹ NH₄NO₃ N and no different to the control, but in the second crop, the N uptake of the alder and gunnera treated plants was greater than that of the control plants and not different to that of plants treated with 200 kg ha⁻¹ NH₄NO₃ N. If this trend were to continue, as was found in the pot experiment, the PMGMs may supply an effective amount of N to field crops grown over a longer time period.

N₂O emissions were low at an average of ≤ 0.3 % of the N added and did not differ between treatments. Low soil moisture levels at a WFPS of ≤ 60 % for most of the experiment may have limited both N₂O emissions and the speed of PMGM decomposition and therefore speed of N release. With limited uptake of N from the PMGMs it is likely that there is a useful amount of PMGM N left in the soil which may yet be available for crop uptake. Therefore, it would be valuable to assess the N uptake from the same plots over another year, as well as that from new additions of dried, mulched PMGMs over a longer growing season.

5.1. Introduction

5.1.1 Differences between pot and field experiments

Though pot experiments give an indication of the effect of green manures in the field, there are many factors which differ between experiments in controlled environments and those in outside cropping. Our pot experiment was designed to test the PMGMs in warm, moist conditions favourable to N₂O production, and field conditions are likely to be drier, cooler and more variable, as well as allowing free drainage which was prevented in the pot experiment. Meta analyses by Chen et al. (2013) and Lehtinen et al. (2014) have found that laboratory and pot experiments are often conducted in conditions more favourable to decomposition and generally report higher N₂O emissions from plant material than field experiments do. The sieving of soil can reduce meso fauna and eliminate macro fauna, which has been found to affect decomposition rates of plant material (Kumar and Goh 1999, Frouz 2018). Therefore, in the field, rates of N₂O emissions, as well as speed of plant matter decomposition affecting N provision to crops are likely to be different to those in the pot experiment.

The application of PMGMs by mulching, instead of incorporation, allows their use in no-till systems and addition while the crop is growing. The drying of PMGMs enables storage giving greater flexibility in timing of applications and a reduced volume of material would increase the practicability of storage and transport. It is important to understand the effect of these methods on N mineralisation and pathways, crop production, and greenhouse gas emissions.

5.1.2 Impacts of reduced tillage on C sequestration and soil functioning

Reducing or eliminating tillage is one strategy recommended to promote soil C sequestration (Jarecki and Lal 2003, Lal 2004, Stockmann et al. 2013). Conventional tillage commonly consists of inversion of the soil to a depth of 20 to 30 cm, as well as surface cultivation to create a fine texture for seed drilling or planting (Abdalla et al. 2013). In contrast, no till, reduced till or conservation tillage uses varying levels of tillage from none to a surface cultivation of less than 15 cm which does not invert the soil (Powlson et al. 2012, Rial-Lovera et al. 2017). Conventional tillage brings soil C from deeper layers to the surface where it is more prone to mineralisation, and can also cause C loss through increased soil erosion due to exposure of fine particles to wind and rain (Jarecki and Lal 2003, Powlson et al. 2011, Bhattacharya et al. 2016). Soil under low or no till management has been shown to have a greater amount of C within microaggregates due to a lower level of macroaggregate turnover which leads to the formation of more stable microaggregates (Six 2000). Many individual studies and meta analyses have found that soils under reduced tillage retain greater levels of C (e.g. Six 2000, West and Marland 2002, Coppens et al. 2006, Abdalla et al. 2013, Chen et al. 2020).

It is not certain that no till or low till systems always increase soil C (Bhogal 2007, Luo et al. 2010). It has been suggested that previously observed effects of C sequestration may be due to shallow sampling, with differences between tilled and no tilled land limited to the top 15 cm, and therefore C sequestration effects may have been overestimated (Poeplau and Don 2015). A meta-analysis of 69 paired sites showed no increase in C sequestration when soil was sampled at depths of over 40 cm, with conversion from conventional tillage to no tillage changing the distribution of soil C rather than the overall amount (Luo et al., 2010).

No or low tillage is considered beneficial for soil functioning, as machinery use can cause soil compaction (Whalen 2014, Roger-Estrade et al. 2010), and the disruption of soil structure can reduce populations of soil fauna including earthworms, mycorrhizal fungi and meso and micro fauna (Kladivko 2001, Roger-Estrade et al. 2010, Lemtiri et al. 2016, Chen et al. 2020). The effects of tillage, however, are complex and can include beneficial effects such as a reduction of some agricultural pests. Roger-Estrade et al. (2010) warn against a simple comparison of tillage against no till and emphasises that the choice of practice needs to be made in consideration of other agricultural factors such as residue management, soil ecology and crop rotations. Though studies vary on its effects, no till is one possible strategy to increase C sequestration and soil functioning, and mulching of green manures rather than incorporation would allow the flexibility to choose no till methods in conjunction with green manure use.

5.1.3 Effect of method of PMGM application on decomposition rates

Many studies show a faster rate of decomposition of plant matter when incorporated, than when applied to the soil surface (Beare et al. 1992, Schomberg et al. 1994, Coppens et al. 2007, Hoyle and Murphy 2011, Chen et al. 2014). For example, Coppens et al. (2006) reported 18.4 % mineralisation of C in mulched material compared to 54.7% mineralisation of C in incorporated material after 9 weeks. The rate of decomposition affects the concentration of mineral N in the soil, which in turn has implications for N₂O emissions and losses of NO₃⁻ by leaching (Coppens et al. 2006, Campiglia et al. 2011, Chen et al. 2014). Surface application of plant matter may restrict access of microbes to the plant tissue due to lack of contact with soil compared to incorporated material (Beare et al. 1992, Whalen 2014). Level of moisture is considered to be a major factor reducing the decomposition rates of mulched plant material. Coppens et al. (2007) used the PASTIS_{mulch} model to conclude that 90% of the difference in the decomposition rates of mulched verses incorporated green manure was due to the lower moisture content of mulched material inhibiting decomposition. Flessa et al. (2002) compared the decomposition of mulched and incorporated plant tissue and concluded that an adequate population of decomposing microbes was present in the mulch, and that humidity was likely

to be the most important factor in influencing decomposition rates. It follows that fresh PMGM material may decompose faster than dried, due to its higher moisture content. In addition, there may be an interaction effect of the method of addition of PMGMs, the C:N ratio, and levels of moisture within the soil and on the soil surface. For example, Coppens et al. (2006) found that for a residue with a C:N ratio of 29, decomposition of incorporated residues was higher than mulched residues, but incorporation resulted in N immobilisation, whereas mulching did not. In addition, the mulched material reduced evaporation, preserving soil water and so increased the risk of leaching.

5.1.4 Gaseous N loss

Whether plant matter is mulched or incorporated influences emissions of N_2O and NH_3 (Ruijter et al. 2010, Shan and Yan 2013, Nett et al. 2015). The rate of decomposition affects concentrations of NO_3^- and available C which in turn can lead to increased N_2O emissions by denitrification. Machinery used for incorporation can exacerbate this by disruption of plant tissues which increases availability of N and C to microbial action (Hansen et al. 2019). Although mulches may decompose more slowly than incorporated material, their effect of maintaining moisture levels within the soil, can lead to increased soil N_2O emissions due to anaerobic conditions (Flessa et al. 2002, Shan and Yan 2013). A meta-analysis by Flessa et al (2013) found higher N_2O levels from mulched than incorporated material, though data from mulched material were limited to 6 studies, compared to 68 studies of incorporated material. One factor which may reduce emissions from incorporated material is the greater residence time of N_2O in the soil profile which allows more opportunity for complete denitrification of N_2O to N_2 (Chapuis-Lardy et al. 2007, Hansen et al. 2019).

As N_2O production is influenced by the many factors affecting denitrification and nitrification, as well as those affecting decomposition rates, the effect of mulching and incorporation is not straightforward. For example, the effect can vary with soil type, and in light soils incorporation has been shown to result in higher N_2O emissions than from mulched material (Hansen et al. 2019). NH_3 losses are not considered to be a major factor when the N source is incorporated, but can be high when materials are mulched on the soil surface (Larsson et al. 1998, Hansen et al. 2019, de Ruijter and Huijsmans 2019). Therefore, the method of incorporation can have opposing effects on N_2O and NH_3 . For example, De Ruijter et al. (2010) found that mulched vegetable crop residues lost most N as NH_3 , but when incorporated lost more through denitrification.

5.1.5 Objectives and experimental design

The aim of this field experiment was to assess the effects of the PMGMs alder, gunnera and gorse, and different methods of processing and application on DM yield, N uptake, and gaseous N emissions

(N₂O and NH₃). N was added to field plots as applications of 200 kg ha⁻¹ N supplied as the PMGMs, alongside a range of NH₄NO₃ application rates from 25 to 200 kg ha⁻¹ N and a control (no addition). Gunnera and gorse were applied as dried, mulched material, and alder as four different combinations of fresh, dried, mulched and incorporated material. Alder was chosen to test the different application methods because of its faster N uptake during the pot experiment, and hence its potential as an alternative organic N supply for agricultural crops within a growing season. A “crop” of *Phacelia tanacetifolia* was sown after addition of the treatments and the crop biomass production and N uptake were measured over a period of 105 days. *P. tanacetifolia* was chosen as a reliable non-legume with a fast growth rate, which would respond to N fertilisation and serve as a proxy crop (Smither-Kopperl 2018). It was chosen over actual crop species to prevent the risk of crop failure which would have been greater with the crops suitable for sowing in late June, such as brassicas, which are prone to many diseases (AHDB 2017). As in the pot experiment (Chapter 3), the dry biomass yields and plant N uptakes were used to calculate fertiliser equivalents (FEs) and apparent nitrogen recovery efficiencies (NREs) to assess the N fertilisation effect of the PMGMs in comparison to NH₄NO₃. Gas samples were regularly taken from static chambers and analysed for N₂O, from which fluxes and total N₂O emissions were calculated, enabling assessment of the N₂O on an area basis, per dry matter yield of the “crop” and calculation of EFs. Unfortunately, methodological problems prevented the measurement of NH₃. Soil was regularly sampled and analysed for extractable DOC, NO₃⁻ and TN.

5.2. Materials and Methods

5.2.1 Green manures

The green manure material was collected between 10th and 30th May 2017 from live plants. Alder and gorse were collected from plants at Henfaes Research Station, in Abergwyngregyn, (53°14'N, 4°01'W), and gunnera from privately owned land at 52°37'N 3°50' W. The alder and gunnera was of leaves only, and the gorse consisted of leaves and stems of ≤ 5 mm width. The gunnera, gorse, and half of the alder leaves were spread out on benches to dry in a well-ventilated greenhouse. The remaining half of the alder leaves were stored fresh at 4 °C. Samples of the fresh alder were dried at 80 °C for 48 hours, and the weight of samples before and after drying was used to determine moisture content. To determine total C and N content of all green manures, dried samples were ground to < 1 mm, using a FOSS CT 193 Cyclotec mill (Hilleroed, Denmark) and analysed using a TruSpecR analyser (Leco Corp., St Joseph, MI). Characteristics of the green manure material are shown in Table 5.1. Dried gunnera and alder were crumbled by hand to pieces of ≤ 1 cm, and gorse was shredded using an electric garden shredder. The fresh alder leaves had been shredded while being stripped from the branches, leaving leaf fragments which were mostly between 2 and 4 cm in diameter. Fresh alder leaves had a dry matter content of 33 % of FW. The C and N contents of the alder leaves were altered during drying, possibly due to some decomposition which could not be prevented, resulting in differences between the C and N content of the fresh and dry alder leaves.

Table 5.1. Green manure characteristics. Means \pm SEM ($n = 4$)

Plant	C (g kg ⁻¹ dry leaf)	N (g kg ⁻¹ dry leaf)	C:N ratio
Alder (dried)	530 \pm 1	41.2 \pm 0.3	12.0 \pm 0.1
Alder (fresh)	507 \pm 2	27.5 \pm 0.3	18.5 \pm 0.2
Gunnera (dried)	458 \pm 1	30.2 \pm 0.4	15.2 \pm 0.2
Gorse (dried)	525 \pm 2	26.7 \pm 1.3	19.8 \pm 1.1

5.2.2 Soil and site

The experiment was conducted at Henfaes Research Station, Abergwyngregyn, (53°14'N, 4°01'W), 10 m above sea level, on land which had previously been sheep-grazed grassland. The area has a temperate climate and an average annual rainfall of 1,060 mm, and the soil is a Eutric Cambisol (FAO) with a sandy clay loam texture (Sánchez-Rodríguez et al. 2018). Other soil properties are shown in Table 5.2. Values for pH, P, K and Mg were obtained from analyses by NRM laboratories (Bracknell,

UK), from samples taken at 0 to 10 cm depth in February 2017. Other background soil data were taken from analyses of samples from the control on day 1 as described below.

Table 5.2 Background Soil characteristics. Results are expressed on a dry soil weight or volume basis. Means \pm SEM, ($n = 5$, except pH $n = 3$). Where SEM is not stated, the value is a single measure obtained from NRM laboratories.

Soil Property	
pH	5.6
Total soil C (g C kg ⁻¹)	26.5 \pm 0.9
Total soil N (g N kg ⁻¹)	2.6 \pm 0.2
Soil C:N ratio	11.0 \pm 0.2
DOC (mg C kg ⁻¹)	135 \pm 7
TN (mg N kg ⁻¹)	30 \pm 3
NO ₃ ⁻ (g N kg ⁻¹)	17 \pm 3
Available P (mg L ⁻¹)	48
Available K (mg L ⁻¹)	169
Bulk Density (g cm ⁻³)	1.116 \pm 0.09

5.2.3 Experimental set up

Six weeks prior to the experiment, the plot was mown, clippings removed, and the remaining grass sward treated with glyphosate herbicide. Four weeks prior, the soil was ploughed, and it was tilled to a depth of 10 cm on the day that the treatments were added. The soil was tested and subsequently amended with Keiserite (MgSO₄·H₂O) at 100 kg ha⁻¹, and K₂O at 140 kg ha⁻¹ to adjust S and K, respectively, as recommended by RB209 (Defra 2010) for a spring cereal crop for the nutrient levels shown in Table 5.2. P addition was not required. Calcifert® was added at 900 kg ha⁻¹ as recommended for arable crops for a target pH of 6.5 (Calcifert 2013). The experimental area was divided into 44 plots of 1.2 by 1.3 m, divided from each other by 0.5 m buffer strips which were covered with woven plastic landscape fabric to prevent weed growth (Fig. 5.1). The plots were arranged in 4 by 11 rows in a random block design of the 11 treatments with four replicates. Treatments are shown in Table 5.3. By including a range of NH₄NO₃ application rates of up to 200 kg N ha⁻¹ as well as a control with no added N, we could expect the N uptake from the PMGMs to fall within the range produced by the crops treated with the control or NH₄NO₃ applications, allowing for calculation of the FEs as described below. For the incorporated treatments, fresh and dry alder were dug in by hand to a depth of 10 cm.

Mulched green manures were applied on the soil surface. NH_4NO_3 was applied dissolved in water using a watering can fitted with a rose, and the equivalent amount of water was applied to all other plots by the same method.

All plots were sown with *Phacelia tanacetifolia* at a rate of 6.7 g m^{-2} , by a mechanical drill in rows spaced 13 cm apart (Fig. 5.1). The sowing was late in the season on 21st June (day 0) due to the need to harvest, process and analyse the green manures (from spring 2017 growth). The day of sowing is hence referred to as day 0, and experimental timing measured from this point. Throughout the experiment, air and soil surface temperatures were recorded hourly by automatic sensors located at air and ground levels approximately 100 m from the experimental plot.



Fig. 5.1. Experimental area showing plots divided by buffer strips and fitted with gas sampling chambers.

Table 5.3. Treatments

Code	Treatment	N (kg N ha^{-1})	Material (kg ha^{-1})
Control	No addition	0	0
AN25	NH_4NO_3	25	71.43
AN50	NH_4NO_3	50	142.86
AN100	NH_4NO_3	100	285.71
AN200	NH_4NO_3	200	571.43
Alder F Inc	Alder Fresh Incorporated	200	20,205
Alder F M	Alder Fresh mulched	200	20,205
Alder D Inc	Alder Dry incorporated	200	4,854
Alder D M	Alder Dry mulched	200	4,854
Gorse D M	Gorse Dry mulched	200	7,436
Gunnera D M	Gunnera Dry mulched	200	6,660

5.2.4 Soil sampling and analyses

Soil cores were taken from each replicate to a depth of 10 cm, no closer to any plot edge than 20 cm, on days 1, 3, 7, 13, 20, 27, 34, 41, 55, 69, and 97, and from depths of 0 to 15 cm and 15 to 30 cm on days 104, and 209. Subsamples were taken to determine water content by weight by drying at 105 °C for 24 hours. Soil extracts were prepared using a 6 g subsample of fresh soil in 25 ml 0.5 M K₂SO₄, shaken for 60 minutes at 200 rev min⁻¹, and centrifuged for 10 min at 2,000 g. The supernatant was filtered and stored at -20 °C until analysis.

Extracts were analysed for dissolved organic carbon (DOC) and total dissolved nitrogen (TN) using a Multi N/C 2100S analyser (AnalytikJena, Jena, Germany). To determine microbial biomass C for control samples on day 1 and for all samples on days 28 and 97, samples were fumigated in CHCl₃ vapour using the method described by Vance et al (1987), extracted as above and analysed for DOC and TN. Microbial biomass C was calculated by subtraction of unfumigated soil DOC from fumigated soil DOC, using a *k_{ec}* conversion factor of 0.45 (Joergensen, 1996). Extracts were also analysed for NO₃⁻ using a PowerWave XS BioTek spectrophotometer (Winooski, VT, US) using Gen 5 software, following the methods described in Miranda et al. (2001). At the time of writing, analyses for NH₄⁺ has been prevented due to restricted laboratory access caused by the COVID 19 pandemic. Water filled pore space (WFPS) was calculated from the soil water content and bulk density. Dried samples of the control soils from day 1 were analysed for C and N using a TruSpecR analyser (Leco Corp., St Joseph, MI) for data on background soil characteristics.

5.2.5 Biomass sampling

40 days after sowing (day 40), above ground plant biomass was sampled from a quadrat of 30 by 25 cm, which included two 30 cm rows of *P. tanacetifolia*. This date was chosen as the *P. tanacetifolia* had begun to develop flower buds, and it was necessary to measure at this growth stage prior to N being mobilised to the developing seeds from which it could be lost by seed dispersal. The quadrat was placed no closer to any plot edge than 20 cm, and no closer to the gas sampling chamber than 10 cm (shown in Fig. 5.1 and described below). The plants were cut to within 2 cm of the soil surface. Biomass was also sampled by the same method within each chamber to check for influences on biomass production and hence N uptake by the presence of the chamber.

The harvested biomass was dried, weighed and analysed for C and N as described for green manure tissue. After sampling, the remainder of the plot was cleared of vegetation, as for the sampled areas. *P. tanacetifolia* was then re-sown by hand, at the same rate, and plants were sampled after a further 65 days by the same method. The first crop contained very little weed growth (approximately < 1%).

The second crop contained weed biomass averaging 27 % of the biomass dry weight. None of the weeds were leguminous or (hemi)parasitic plants. Weeds were separated from *P. tanacetifolia* and weighed and analysed for C and N separately. After harvesting, the soil was left bare to allow testing of deeper soil levels for evidence of N leaching without the presence of a cover crop.

5.2.6 Gas sampling and analyses

Static gas sampling chambers, consisting of four vertical sides of 40 cm width and of 25 cm height were used to sample gas from all plots except those of treatments AN25 and AN50. Immediately after the first sowing of *P. tanacetifolia*, chambers were positioned within the plots, over 20 cm from any plot edge, and inserted into the soil leaving sides at a height of 20.35 ± 0.5 cm above the soil surface (Fig. 5.2). For sampling, the chambers were fitted with airtight lids, each of which contained a suba-seal™ through which the samples could be drawn out with a syringe. Chambers fitted with lids can be seen in Fig. 5.1 above. Gas was sampled between 10 am and 12 noon BST, on days 1, 3, 7, 13, 20, 27, 41, 69, and 97. Gas samples of 20 ml were taken at timepoints 0, 30 and 60 minutes after the lids were fitted and stored in pre-evacuated 20 ml glass vials until analysis. The head-space volume of the chambers above the soil surface was calculated to be 0.0326 m^3 and this volume was used in the gas flux calculations.

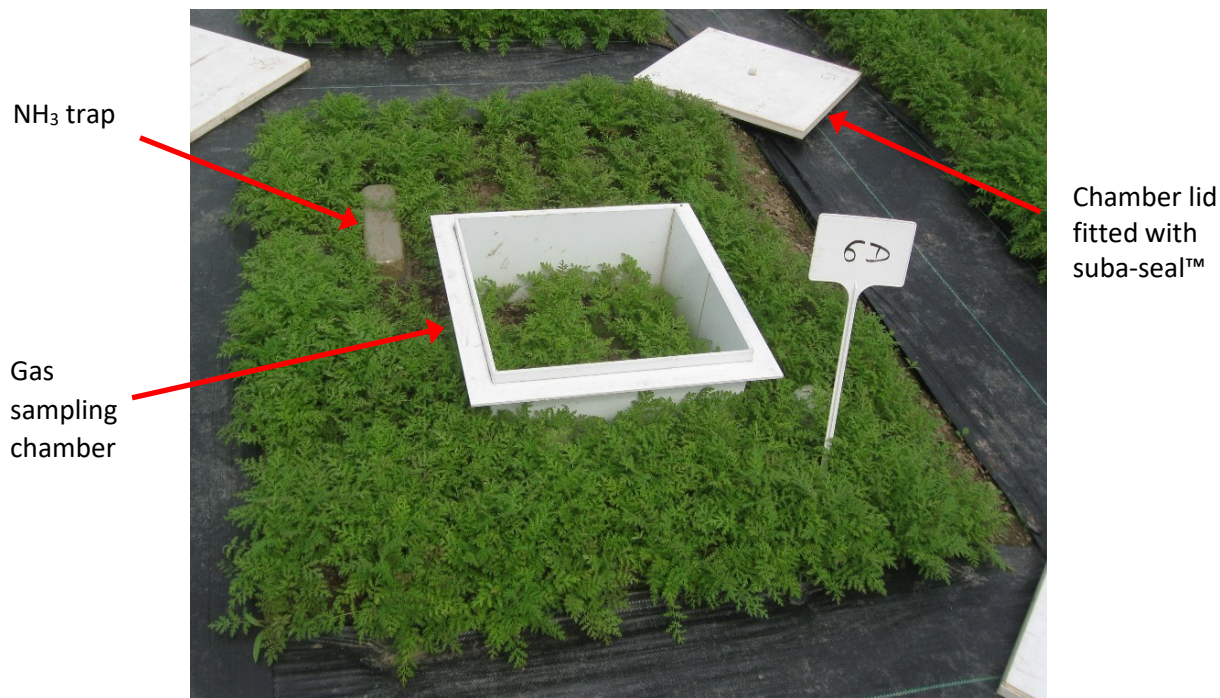


Fig. 5.2. One replicate plot, showing growth of *P. tanacetifolia* and gas sampling apparatus.

Gas was analysed using a Perkin Elmer 580 Gas Chromatograph fitted with an ^{63}Ni Electron Capture Detector (ECD) (Beverly CT, USA), and N_2O concentration was calculated against a series of certified N_2O standards. The molecular volume was corrected for temperature (at time of sampling from the hourly automatic sensor) and pressure, and fluxes calculated by linear interpolation of the headspace gas concentration from the three samples, following the method set out in Scheer et al. (2014). Flux calculations using the three time points were accepted if the R^2 of this regression was ≥ 0.8 . Of those with an $R^2 < 0.8$, if the sample at the starting time point had an N_2O concentration as expected for ambient air, and either the 30 or 60 minute samples gave a value within the expected range, fluxes were calculated between these two samples and the outlier value excluded. Any flux rates which did not meet these criteria were discarded. Cumulative N_2O was determined by calculating the area under the curve of fluxes against time, by use of the trapezoidal rule (Li et al. 2016). The emission factors, (EFs) were calculated by subtracting the cumulative N_2O emissions for the control treatment from the cumulative emissions of the treatment replicates (paired by block) and dividing by the amount of N added as described in De Klein and Harvey (2015). N_2O emissions on a crop yield basis were calculated by division of emissions by the biomass dry weight. Measurement of NH_3 volatilisation was undertaken using vials of H_2SO_4 placed within plastic chambers over the soil surface (Fig 5.2). Unfortunately, insects trapped within the H_2SO_4 made the results invalid.

5.2.7 Calculations and statistical analyses

N uptake in biomass per cumulative degree day (CDD) was calculated using the total of the mean daily ground temperatures above 5°C as in Bhogal et al. (2016). The FEs for biomass and N uptake were calculated by use of linear regressions of biomass and N uptake against NH_4NO_3 fertiliser response as in Sánchez-Rodríguez et al (2018). The range of NH_4NO_3 application rates used in the regressions were selected so that the green manure responses were within a fertiliser response range which was linear. For the N uptake FE this was using the 0 (control), 25 and 50 kg ha^{-1} AN-N treatments, and for the biomass FE this was using the 0, 25, 50 and 100 kg ha^{-1} AN-N. Apparent nitrogen recovery efficiencies (NREs) were calculated as in Ovsthus et al. (2017) by subtracting the N uptake of the control replicate from the N uptake of each treated replicate (paired by block), dividing by the amount of N added and converting to a percentage. All statistical analyses were performed using SPSS v25 (IBM Corp., Armonk, NY). Data were tested for homogeneity of variance using Levene's test. One way analyses of variance (ANOVA), and the Tukey's honest significant difference test were conducted to determine significant differences between treatments. Relationships between data were analysed using linear regression, and linear multiple regression as appropriate.

5.3. Results

5.3.1 Biomass yields

The AN200 was the only treatment which produced a higher total dry biomass uptake (in crop 1 + crop 2) than the control (Fig. 5.3). Biomass production was generally higher in the first crop than the second, despite a longer growing period for crop 2 of 65 days (713 CDDs) compared to 40 days (566 CDDs) for crop 1 (Fig. 5.4). The comparative growth between the treatments differed between the two crops. In crop 1, AN200 treated plants had a greater biomass than the control, but there was no difference between the control and the PMGM treated plants. In crop 2 however, fresh alder (incorporated and mulched) and dry incorporated alder treated plants, as well as the AN200 had a greater biomass than the control. The decline in growth of the second crop compared to the first was greater from the NH_4NO_3 treatments than from the PMGMs. The NH_4NO_3 treated grass biomass dry weights were less than 50 % of those of crop 1, whereas the crop 2 dry biomass from alder and gunnera treatments were over 50 % of the first crop, and fresh, incorporated alder over 75 % of crop 1.

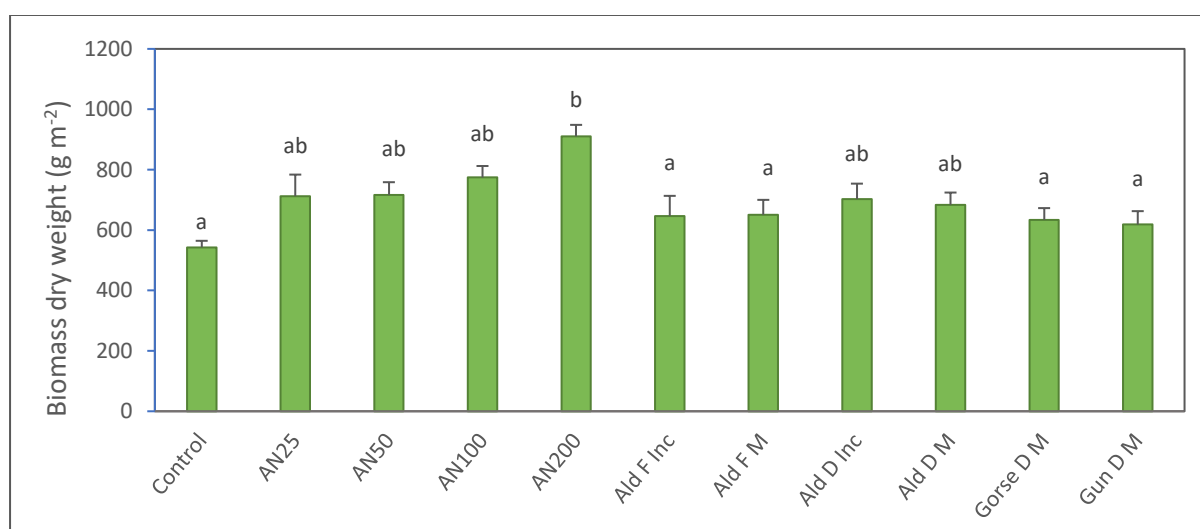


Fig. 5.3. Total above ground biomass harvested from crop 1 + crop 2. Means \pm SEM ($n = 4$). Letters denote significant differences between treatments ($P < 0.05$).

The data presented in Figs. 5.3 and 5.4 are the biomass data from the harvested quadrats. However, the biomass production was higher within the gas sampling chambers than that shown for the quadrat samples taken outside of the chambers. This is likely to be due to the sheltering effect of the chamber walls. On average the dry biomass production within the chamber was 84 % more than that measured outside the chambers, the increases ranging from 11 to 135 %. There was no difference in the increase in biomass within and outside the chambers between the different treatments. Data shown in Fig. 1, Appendix 2.

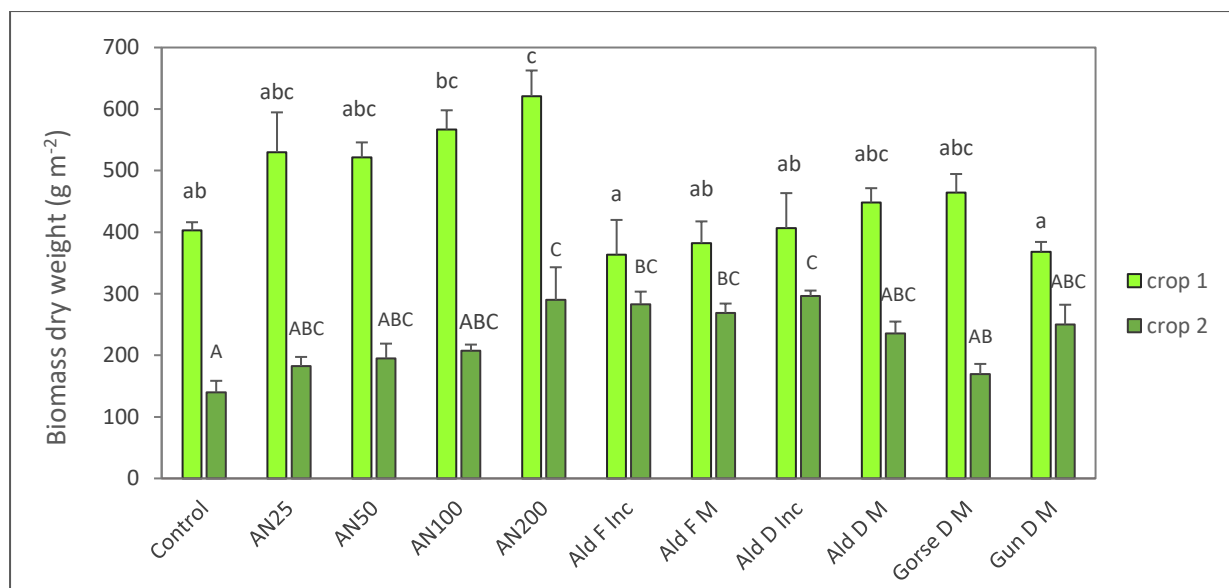


Fig. 5.4. Above ground biomass harvested from crops 1 and 2. Means \pm SEM ($n = 4$). Letters denote significant differences between treatments from the same crop ($P < 0.05$), with lowercase letters for crop 1 and uppercase letters for crop 2. A proportion of crop 2 is made up of weed growth, (Appendix 2, Table 1).

5.3.2 N uptake

The total N uptake of crop 1 plus crop 2 is shown in Fig. 5.5. To enable comparison between treatments of the effect of accumulated time and temperatures, Fig. 5.6 shows N uptake for each crop per CDD over 5 °C (at the soil surface). Again, the PMGM treated plants did not differ from the control in crop 1, but in crop 2, shoot growth from the alder treatment (all methods of addition) and gunnera treatment had higher N uptake than the control, and the fresh mulched and dry incorporated alder treated plants had a higher N uptake than from 100 AN. (See Table 2, Appendix 2 for % N in above ground biomass). For crop 1, a regression of C:N ratio against N uptake of dry mulched alder, gunnera and gorse showed a negative relationship ($P = 0.01$, Fig. 5.7)

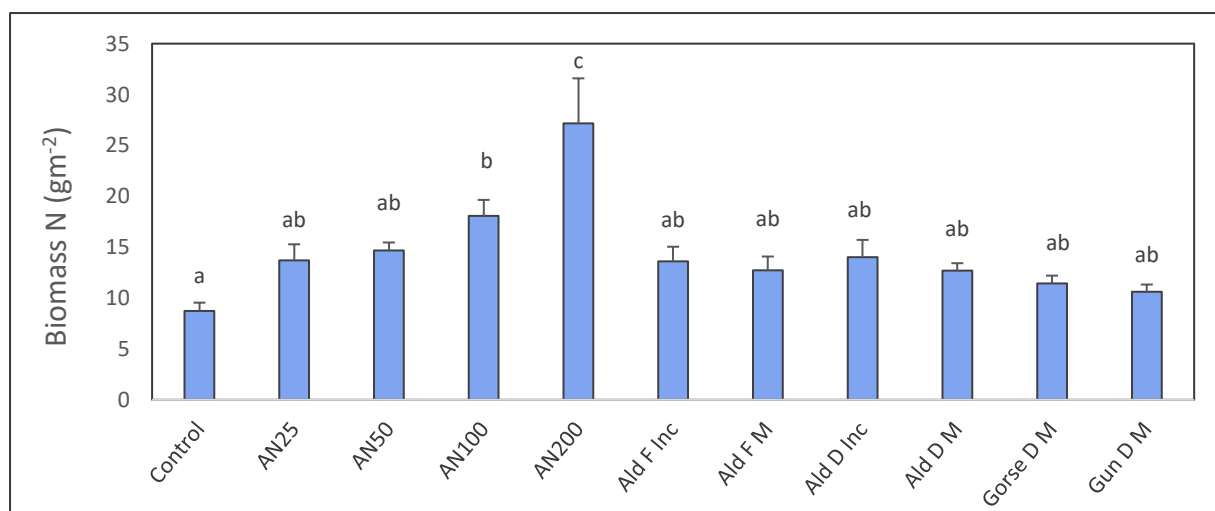


Fig. 5.5. Total N in above ground biomass. Means \pm SEM ($n = 4$). Letters denote significant differences between treatments ($P < 0.05$).

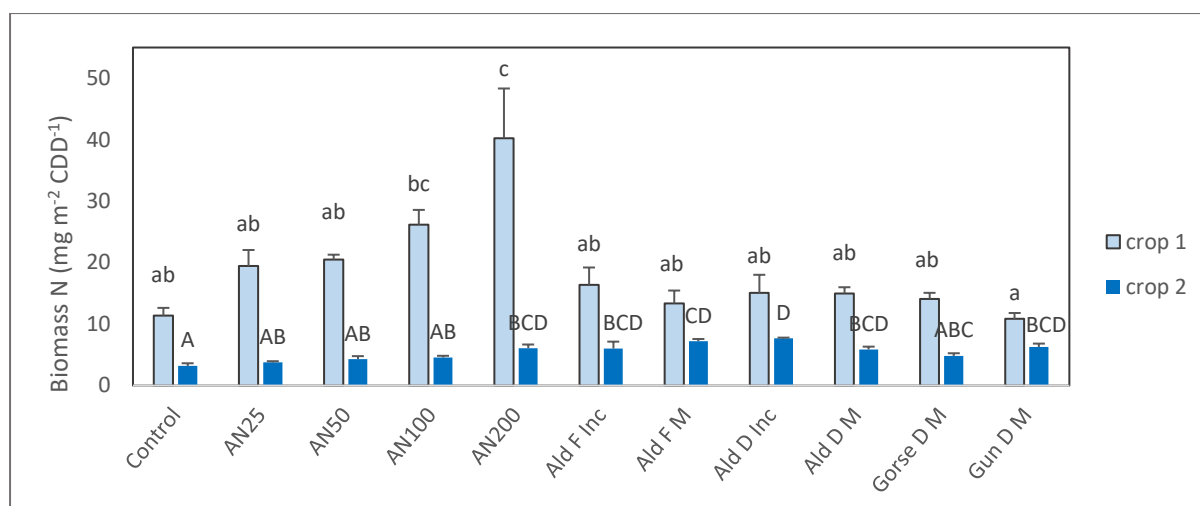


Fig. 5.6. Total N in above ground biomass from crops 1 + 2 per CDD above 5°C. Means \pm SEM ($n = 4$). Letters denote significant differences between treatments from the same crop ($P < 0.05$).

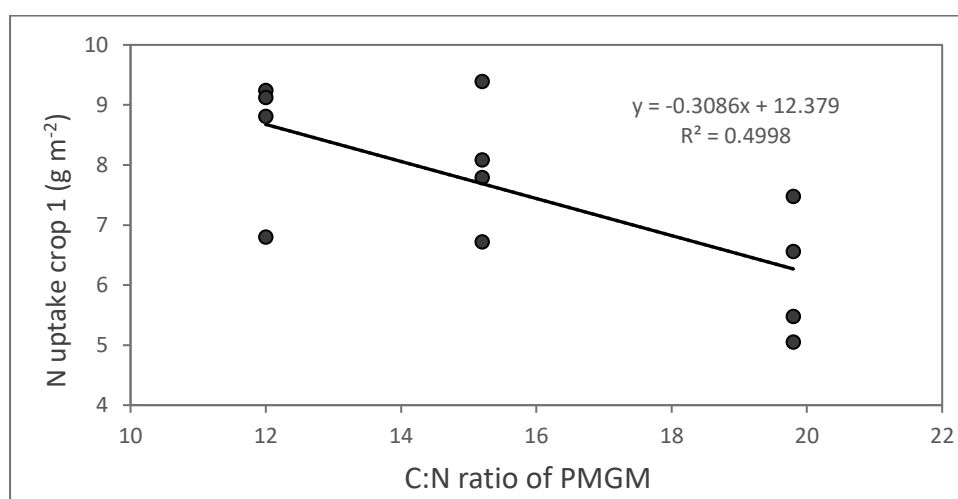


Fig. 5.7. Relationship between the C:N ratios of the PMGMs (dried, mulched), and the N uptake of crop 1 above ground biomass. Data points are individual replicates.

5.3.3 Fertiliser Equivalent values and Apparent Nitrogen Recovery for PMGMs

The FEs for N content of the PMGM treated plants ranged from 14 to 39 kg N ha⁻¹ NH₄NO₃ and the dry weight biomass FEs from 31 to 58 kg ha⁻¹ (Table 5.4). There was a wide variation between replicates for both these measurements which resulted in no significant differences between the FEs of the different PMGMs. The apparent NREs show an uptake by the AN200 treated plants of 92 ± 23 % of the N supplied, but the PMGM treated plants only took up between 10 and 26 % of the PMGM N (Fig 5.8). Over 80 % of the AN200 supplied N was shown to have been taken up during the first 40 days, with only 10 % taken up during the remaining 65 days (Fig 5.9). In contrast the PMGM N uptake shows no such difference in uptake between crops 1 and 2. In crop 2 the alder D Inc treated biomass had the

highest N uptake of 16 % of the N added which was higher than the gorse N uptake of only 4 %, but there were no other differences between treatments.

Table 5.4. FEs of N uptake in biomass for crop 1 and total (crop 1 + crop 2), and total biomass dry weight (crop 1 + crop 2). Means \pm SEM ($n = 4$).

Treatment	Crop 1 N uptake (kg N ha ⁻¹)	Total N uptake FE (kg N ha ⁻¹)	Total Biomass FE (kg ha ⁻¹)
Ald F Inc	24 \pm 13	36 \pm 11	38 \pm 24
Ald F M	10 \pm 10	30 \pm 10	39 \pm 18
Ald D Inc	18 \pm 14	39 \pm 13	58 \pm 19
Ald D M	17 \pm 5	29 \pm 6	51 \pm 15
Gorse D M	13 \pm 5	20 \pm 6	33 \pm 14
Gun D M	-2 \pm 5	14 \pm 5	31 \pm 13

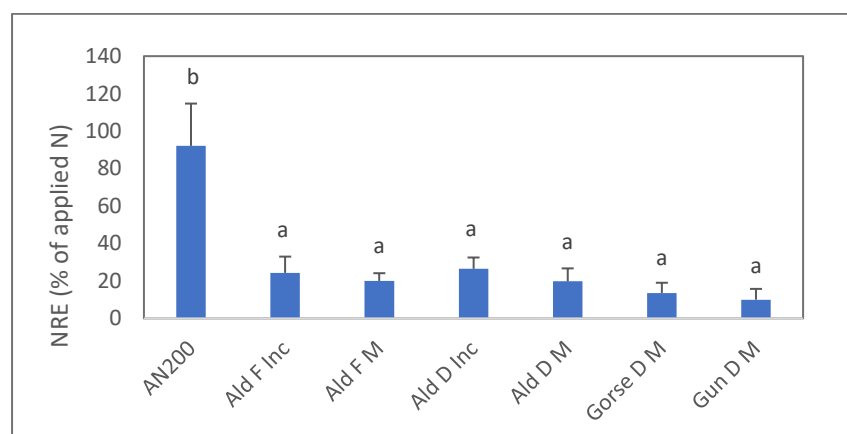


Fig. 5.8. Total NREs in crop 1 + crop 2. Means \pm SEM ($n = 4$). Letters denote significant differences between treatments ($P < 0.05$).

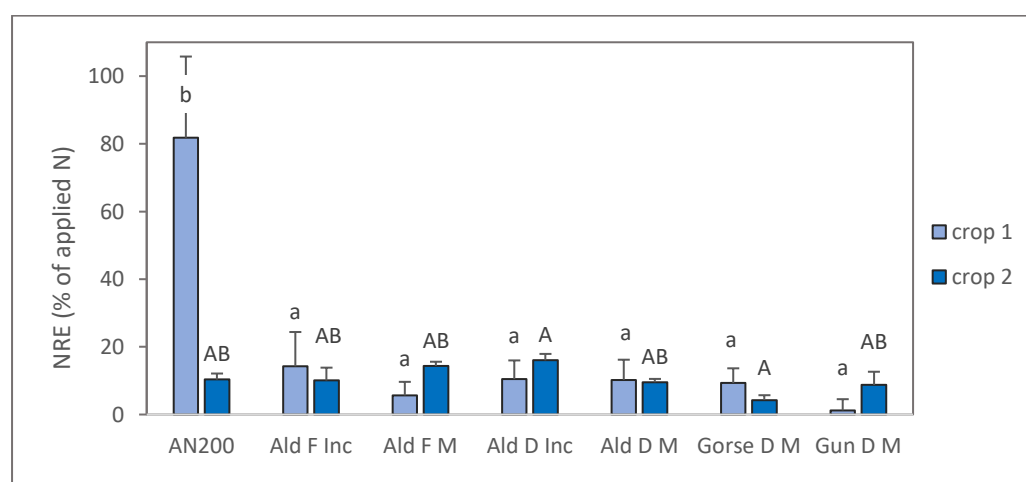


Fig 5.9. NREs in crops 1 and 2. Means \pm SEM ($n = 4$). Letters denote significant differences between treatments within the same crop ($P < 0.05$).

5.3.4 Soil Nitrogen Dynamics

The AN200 treated soil had higher TN concentrations than all other treatments until day 27, after which TN from all treatments dropped to below 50 mg kg^{-1} dry soil ($P < 0.05$) (Fig 5.10). The highest TN concentration was $293.3 \pm 42.4 \text{ mg kg}^{-1}$ dry soil from the AN200 treatment on day 7. On day 41, the alder D Inc treatment had higher TN than all other treatments at $34.5 \pm 3.9 \text{ mg kg}^{-1}$ dry soil, except alder D M, ($P < 0.05$), after which there were no differences in TN between treatments. TN measured in January 2018 (day 209) from samples taken to 0 to 15 cm and 15 to 30 cm showed no differences between treatments, and all samples were below 40 mg kg^{-1} dry soil. All TN data are shown in Table 4, Appendix 2. Soil NO_3^- concentrations were higher in AN200 treated soil than in the control or PMGM treated soil from days 1 to 13 ($P < 0.05$). From day 41 all treatments had NO_3^- concentrations below $10 \text{ mg NO}_3^- \text{ N kg}^{-1}$ dry soil, and there were no differences between treatments (Fig 5.11, Table 5, Appendix 2).

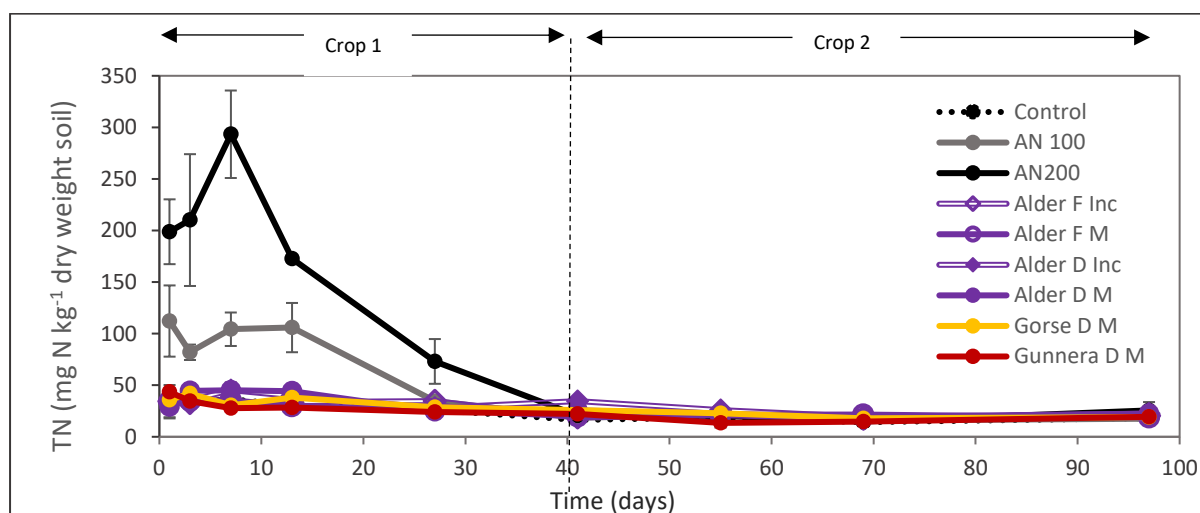


Fig. 5.10. TN concentration in soil at 0 to 10cm depth, throughout the experiment. Means \pm SEM ($n = 4$)

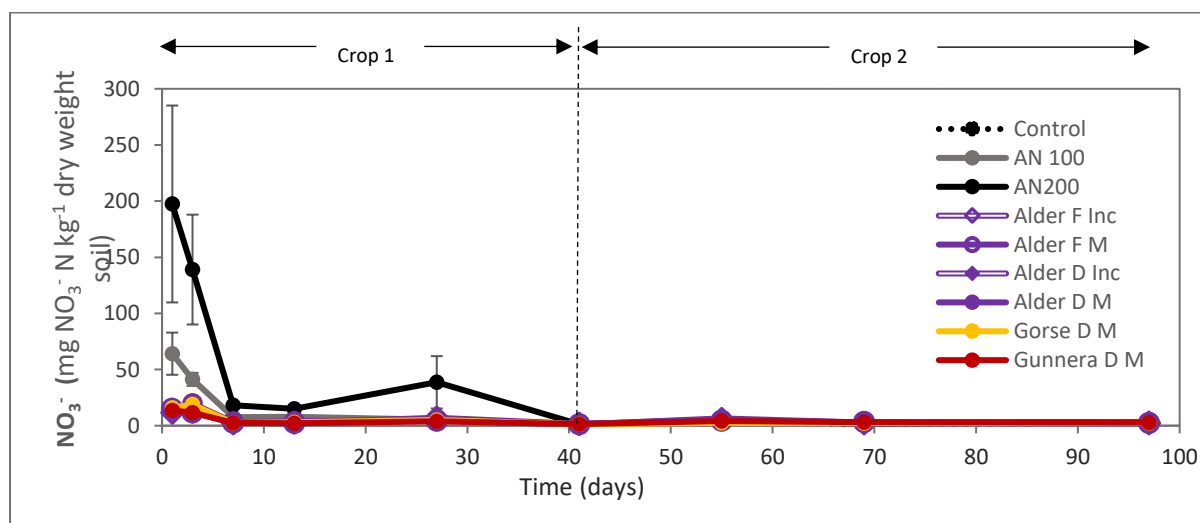


Fig. 5.11. NO_3^- concentration in soil at 0 to 10cm depth throughout the experiment. Means \pm SEM ($n = 4$)

5.3.5 Other soil properties.

DOC concentrations did not vary between treatments (Fig. 5.12). There were no differences in microbial biomass C between treatments measured on day 27 and 98 (Table 8 Appendix 2). Soil water content varied from 19 to 65 % of dry soil and is shown in Table 7, Appendix 2, and the WFPS is shown in Fig. 5.14. It should be noted that the amount of air space may have varied between treatments, and over time due to disruption by tillage and green manure leaf matter addition. This variation will not be evident in the WFPS values which were calculated using an average bulk density of untreated soil and the water content of each replicate, and therefore do not take account of varying soil architecture due to green manure addition or time elapsed since ploughing. The mean daily temperature ranged from 13 °C to 25 °C (Table 10 appendix 2), and the CDD during the growth of crop 1 was 566 °C and crop 2 was 644 °C using the soil surface average above 5 °C.

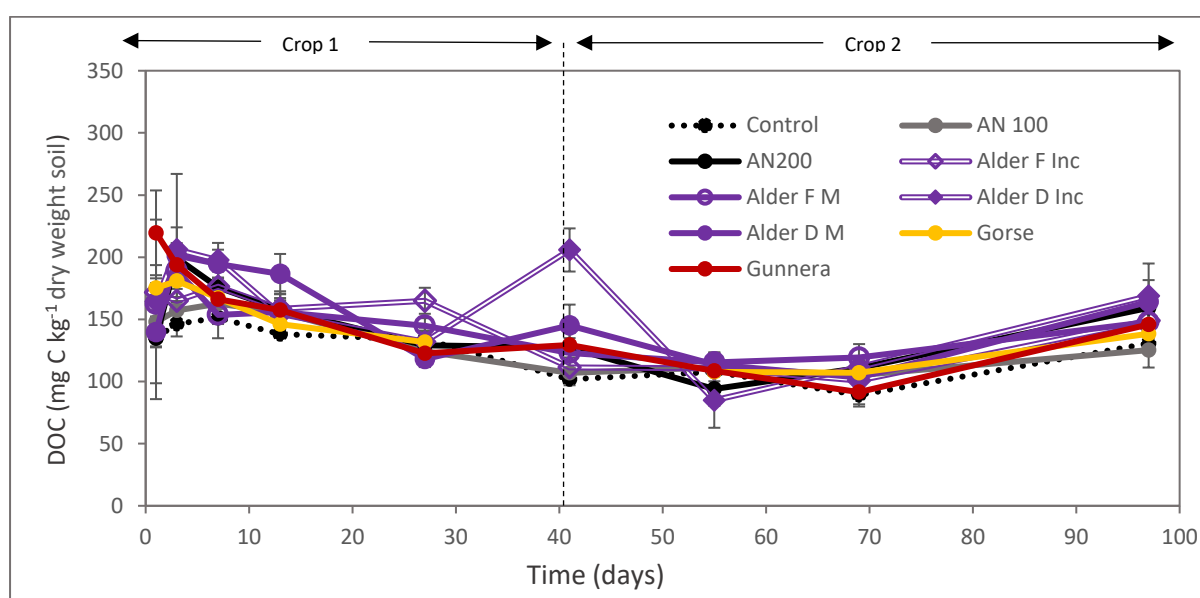


Fig 5.12. DOC concentration in soil throughout the experiment. Means \pm SEM ($n = 4$).

5.3.6 Nitrous oxide emissions

N₂O fluxes are shown in Fig 5.13. Fluxes were highest from all treatments on day 13 and varied more over time throughout the experiment than they did between treatments. All N₂O EFs were below 0.25 % and there were no differences between treatments in the total N₂O-N m⁻², N₂O-N kg⁻¹ dry weight biomass or in the EFs (Fig. 5.15).

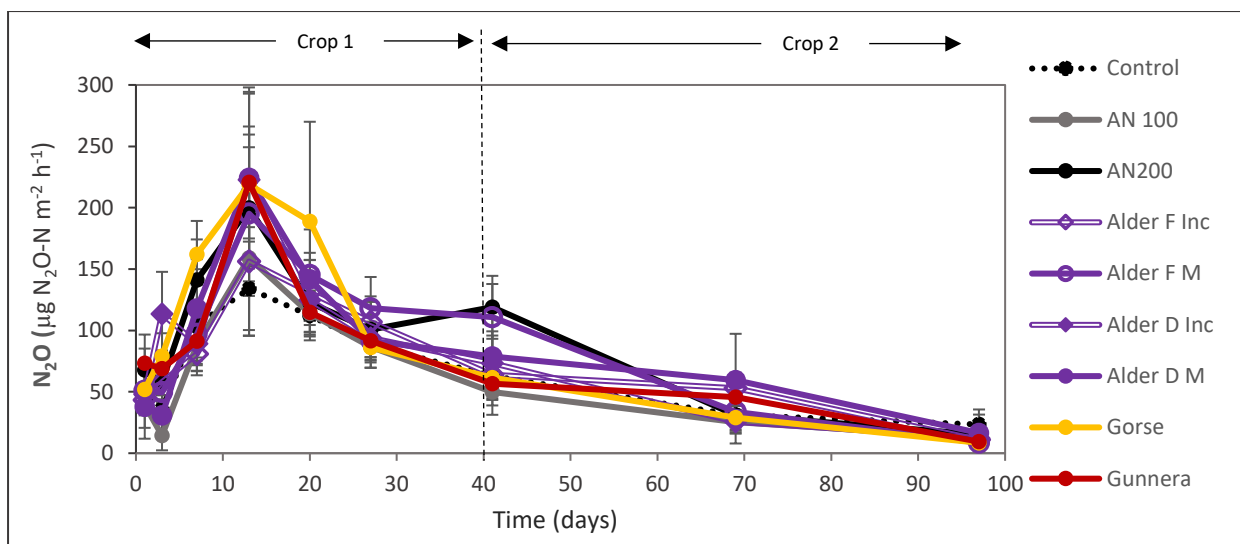


Fig. 5.13. N_2O fluxes from soil throughout the experiment. Means \pm SEM ($n = 4$).

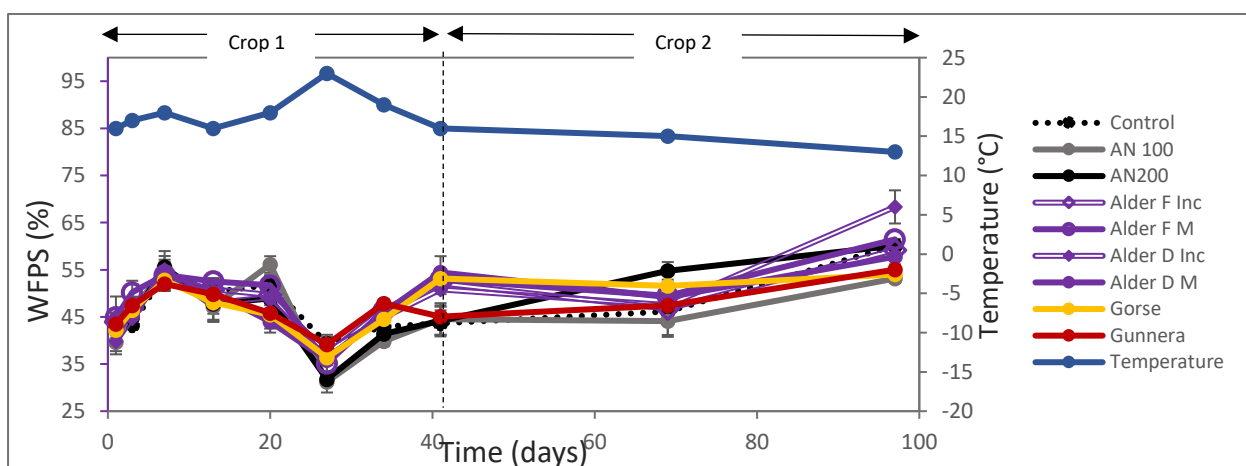


Fig. 5.14. Percent WFPS of soil by treatment (means \pm SEM, $n = 4$), and air temperature for each gas sampling point throughout the experiment.

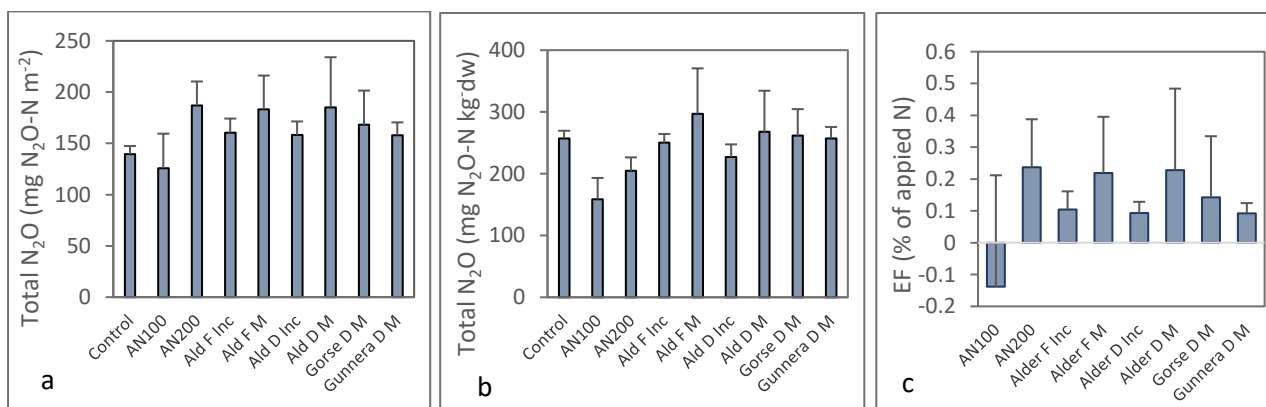


Fig. 5.15. Total cumulative N_2O emissions over the growing period (crop 1 + crop 2) by a) on an area basis, b) yield-scaled intensity, by dry weight of biomass, c) EF (Means \pm SEM, $n = 4$). No significant differences between treatments.

There was no relationship between the C:N ratio of the green manure and the N₂O emissions. The largest influence on the N₂O fluxes appears to have been the wider environmental factors, over any effect of treatment. On day 13, when N₂O was highest, a multiple regression of N₂O against H₂O, DOC, and NO₃⁻ showed H₂O as having the only significant effect ($P = 0.03$), and this accounted for 17 % of the N₂O variability. A multiple linear regression of WFPS, DOC, NO₃⁻ and temperature against N₂O fluxes throughout the experiment, showed temperature to be the most significant factor, ($P < 0.001$) with no effect of NO₃⁻ concentration (Table 9, Appendix 2). N₂O fluxes increased with temperature by 8.33 µg N₂O-N m⁻² h⁻¹ for each additional 1 °C, and by 1.2 µg N₂O-N m⁻² h⁻¹ for each additional 1 % soil WFPS ($P < 0.05$). Emissions also increased with soil DOC concentration by 219 µg N₂O-N m⁻² h⁻¹ for each g C kg⁻¹ soil ($P = 0.05$). These factors together accounted for < 10 % of the N₂O emissions ($R^2 = 0.087$).

5.4. Discussion

5.4.1 N supply to the crop

The PMGM treated soil did not produce higher total plant biomass or total plant N uptake than the control soil, and the N uptake rates from the PMGMs were lower than those in the pot experiment. The FEs show that the plant biomass produced from the PMGM treatments was less than that which would have been produced by the addition of 60 kg ha⁻¹ N supplied as NH₄NO₃. The crop N uptake from the PMGMs was less than that which would have been gained from the addition of 40 kg ha⁻¹ N supplied as NH₄NO₃. Comparison of N uptake with those in the initial period of the pot experiment shows lower rates of N uptake in the field experiment, with FEs for N uptake at the end of the field experiment (after 1279 CDDs), of 14 ± 5, 20 ± 6 and 29 ± 6 kg NH₄NO₃ N ha⁻¹ for gunnera, gorse and alder respectively (dried mulched), compared to 18.3 ± 7.2, 34.1 ± 1.4 and 49.8 ± 6.8 kg ha⁻¹ after 1100 CDDs (94 days) of the pot experiment (Table 3, Appendix 1). FEs for fresh or incorporated materials were no higher.

These comparatively low N uptake rates are despite the PMGMs of the field experiment having lower C:N ratios than those of the pot experiment, with decomposition rates known to increase with lower C:N ratios of plant material (Trinsoutrot et al. 2000, Nicolardot et al. 2001, Chaves et al. 2004). One likely cause of slower decomposition is the dry conditions of the field experiment in which for most of the experiment the WFPS was less than the optimal of 55 to 60 % for plant matter decomposition (De Neve and Hofman 2002, Whalen 2014). The response to N input may also have been limited by a sub optimal pH, which was measured at 5.5 before the addition of Calcifert. Though measurements taken the following spring (table 4, appendix 3) show pH to have risen to over 5.9, the optimal pH for Phacelia is thought to be in the range of 6.4 to 8.5 (Smither-Kopperl 2018). This could have limited both the uptake of N directly and the utilisation of N due to limitation on growth by a sub-optimal uptake of other nutrients.

Though the N uptake during the field experiment was low, the comparative rates of N uptake between the different PMGMs follow a similar pattern to those in the first part of the pot experiment. As in the pot experiment there was a negative correlation between the initial N uptake (crop 1) and the C:N ratio of the PMGMs (dried mulched). In both the pot and field experiments, the gunnera treated plants initially had the lowest N uptake rates of the green manures which were lower than or equal to that of the control, the gunnera FE of the first crop of the field experiment after 566 CDDs being -2.5 ± 4.6 kg ha⁻¹ and that of the pot experiment after 704 CDDs being 2.6 ± 6.4 kg ha⁻¹ (Table 3, Appendix 1). The field experiment PMGMs also provided a delayed supply of N relative to that from the NH₄NO₃, with crop 2 gaining relatively more N from PMGMs than crop 1. Therefore, although the rates of N

uptake are different, the pot experiment data appear to give a useful indication of the relative effects of the PMGMs in the field, despite the differing environmental conditions, and can be used to estimate the likely effect of PMGMs in the field over a longer period than the 105 days of this experiment. The biomass production, and N uptake from gorse treated soil followed the same trends as in the pot experiment, and gorse was the only PMGM treatment not to result in higher N uptake than the control in crop 2. This gives further support to the theory that there is an aspect of the gorse leaf material which prevents N uptake, for example an allelopathic response as discussed in section 3.4.3. Though a longer growing season would have given more useful data, it can be concluded that gorse is a less beneficial PMGM than alder and gunnera.

NH₄NO₃ treatments resulted in fast N uptake as expected, with an N content of the plant tissue of crop 1 of 3.6 % from the AN200 treatment, compared to 2 % in crop 2, (Table 2, Appendix 2) and elevated TN soil concentrations in the first two weeks. Therefore, a split N supply of two or more applications over the growing season is likely to provide a more efficient use of N reducing the risk of leaching if heavy rains occurred during the first week. Though in arable farming NH₄NO₃ is usually applied in two or more applications, for some crops, such as late sown spring wheat, up to 210 kg NH₄NO₃ N is recommended to be applied to the seedbed unless the soil is a light sand (AHDB 2018).

5.4.2 Effect of method of PMGM processing and application

There were no differences in plant N uptake or N₂O emissions between the fresh or dried alder, or the method of application (mulched or incorporated), nor an interaction effect. As discussed in the introduction, fresh and incorporated material may be expected to decompose faster due to availability of water. Two factors which may have negated this were that the dried alder had a lower C:N ratio than the fresh, and that it had been crumbled to smaller pieces, giving a larger surface area and therefore more access to microbes (Nicolardot et al. 2001, Chaves et al. 2004, Moorhead and Sinsabaugh 2006, Lehtinen et al. 2014). It is also possible that there were not large differences in moisture levels between the mulched and incorporated alder leaves. Rainfall, which occurred on 23 days during the experiment, including 6 days out of the first 10 after sowing, will have dampened the mulched material, and there may also have been a wetting effect of dew. The *Phacelia* plants grew quickly and shaded much of the ground surface and so likely preserved moisture. Previous experiments have found that where mulched plant materials were kept moist, decomposition rates were higher than in drier materials (Schomberg et al. 1994, Iqbal et al. 2015) and Hoyle and Murphy (2011) concluded that abiotic factors, for example, temperature and rainfall had a greater effect on decomposition rates than residue type or level of incorporation.

Decomposition rates of mulched material may also be speeded up by photodegradation during the initial period when the material is not shaded by the crop (Whalen 2014). A proportion of the mulched material will also have been incorporated by soil fauna. It has been estimated that 19 to 24 % of plant residue applied as a surface mulch is redistributed by earthworms into the top 15 cm of soil within one year (Whalen and Parmelee 1999, Whalen 2014). It can be concluded that in the conditions of this field experiment, applying the material as a mulch, or a dried material results in no difference in N supply and can be used to take advantage of the potential benefits to soil health. However, it should be noted that in conditions of lower or less frequent rainfall, or where the crop does not provide the same level of shading, mulched materials may decompose more slowly than incorporated material.

5.4.3 Nitrous oxide emissions

The N₂O emissions from the PMGM treatments (≤ 0.3 % of N added) were less than half of those from the pot experiment. These EFs are similar to those of 0.19 ± 0.08 from residues in the meta analyses of field studies by Charles et al. (2017). The lower EFs than the pot experiment can be explained by the drier conditions, and are within the range given by the IPCC for all N sources in dry climates of 0.5 % with an uncertainty range of 0.1 – 1.1 % (Hergoualc’h et al. 2019). Though the site usually receives a high level of rainfall, with a yearly average of 1,060 mm, and monthly averages in nearby Bangor of 63 mm within each month of June and July, and 83 mm within August (Sánchez-Rodríguez et al. 2018, Climate Data 2020), only 16.43 mm fell during the duration of the experiment resulting in low soil moisture levels.

Unlike in the pot experiment, emissions from the AN treatments were as low as those from the PMGMs. The AN 100 kg ha⁻¹ treatment resulted in a negative EF value, but this was not significantly different to zero, and any negative fluxes were small and likely to be due to instrument noise (Cowan et al. 2014). This contrasts with the high EF of 3.6 % from AN 70 kg ha⁻¹ in the pot experiment. The field experiment soil may have been lacking substrate for denitrification, as the soil NO₃⁻ concentrations dropped quickly after the third day after NH₄NO₃ application, whereas in the pot experiment NO₃⁻ in AN70 treated soil was significantly higher than other treatments until day 25. Though the same amount of NH₄NO₃ per unit of soil surface area was added to the pot and field experiments, the pot experiment had a restricted depth of soil, whereas the field experiment soil had a large depth in which the NH₄NO₃ could diffuse, so reducing the NO₃⁻ concentrations.

The low N₂O fluxes on days 1 and 3 however, when NO₃⁻ concentrations were high in AN70 treated soil suggest that other factors were limiting emissions. The negative relationship between the C:N ratios and the N uptake in PMGM treated soil, was not replicated in the N₂O emissions. Though this indicates that low C:N PMGMs released more N for plant uptake than higher C:N PMGMs, the plant

uptake may have prevented an increase in mineral N concentrations in soil, or any increase in mineral N did not result in increased N₂O emissions because of other factors. The multiple linear regression confirmed that the most significant factor was temperature ($P < 0.001$), followed by WFPS ($P < 0.05$) and DOC concentration ($P = 0.05$). Results from Sánchez-Rodríguez et al. (2018), who conducted a field experiment on an adjacent field during the same year, show similar N₂O emissions averaging at 0.45 % of N applied in “digestate”, the product of anaerobic digestion, and also found that the highest fluxes were related to increases in WFPS.

The WFPS for most of the experiment was considerably lower than the optimum of 70 to 80 % for N₂O emissions (Thangarajan et al. 2013, Butterbach-Bahl et al. 2013). As the soil mostly had a WFPS of less than 60 %, it is likely that more N₂O production would have occurred by nitrification than by denitrification (Bateman and Baggs 2005, Thangarajan et al. 2013, Lan et al. 2017). Where conditions promoting denitrification do occur, N₂O production is increased by high concentrations of NO₃⁻ and DOC, as occurred in the pot experiment (Thangarajan et al. 2013, Hansen et al. 2019, Butterbach-Bahl et al. 2013). Where lack of moisture limits denitrification, a high DOC concentration is less of a risk factor for N₂O emissions. However, even at low a WFPS, hotspots of N₂O production by denitrification can occur in microsites with low O₂ levels due to increased microbial activity promoted by green manure decomposition (Groffman et al. 2009, Li et al. 2016). Here, PMGM treated soil did not have higher DOC levels than the NH₄NO₃ treated soil. This may be because of slow decomposition of the PMGMs, higher background DOC in un-sieved soil due to decomposition of the roots of weeds killed off prior to the experiment, or fast consumption of newly available C by soil microbes (Schlesinger and Andrews 2000, Buchkowski et al. 2015, Arcan et al. 2017). Most of the variability in N₂O emissions is left unexplained by the regressions. Some will be due to background variation which has not been recorded in the data. It is also possible that the pattern of N₂O fluxes which peaked on day 13 was related to the amount of time since soil cultivation, with an increase in microbial activity promoted by increased aeration and disruption of soil structure exposing previously unobtainable substrates (Hansen et al. 2019).

The low N₂O emissions from all treatments of this experiment compared to those of the pot experiment are in agreement with the finding of Chen et al. (2013) that field experiments often record lower N₂O emissions than laboratory or pot experiments. This highlights the importance of the interaction of environmental factors with soil amendments. The majority of a growing season's cumulative N₂O emissions can be produced within just a few short events where suitable environmental conditions and substrate availability coincide (Molodovskaya et al. 2012, Charles et al. 2017). This demonstrates the importance of guarding against the risk of sudden increases in temperature and moisture when both NO₃⁻ and C are available which could create a spike in N₂O.

Considering the pot experiment data, the NH_4NO_3 treatments could have resulted in much higher N_2O emissions if warm, wet weather had occurred, within the first few days. Though the period of elevated NO_3^- lasted for a much shorter duration in the field experiment, limiting the window of opportunity for high emissions, a form of N fertilisation which does not result in greatly elevated mineral N at any one time does negate this risk.

5.4.4 Conclusions and further research

Although 200 kg N ha^{-1} supplied as the PMGMs did not supply as much N to the crop as that supplied as $200 \text{ kg N ha}^{-1} \text{ NH}_4\text{NO}_3$, the N uptake and crop biomass production was in line with that of the pot experiment per CDD. Therefore, we conclude that the PMGMs behave similarly in field conditions and that with a longer growing season N supply to the crop is likely to be effective. As the NREs of the PMGMs were between 10 and 26 % at the end of the experiment, most of the added N will either have remained in soil, including in root tissue (not quantified), or have been lost from the soil-plant system. Losses to leaching were unlikely due to low NO_3^- levels and low rainfall, and the TN levels of the 15 to 30 cm deep soil cores taken on day 209 show no differences between treatments. A proportion of N will have been lost to complete denitrification, and losses may have occurred as NH_3 . However, it is likely that much of the PMGM supplied N remained in the soil and therefore, would act as an N supply to future crops.

The dry weather was likely to have reduced the speed of decomposition, lowering both the effectivity of the PMGMs, but also the potential for N_2O emissions. Low soil moisture can be limiting for soil functioning and crop growth and therefore it would benefit cropping to use PMGMs in conditions of higher soil moisture (Kibblewhite et al. 2008, Knox et al. 2010). As these results confirm the lower uptake of N by gorse-treated plants as found in the pot experiment, gorse is unlikely to be an effective PMGM, and future experiments should continue with alder and gunnera only. Further field research is therefore needed to assess whether in conditions of higher moisture, alder and gunnera soil amendments could supply N to field crops with a lower risk of N_2O emission than fertilisation by NH_4NO_3 . Assessment is now needed to ascertain the N supply to crops by PMGMs over a longer period, and of the benefit of N remaining in the soil at the end of this experiment to crops in future years. Use of a commonly grown crop species would also allow comparison with typical UK farm yields. To give a more complete N budget the loss of N to NH_3 volatilisation needs to be quantified, as well as the biomass and N uptake into crop roots.

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Chapter 6: Effect of two years of PMGM additions on crop N fertilisation and climate change impact: A continuation of the field experiment

Contributions: DRC conceived the experiment. CW, DRC and PWH designed the experiment. Experimental work, data processing and statistical analysis was performed by CW. CW wrote the chapter with advice and edits from PWH and DRC.

Abstract

This continuation of the field experiment investigated the cumulative effect of two yearly additions of PMGMs. Additions of organic matter to soil can affect soil properties for many years, influencing the provision of N to crops and gaseous N emissions. The limited N uptake from PMGM treated soil during the first year of the field experiment, combined with the data on N uptake over a whole year from the pot experiment indicated that a substantial amount of PMGM-derived plant available N could be remaining in the PMGM treated plots. The combined pot and field data also indicated that over a longer growing period than that in year one, a field grown crop supplied with a PMGM could achieve a higher fertiliser equivalent (FE). Therefore, it was hypothesised that the combination of N supply from a second addition of PMGMs as well as N remaining from year one, could effectively fertilise a crop of wheat.

In a split plot experiment, we measured dry biomass production, N uptake, N_2O and potential NH_3 emissions from a second application of 200 kg ha^{-1} of alder, gunnera and mineral N to one half of each plot previously treated with the same amendments, as well as the other half with no subsequent treatment. Alongside this we tested repeated applications of the same range of ammonium nitrate application rates used in year 1, and again calculated FEs and NREs. There were no effects of the year 1 additions only on N uptake compared to the control which had received no additions in either year. However, application of gunnera in both years resulted in a FE over the two years of 70 kg N ha^{-1} of NH_4NO_3 N, compared to 14 kg N ha^{-1} in year 1 only ($P = 0.065$). These data indicate an interaction effect of the two gunnera additions, which may be due to the second year's amendments promoting N release from previously undegraded or stabilised material added in year 1, or that the material added in the second year degraded faster due to the presence of the previously added material; a "home field advantage" effect.

N_2O emissions from the PMGMs were low, with average emission factors (EFs) of $< 0.4 \%$, but due to dry weather, as in year 1, the effect of PMGMs in the field in wetter conditions is still unknown. Potential NH_3 emissions were also low during the first 26 days, after which data were not available. These results show a promising long term fertilisation value of PMGMs, but provision of N must be

considered in relation to the overall climate change mitigation value when GHG gas emissions and effects on soil C are also taken into account.

6.1 Introduction

Crops can continue to take up nutrients derived from green manures for years after the addition to soil (Lupwayi and Soon 2015, Celestina et al. 2019), and alterations of the soil characteristics due to amendments can affect N dynamics, crop growth and GHG emissions in future years (Pugesgaard et al. 2017, Hansen et al. 2019). Therefore, measurements taken in the year of addition only give a partial picture of the overall contribution to N supply. In this second year of the field experiment we aimed to assess the crop production, N fertilisation value and N₂O emissions from two successional years of PMGM treatments, as well as the effect of the current year's additions over a longer growing season than in the first year. A second year of the field experiment also allowed measurement of NH₃ emissions and root N uptake giving a more complete N budget than in year 1.

6.1.1 Long term N fertilisation effect of green manures

The rate of release of plant available N is highly variable and influenced by the characteristics of the material, as well as environmental factors. Uptake of N when over a year has past since the addition can be very low, for example, Janzen et al. (1990) found that less than 2% of N added as green manure was recovered in the second year of wheat production and Ovsthus et al. (2017) found no effect from organic fertilisers in the second year after addition. However, Hu et al. (2015) found that N uptake increased over time from high C:N ratio (51.9) maize residues from only 3.2 % in the first year to 7.1 % of residue N in the third year after addition. High N forage pea residues were found to result in a much higher crop N uptake, but declining over time with 45 % uptake in year 1, 20 % in year 2 and a further 10 % in the third year (Lupwayi and Soon 2015).

6.1.2 Potential for N uptake in a second year of the field experiment

In year 1 of the field experiment, plants growing in PMGM treated soil, supplied at rates of 200 kg N ha⁻¹ took up less N than those in soil treated with 200 kg ha⁻¹ NH₄NO₃ N. However, the change in rates of N uptake over time were similar to those found in the same period at the beginning of the pot experiment, which increased from the PMGM treatments relative to the NH₄NO₃ N over time. We therefore hypothesised that during a longer growing season in the field, plant uptake of PMGM N would also increase relative to that from NH₄NO₃ N. That the N uptake from PMGM treated soil in year 1 was limited due to the short growing season, may also have resulted in a significant amount of plant available N remaining from PMGM additions in year 1. This experiment assesses the N fertilisation effect of a second year's application of PMGMs in comparison to NH₄NO₃ N on a crop of spring wheat.

6.1.3 N Supply to a wheat crop

Whereas in year 1, the sowing was delayed until PMGM materials could be harvested from spring growth, adding pre-harvested, dried PMGM material allows an earlier sowing date and therefore a longer growing season. Spring wheat, “Mulika” has a growing season in ideal conditions in the region of 131 days (AHDB 2016), giving a further 26 days for N uptake than in the *Phacelia* in year 1. A healthy wheat crop takes up in the region of 279 kg N ha⁻¹, with the highest rate of uptake during the construction phase, usually between 33 and 71 days after sowing, and some N uptake continues until the crop is ripe (AHDB 2016). For late drilled spring wheat, recommended applications of N range from 0 to 40 kg ha⁻¹ for soils with an SNS index of 6, to 210 kg ha⁻¹ for with an SNS index 0, all of which is applied to the seedbed RB209 (2018). In fertiliser fed cereal crops approximately half of the N taken up by the plants is thought to be derived from N supplied in the current year, with the rest of the N being derived from the soil or other sources such as N deposition (ADHB 2015, Yan et al. 2020). Therefore, for an ideal uptake of 279 kg N ha⁻¹, the PMGMs are required to supply approximately 140 kg ha⁻¹ N.

The N uptake which could occur over the 131 days of a wheat growing period from the PMGMs was roughly estimated by use of N uptake data from the pot experiment, which from alder and gunnera into above ground biomass during the first 129 days was 51 and 34 kg ha⁻¹ for alder and gunnera respectively. The amount of PMGM N remaining in soil from the year 1 additions as estimated by the amount of N added as alder and gunnera minus the amount taken up by the *Phacelia* plants, results in a further 160 and 181 kg ha⁻¹ of N (respectively) which may have been available for plant uptake. There will have been some losses of N since the harvest in year 1 by denitrification, and possibly by leaching. However, the soil TN concentrations measured in January 2018 at 0 to 15 cm and 15 to 30 cm depth, 209 days after sowing were no higher at either depth for alder or gunnera (dried mulched) than for the control, suggesting that there was no or minimal N loss during winter by leaching. It is possible that some N loss by leaching may have occurred in spring before the experiment as temperatures warmed, however a large amount of N was likely to be remaining in the soil. Considering the amounts of plant N uptake which have been found to occur from green manures added in previous years, as noted above, the N uptake from the PMGM treated plots was unlikely to be as high as that from a repeated addition of 200 kg ha⁻¹ of NH₄NO₃ N. However, calculation of FEs and NRE's as in year 1 enabled useful measurements of the effectivity of repeated additions.

6.1.4 Gaseous N emissions

A second year of the field experiment allowed investigation of the effects of two successional additions of PMGMs on N_2O emissions. A second aim was to give opportunity for assessment of N_2O production in the field under wetter conditions than those that occurred with unusually low rainfall in year 1. The pot experiment, in which WFPS was deliberately high at 70 % showed low emissions from PMGM treated soil, in which high NO_3^- and DOC did not occur simultaneously, so preventing all factors necessary for N_2O production by denitrification. In year 1 of the field experiment, DOC concentrations in soil were not higher in PMGM treated soil than in fertiliser treated or control soil. However, it is possible that repeated additions of PMGMs, could cause concentrations of DOC and NO_3^- to rise, which if coinciding with high moisture levels could create conditions for high N_2O production by denitrification.

Loss of N as NH_3 by volatilisation is generally higher from mulched plant material than when incorporated (de Ruijter et al. 2010, Nett et al. 2016). Data on volatilisation from additions of plant material are limited compared to the many studies on that from mineral N fertiliser (Pan et al. 2016). However large losses of N by volatilisation have been recorded (Janzen and McGinn 1991), for example, Larsson et al. (1998) estimated a loss of up to 39 % of N by volatilisation from a grass mulch. In addition to loss of N, volatilised N is deposited elsewhere, where it can be a pollutant if nitrified to NO_3^- or converted to N_2O (Moorby 2007, Reay et al. 2012).

6.1.5 Objectives and experimental design

In this second year of the field experiment, gorse was discontinued due to its lower effectiveness as an N fertiliser and possible allelopathic effect shown in the pot experiment and field experiment year 1. Addition of the PMGMs as mulched and dried material was chosen for the practical advantages, as drying and mulching showed no disadvantages in N fertilisation or N_2O emissions in the first year. Therefore, we continued the experiment using the plots in which alder and gunnera had been added as dry mulched material, alongside the fertiliser response plots. The experiment used a split plot design with and without repeated additions of the treatments. This enabled measurement of the N uptake from the year 1 additions only compared to repeated additions in year 2. As in year 1, we assessed the performance of the PMGMs by calculation of NREs and of FEs, by comparison to the fertiliser response plots. We also took measurements of NH_3 volatilisation and root growth for which there were no data in year 1.

6.2 Materials and methods

6.2.1 PMGM material

Alder and gunnera leaves were collected from live plants in September 2017; alder from Henfaes Research Station, in Abergwyngregyn, (53°14'N, 4°01'W), and gunnera from privately owned land at 52°37'N 3°50' W. They were dried in a well ventilated greenhouse and stored at room temperature. Leaf tissue was analysed for C and N content as in year 1 (Chapter 5, section 5.2.1). Characteristics of the green manure material are shown in Table 6.1.

Table 6.1. Green manure characteristics. Means \pm SEM ($n = 4$)

Plant	Carbon (g kg ⁻¹ dry leaf)	Nitrogen (g kg ⁻¹ dry leaf)	C/N ratio
Alder	472.3 \pm 0.6	28.44 \pm 0.04	16.60 \pm 0.04
Gunnera	375.5 \pm 1.6	16.90 \pm 0.10	22.22 \pm 0.12

6.2.2 Soil and site

The site is described in Chapter 5. The experiment begins on the plots of year 1, which were selected by treatment for continuation of the study: control, AN of various levels, alder (dry mulched) and gunnera (dry mulched). The plots had been left with *Phacelia* stubble, but otherwise bare ground since the sampling and removal of the remaining plants. Soil characteristics varied as a result of the previous treatments.

6.2.3 Experimental set up

Analyses from NRM laboratories (Bracknell, UK) for P, K Mg and pH of samples taken on 23 March 2018, from each plot were used to determine requirements for non N nutrients, and pH adjustment. Nutrient requirements did not differ between the replicates and were applied two days prior to sowing (25/4/2018), at a rate of 100 kg ha⁻¹ Kesperite, 100 kg ha⁻¹ K₂O, and 600 kg ha⁻¹ Calcifert, for a target pH of 6.5 (Calcifert 2013). The plots were then hand cultivated to a depth of 5 cm using a garden rake.

It was possible to begin the experiment earlier than in year 1, as the green manure had been collected and dried from the previous season's growth. A sowing date of 27/04/2018 was chosen to enable a longer growing season than in year 1 (sown on 21/06/17), with more time for PMGM decomposition. However, due to pest damage the season was cut short, and biomass sampling took place on 16th August amounting only 111 days from sowing to harvest. All the plots being continued from year one were divided into two halves giving an area of 1.2 m by 0.65 m. The plots were monitored for the effects of the year one treatment only, and the effect of the same treatment repeated as shown in table 6.2. The AN treatments of 25, 50 and 100 kg ha⁻¹ N were monitored for repeated applications

only to enable calculation of FEs of the PMGMs. Each half of a plot was randomly assigned to either receive or not receive the repeated treatment, with two of each replicate receiving or not receiving additional treatment located on the northward or southward sides of each plot. Treatments were added on 27/4/2018 (day 0) by the methods as set out in year 1 for dry mulched PMGM and NH_4NO_3 .

Table 6.2. Treatments added to plots

Code	Type of amendment	N added in year 1 (kg N ha ⁻¹)	N added in year 2 (kg N ha ⁻¹)	Dry weight of PMGM or NH_4NO_3 (kg ha ⁻¹)
Control	None	0	0	0
AN 25 + 25	NH_4NO_3	25	25	71.429
AN 50 + 50	NH_4NO_3	50	50	142.857
AN 100 + 100	NH_4NO_3	100	100	285.714
AN 200 + 0	NH_4NO_3	200	0	0
AN 200 + 200	NH_4NO_3	200	200	571.429
Ald + 0	Alder (dry, mulched)	200	0	0
Ald + Ald	Alder (dry, mulched)	200	200	7032.040
Gun + 0	Gunnera (dry, mulched)	200	0	0
Gun + Gun	Gunnera (dry, mulched)	200	200	11832.394



Fig 6.1. Two adjacent treatment plots, which had both received the same treatment in year 1, showing developing wheat plants, gas sampling chambers and NH_3 traps.

Spring wheat (*Triticum aestivum*) “Mulika” was sown by hand at a rate of 200 kg ha⁻¹ (with 9 rows per plot, at 13 cm spacings), across all plots, including unmonitored areas. Gas sampling chambers were added to plots as in year 1 (chapter 5, section 5.2.6) no closer to the plot edge than 10 cm and no closer to the adjoining treatment than 15 cm (Fig. 6.1). After sowing, the plots were covered with wire

gabions to exclude predation by Corvids (Fig. 6.2). On day 76 (12th July), all plots were surrounded and covered with netting (approx. 10 mm holes) to exclude rabbits and birds (Fig. 6.3). Throughout the experiment, daily precipitation and hourly temperatures were recorded by automatic sensors located at air and ground levels approximately 100 m from the experimental plot. The cumulative degree days (CDDs) were calculated as the sum of the daily degrees °C above 5 °C.



Fig 6.2. The experimental area, with gabions to exclude rabbits and Corvids.

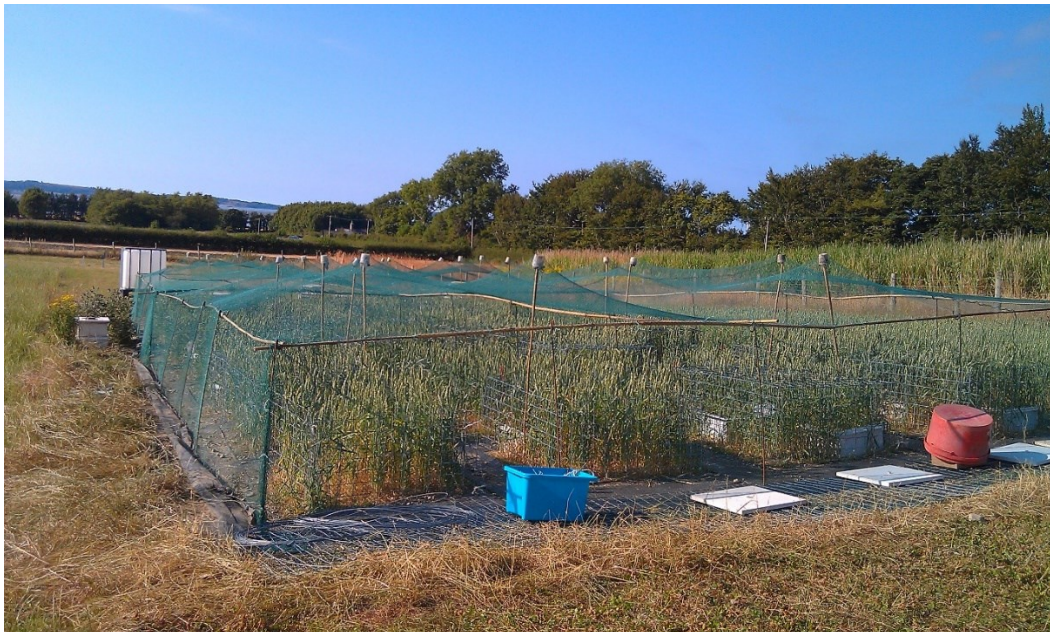


Fig. 6.3. The experimental area with netting to exclude seed eating birds.

6.2.4 Biomass monitoring and analyses

From soon after emergence of the crop, and then approximately every 2 weeks (26, 40, 54, 74 and 97), a range of measurements were taken to assess crop development as follows. Plant height was monitored by selection of five plants at random, ≥ 25 cm away from the adjoining treatment and ≥ 15 cm away from any edge, and measured to the top of the top leaf. Although crop height is often measured to the base of the flag leaf (AHDB 2016), this measure was chosen to allow a measurement of crop growth before stem elongation took place. A measure of leaf chlorophyll content was taken using a chlorophyll meter (SPAD-502Plus Marunouchi, Chiyoda, Tokyo, Japan). Ten plants were selected at random and meter readings taken from the first fully expanded leaf from the top of the plant and about halfway between the tip and base of the leaf, as recommended for wheat by Debaeke et al. (2006). The life cycle stages of the plants were noted according to AHDB (2016).

Biomass was sampled on day 111 (16th August), when grain was almost ripe, and plants at growth stages GS87 to GS91 (ADHB 2016). This was earlier than had been planned due to the observation in early August of small piles of wheat chaff indicating damage by rodents. Samples were taken within quadrats measuring 20 cm by 40 cm which were placed at least 25 cm from any adjacent treatment, and 20 cm from the buffer strip. Counting the numbers of ears and stems allowed an assessment of damage by rodents which typically remove the ear leaving the stem in place. Stems with ears missing were fewer than 2 %, with no difference in loss between treatments. Grain was extracted from the ears, and separated from chaff by hand, and winnowed using air from a fume hood compressed air tap to separate chaff from grain. Root growth of plants was measured under all treatments except AN 25 + 25 and AN 50 + 50 as follows. 8 cm diameter cores were taken at 10 to 15 cm and 15 to 30 cm depth on day 130 (4th September). Over 70 % of wheat root biomass is typically found in the top 30 cm of soil (ADHB 2016). Subsamples of soil from the upper and lower cores were stored at 4 °C for analyses. The rest of the soil was washed from the roots, and roots retained.

All biomass (above and below ground) was dried at 80 °C for over 24 hours and weighed. 20 g subsamples of grain were counted using an automatic seed counter to calculate the thousand grain weight (TGW). Grain and roots were ground using a Retch mixer mill MM 200 (Haan, Germany). Stems and chaff were combined and ground using a FOSS CT 193 Cyclotec mill (Hillerød, Denmark). Biomass C and N contents were analysed as in year 1 (chapter 5, section 5.2.5).

6.2.5 Soil analyses

Soil cores to 10 cm depth were taken on days 1, 3, 7, 13, 20, 27, 41, 69, 97, 116, and 125, as well as to 30 cm depth on day 130 for root biomass as described above. Soil extracts were prepared and analysed for TN, DOC and NO₃⁻ as in year one (chapter 5, section 5.2.4). At the time of writing, analyses

for NH_4^+ has been prevented due to restricted laboratory access caused by the COVID 19 pandemic. The samples taken on day 125 after the wheat had been harvested were fumigated and analysed for microbial biomass as described in section 5.2.4. To gain a measure of “readily mineralisable” N which may be available for crops during a further year of cropping, samples from day 125 were analysed for the amount of N which was converted to NH_4^+ during 7 days in anaerobic conditions (Chadwick et al. 2000, Mariano et al. 2013, McDonald et al. 2014). This was determined by the method described by Schindelbeck et al. (2016) as follows. 25 ml of 1 M KCl was added to 6 g samples of fresh soil. A further set of 6 g samples of fresh soil were incubated in 12.5 ml H_2O for 7 days, after which 12.5 ml 2 M KCl was added so that soil was in a solution of 25 ml of 1 M KCl. Both solutions were shaken for 60 minutes at 200 rev min^{-1} , and centrifuged for 10 min at 2,000 g . The supernatant was filtered and stored at -20 °C until analysis for NH_4^+ as in year 1. The “readily mineralisable N” was calculated by subtracting the concentration of NH_4^+ in the non-incubated samples from that of the incubated samples. To assess N leaching, samples from the cores taken on day 130 at depths of 0 to 15 cm and 15 to 30 cm were extracted and analysed for TN. WFPS was calculated using the soil water content and bulk density.

6.2.6 Gas measurement and calculations

Gas was sampled for N_2O analyses between 10 am and 12 noon BST, on days 1, 3, 7, 13, 20, 27, 41, 69 and 112 by the method described for year 1 in section 5.2.6. By day 69 the wheat plants had grown higher than the chamber walls. To enable sampling, two more chambers were stacked on top of the first and the joins sealed with plastic tape, allowing lids to be fitted to the top chamber. Gas was sampled, analysed and fluxes calculated as in year 1 (section 5.2.6) using the head-space volumes of the chambers above soil level of 0.0326 m^3 and 0.113 m^3 for the single chambers and triple chambers respectively.

The emission factors (EFs) over both years were calculated using total N emissions over the two years of the field experiment. The cumulative N_2O emissions for the control treatment (year 1 + year 2) were subtracted from the cumulative emissions of the treatment replicates (year 1 + year 2, paired by block) and presented as a percentage of N added (De Klein and Harvey 2015). The EFs for year 2 only, from additions of AN200, Alder and Gunnera, were calculated by subtracting the cumulative year 2 N_2O emissions from the treatments AN 200 + 200, Ald + Ald and Gun + Gun, from the cumulative emissions of the treatments with the same type of N added in year 1 only (AN 200 + 0, Ald + 0 and Gun + 0, paired by block) and dividing by the amount of N added. Total N_2O emissions were also calculated per grain yield and per grain N production.

Potential ammonia volatilisation was measured by trapping NH_3 in H_2SO_4 and measuring the NH_4^+ content of the resulting solution as follows. Lidless plastic tubs of 8.5 cm by 28.6 cm, and a height of

6 cm were inserted between the wheat rows, with the opening facing downwards, and pushed 5 mm into the soil trapping the air above a soil surface area of 239.1 cm² (when rounded corners of the tubs were accounted for). 20 ml scintillation vials containing 5 ml of 0.01 M H₂SO₄ were placed inside the chambers. The H₂SO₄ was removed and replaced on days 13, 26, 40, 69, 96, and 112. H₂SO₄ solutions were analysed for NH₄⁺ as in Mulvaney (1996). The amount of NH₃ N emitted from the area of each trap was assumed to be equal to the amount of NH₄⁺ N trapped in solution. From this the potential emissions per m² were calculated and then EFs calculated as for N₂O. Traps with insects in were discarded resulting in fewer reps and missing values in some cases, so that all analyses after day 26 were discarded.

6.2.7 Biomass and N uptake calculations

Total N content of the biomass was calculated by using the % N of the various plant parts (stems + chaff, grain and roots) and the biomass dry weight. Apparent nitrogen recovery efficiencies (NREs) over both years were calculated using the total N which had been added and recovered in both years as follows. The cumulative N uptake of the control plants (year 1 + year 2) was subtracted from the cumulative N uptake of the N fertilised plants (year 1 + year 2, paired by block) and presented as a percentage of N added over both years. NREs for year two only of additions of AN200, Gunnera and Alder were calculated by subtracting the N uptake of the paired replicate which had received the treatment in Year 1, but not in year 2, from the N uptake of the replicate treated in both years and presenting this as a percentage of the N added in year 2.

The Fertiliser Equivalent (FEs) could not be calculated for year 2 of the field experiment alone, as the various AN treatment plots all began with legacy effects from year 1 treatments. Therefore, the FEs were calculated for alder and gunnera for the cumulative effect of year 1 + year 2. A regression was drawn up of total above ground N uptake over both years against yearly addition of NH₄NO₃ N. The regression was used to calculate the amount of NH₄NO₃ N required per year to obtain the equivalent total above ground N uptake which resulted from the Ald + Ald and Gun + Gun treatments.

6.2.8 Statistical analyses

All statistical analyses were performed using SPSS v25 (IBM Corp., Armonk, NY). Data where variance was not homogenous by Levene's test were log-transformed before analyses. One way analyses of variance (ANOVA), and the Tukey's honest significant difference (HSD) test were conducted to determine significant differences between treatments. A repeated measures ANOVA was used to determine differences between treatments in DOC over the whole year 2 experimental period. Two way ANOVAs were used to test for differences between the type of N added and whether N was added in year 1 only or in both years, using the treatments of AN200 + 0, AN 200 + 200, Ald + 0, Ald + Ald,

Gun + 0 and Gun + Gun. Paired t. tests were used to test for differences between the FEs of alder and gunnera treatments in year 1 and the FEs of alder and gunnera added in both years. Relationships between data were analysed using linear regression, and multiple linear regression as appropriate.

6.3 Results

6.3.1 Weather conditions

The CDDs totalled 1475 over the 111 days from sowing to harvest, and there was 83.5 mm of rainfall, which fell on 33 days, with no rainfall between days 55 and 76 (Fig 2, Appendix 3).

6.3.2 Biomass yields

There were no differences between the individual treatments in the total above ground biomass dry weight, or the dry weights of the plant components: grain, stems and chaff, roots, or root:shoot ratio (Figs 6.4 and 6.5, Table 1, appendix 3). A two way ANOVA between the quantity of N added and the type of N added showed a higher grain weight with an addition of 200 kg ha⁻¹ N in year 2, compared to treatments with N added in year 1 only ($P < 0.05$) but showed no differences in grain weights for the type of N added. However, Fig 6.5 shows that the grain weights of alder treated plants are very similar, with or without an additional treatment in the second year, and therefore the additional biomass from two years of treatment is likely to be due to the NH₄NO₃ and gunnera year two treatments. The grain weights were equivalent to a harvest ranging between 5.1 and 7.8 tonnes ha⁻¹, with a thousand grain weight (TGW) between 37.0 and 43.1 g (Table 3, appendix 3).

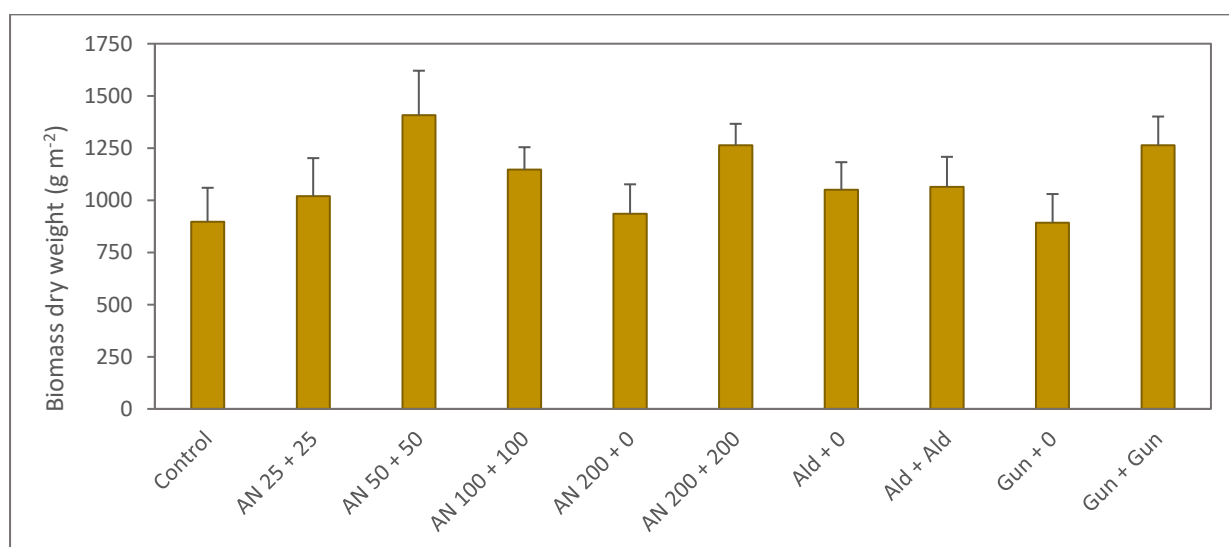


Fig 6.4. Total above ground biomass dry weights of wheat plants harvested 111 days after sowing. Means \pm SEM ($n = 4$). No significant differences between treatments.

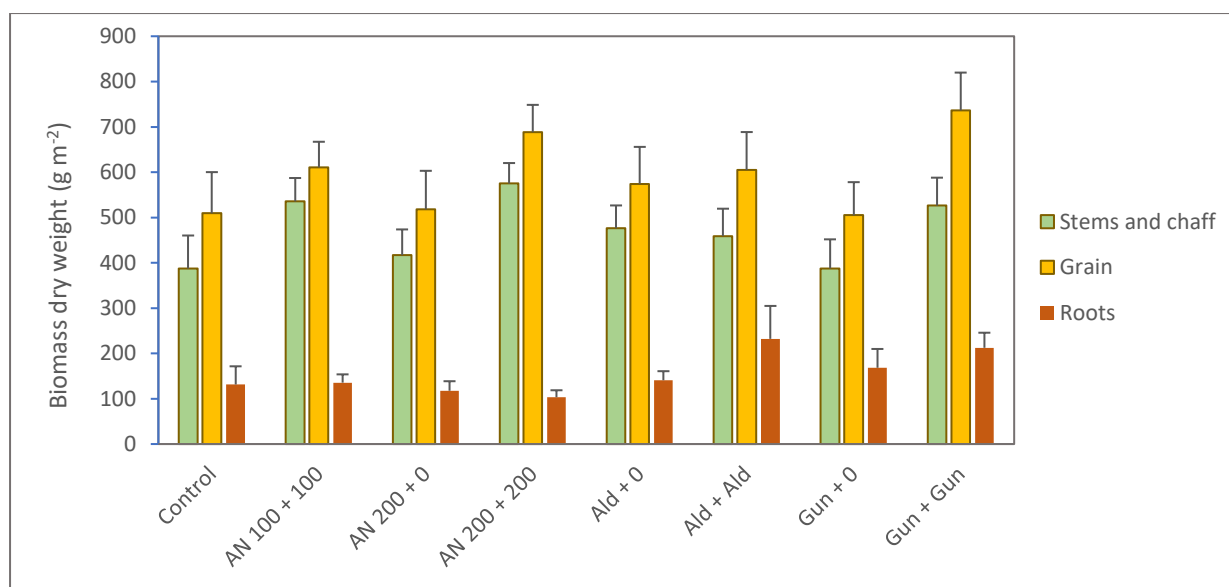


Fig. 6.5. Biomass of dry weights of plant parts harvested 111 days after sowing. Means \pm SEM ($n = 4$). (Root data not available for AN 25 + 25 and AN 50 + 50). No significant differences between treatments.

6.3.3 N uptake

N uptake of the above ground biomass and plant components are shown in Figs. 6.6 and 6.7. A two way ANOVA between the quantity of N added and the type of N added showed that addition of N in both years increased the total N uptake of the wheat plants ($P < 0.01$), and within grain ($P < 0.05$), over that applied in year 1 only, but there was no difference in N uptake between the types of N supplied. However, as for the biomass weights, the increase in N resulting from additions in both years must be due to those from NH_4NO_3 and gunnera as the N uptake between one or both years of alder addition is almost identical. Grain N content ranged from 1.9 % to 2.1 % but with no differences between treatments (Table 2, Appendix 3)

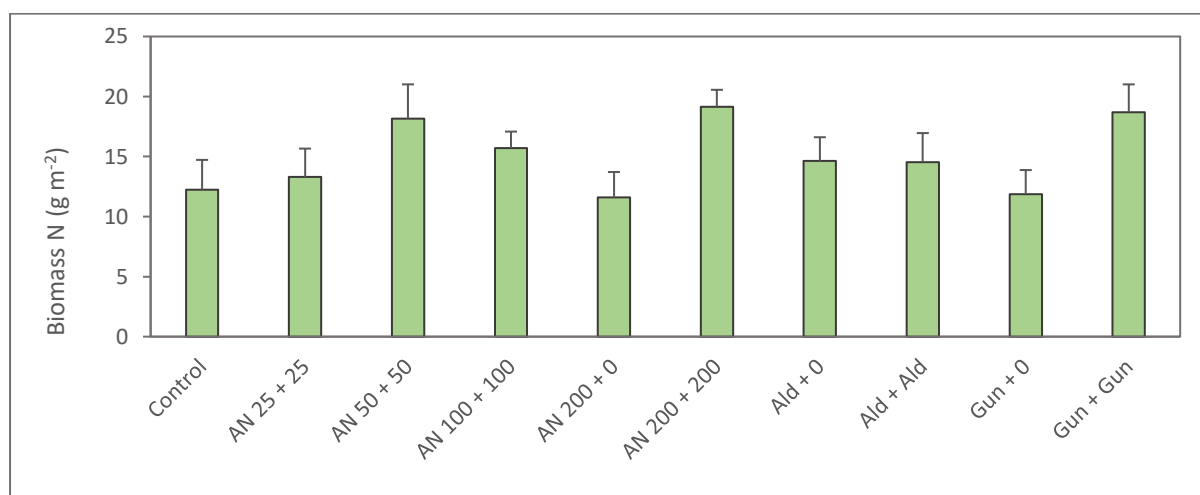


Fig. 6.6. Total N in above ground biomass of wheat plants, 111 days after sowing. Means \pm SEM ($n = 4$).

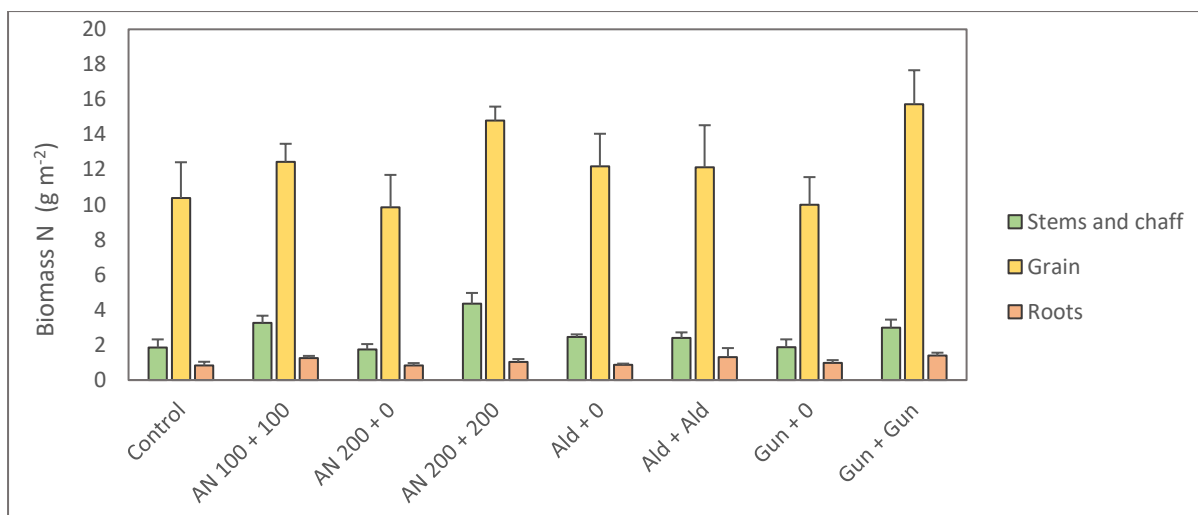


Fig. 6.7. N content of wheat plant parts harvested 111 days after sowing. Means \pm SEM ($n = 4$). (Root data not available for AN 25 + 25 and AN 50 + 50).

6.3.4 N fertilisation effect of PMGMs

NRE values representing the percentage of N supplied over both years which was taken up by plants over both years are shown in Fig 6.8. NREs resulting from the Ald + Ald, Gun + 0 and Gun + Gun treatments were significantly lower than the AN 200 + 0 treatment at the 0.1 significance level.

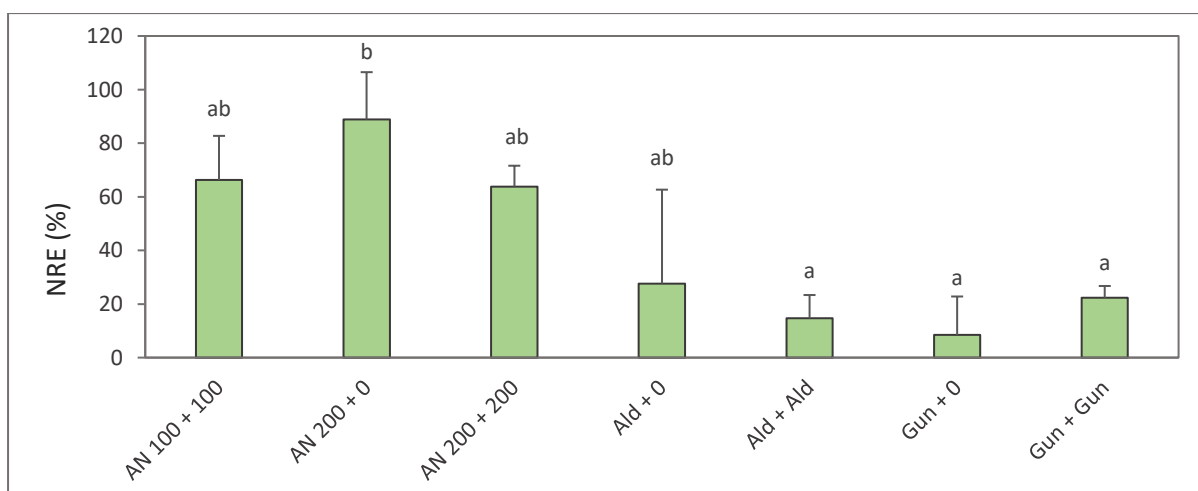


Fig. 6.8. Nitrogen Recovery Efficiencies (NREs). Percent of N supplied taken up into biomass over the two years of the field experiment (Phacelia crop + wheat crop). Means \pm SEM ($n = 4$). Letters denote significant differences at $P < 0.1$.

Fig. 6.9(a) shows the NREs for year 1 only, year 2 only and both years, and 9(b) shows the Fertiliser Equivalents (FEs) for above ground biomass N uptake over both years. There were no differences between the NREs of different types of N supplied in year 2. However, Figs 6.9 a and b indicate a trend of increased N uptake for a repeated addition of gunnera. A paired t.test showed that the FE for the

gunnera treatment over both years was higher at the $P=0.1$ level, than an application in year 1 only ($P = 0.065$), but there was no difference for N uptake over both years compared to in year 1.

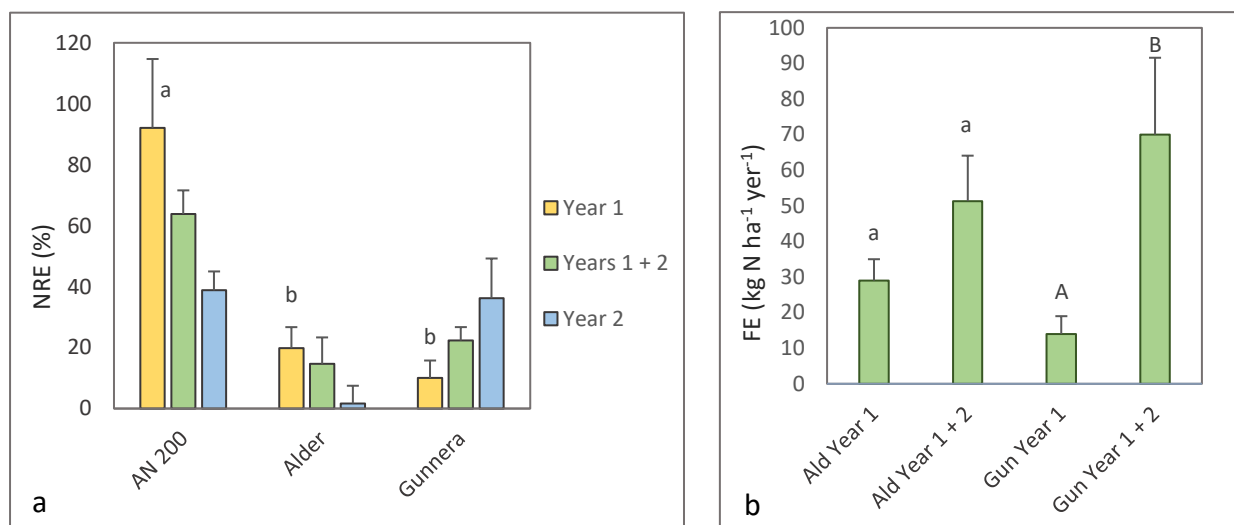


Fig. 6.9. Comparison of N uptake during years 1 and 2. **a)** Nitrogen Recovery Efficiencies (NREs). Percent of N supplied taken up into above ground biomass in year 1 only, in year 1 + 2, and in year 2 only. Means \pm SEM ($n = 4$). Letters denote significant differences at $P < 0.05$. There were no significant differences between treatments in years 1 + 2 or year 2. **b)** Fertiliser equivalents (FEs) for year 1 only and over both years. Means \pm SEM ($n = 4$). FEs represent the amount of NH_4NO_3 N required per year to produce the same amount of N uptake into the above ground biomass as the addition of 200 kg ha^{-1} N supplied as gunnera or alder. Letters denote significant differences between year 1 only and year 1 + 2 at $P < 0.1$

SPAD readings, a measure of leaf chlorophyll content are shown in Fig. 6.10. The treatment of Gun + Gun showed a different pattern in leaf chlorophyll as measured by SPAD readings, with a lower SPAD than all other treatments (except AN25; data not shown) on day 26, and lower than AN 100 + 100 and AN 200 + 200 on day 40 (Fig. 6.10; $P < 0.05$). On days 54 and 74 however, the Gun + Gun leaf SPAD readings were no different to those from AN 100 + 100 and AN 200 + 200. On day 74 only the control and AN 25 + 25 had lower SPAD readings than AN 200 + 200. There were no differences in the growth stages of plants between treatments when surveyed.

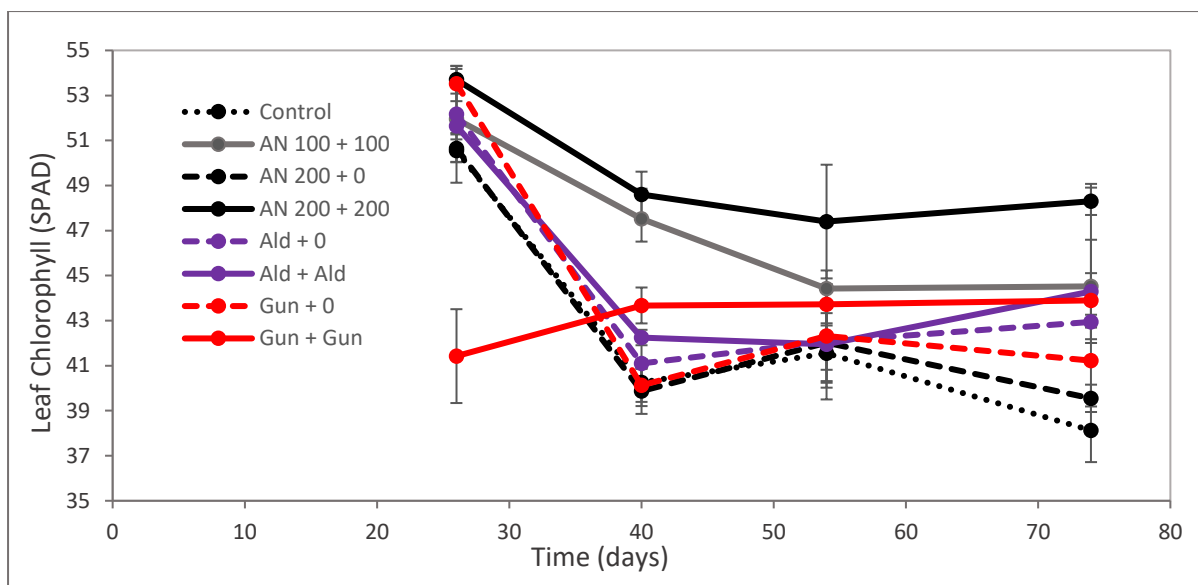


Fig 6.10. Leaf chlorophyll as measured by SPAD meter readings representing chlorophyll content. Taken from the highest fully expanded leaf of 10 randomly selected plants within each plot. (Means + SEM, $n = 4$).

6.3.5 Soil properties

Soil NO_3^- and TN are shown in Figs 6.11 and 6.12 respectively. AN200 treated soil had higher NO_3^- concentrations than PMGM treated soil until and including day 7, and higher TN from days 3 to day 27. TN, measured at depths of 0 to 15cm and 15 to 30 cm, after the wheat harvest on day 130 was under 35 mg kg^{-1} for all measurements and there were no differences between treatments or soil depth (Table 6.3). There were no differences in readily mineralisable N measured on day 125 (Table 6.3).

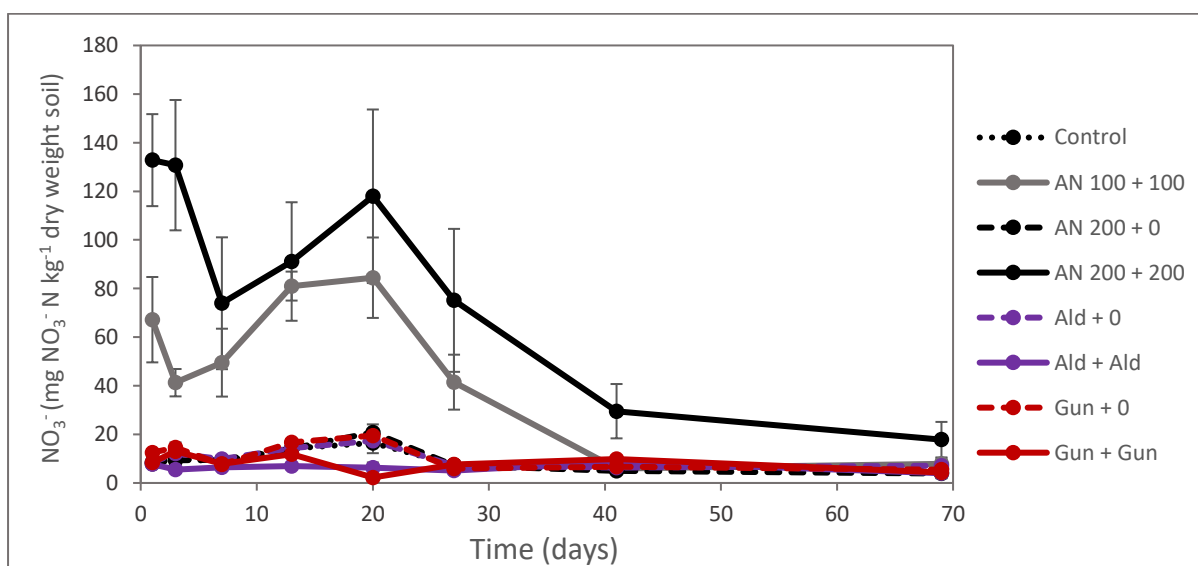


Fig. 6.11. NO_3^- concentration in soil to day 69. Means \pm SEM ($n = 4$).

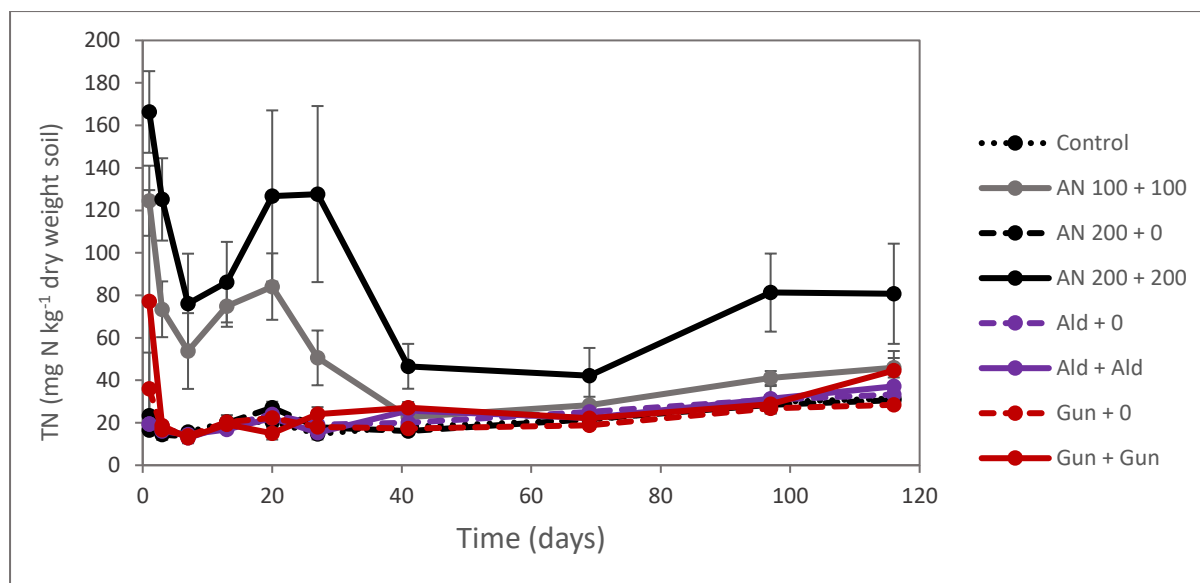


Fig. 6.12. TN concentration in soil throughout the experiment. Means \pm SEM ($n = 4$).

Table 6.3. N content of soil after wheat harvesting. TN sampled on day 130, and readily mineralisable N on day 125. Means \pm SEM ($n = 4$). No significant differences.

Treatment	TN 0 to 15cm depth (mg kg ⁻¹ DW)	TN 15 to 30 cm depth (mg kg ⁻¹ DW)	Readily mineralisable N 0 to 10 cm (mg kg ⁻¹ DW week ⁻¹)
Control	23.0 \pm 1.3	22.7 \pm 1.6	28.8 \pm 6.4
AN 25 + 25	20.6 \pm 8.1	27.2 \pm 4.2	N/A
AN 50 + 50	24.7 \pm 3.3	30.4 \pm 5.6	N/A
AN 100 + 100	24.9 \pm 7.8	30.4 \pm 2.5	36.7 \pm 2.3
AN 200 + 0	22.3 \pm 1.5	22.5 \pm 1.0	25.2 \pm 9.3
AN 200 + 200	29.5 \pm 4.3	31.3 \pm 3.2	87.6 \pm 64.6
Ald + 0	27.0 \pm 3.3	26.9 \pm 4.0	125.2 \pm 68.5
Ald + Ald	24.0 \pm 1.1	25.1 \pm 2.2	61.8 \pm 17.4
Gun + 0	22.3 \pm 1.3	23.1 \pm 1.2	23.2 \pm 2.5
Gun + Gun	26.7 \pm 4.3	32.3 \pm 7.3	56.0 \pm 14.3

Additions of PMGMs in year 2 increased DOC concentrations (Fig. 6.13). A repeated measures ANOVA of DOC throughout the experiment showed that the Gun + Gun treated soil had higher DOC concentrations than all other treatments except Ald + Ald ($P < 0.05$). On day 41 Gun + Gun and Ald + Ald treated soil had higher DOC concentrations than the AN200 treated soil ($P < 0.05$). There were no differences in microbial biomass C measured on day 125 (Fig 1, appendix 3). pH was higher in Gun + Gun and Gun + 0 treated soil at above 6.7 than in AN 200 + 200 treated soil where it was 5.7. There were no other differences between treatments (Table 4, Appendix 3)

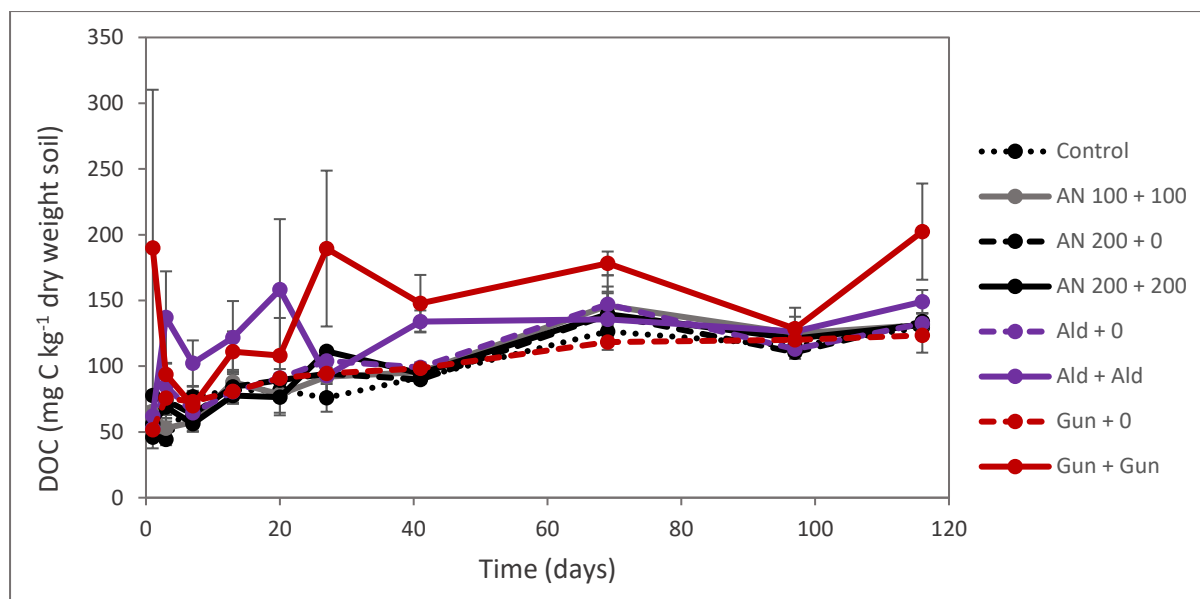


Fig 6.13. Soil DOC concentration throughout the experiment. Means \pm SEM ($n = 4$).

6.3.6 Gaseous N emissions

Potential NH_3 emissions per unit area from AN 100 + 100 and AN 200 + 200 treated soil during the first 26 days, were higher at over $0.4 \text{ mg NH}_3 \text{ N m}^{-2}$ than for all other treatments which were less than $0.15 \text{ mg NH}_3 \text{ N m}^{-2}$ ($P < 0.01$, Table 5, Appendix 3). The emissions during the 26 days from all treatments were lower than 1 mg N m^{-2} and the EFs of AN 200 + 200, Ald + Ald and Gun + Gun (paired by block) were all $< 0.01 \%$. Data after day 26 are not available due to insects contaminating the samples.

N_2O fluxes are shown in Fig. 6.14. There were no differences between treatments in the total N_2O emissions by unit area, grain weight or plant N uptake, and no differences in the emission factors (Fig. 6.16 and 6.17). A multivariate regression of N_2O fluxes against WFPS, DOC, NO_3^- and temperature throughout the experiment was significant for WFPS ($P = 0.003$) and temperature ($P = 0.012$), but not for NO_3^- . On average N_2O increased by $1.6 \text{ mg N}_2\text{O m}^{-2} \text{ h}^{-1}$ for each additional % of WFPS, and $4.1 \text{ mg N}_2\text{O m}^{-2} \text{ h}^{-1}$ for each 1°C temperature increase. The predictors accounted for $< 7 \%$ of the variability. Temperature and soil WFPS are shown in Fig. 6.15.

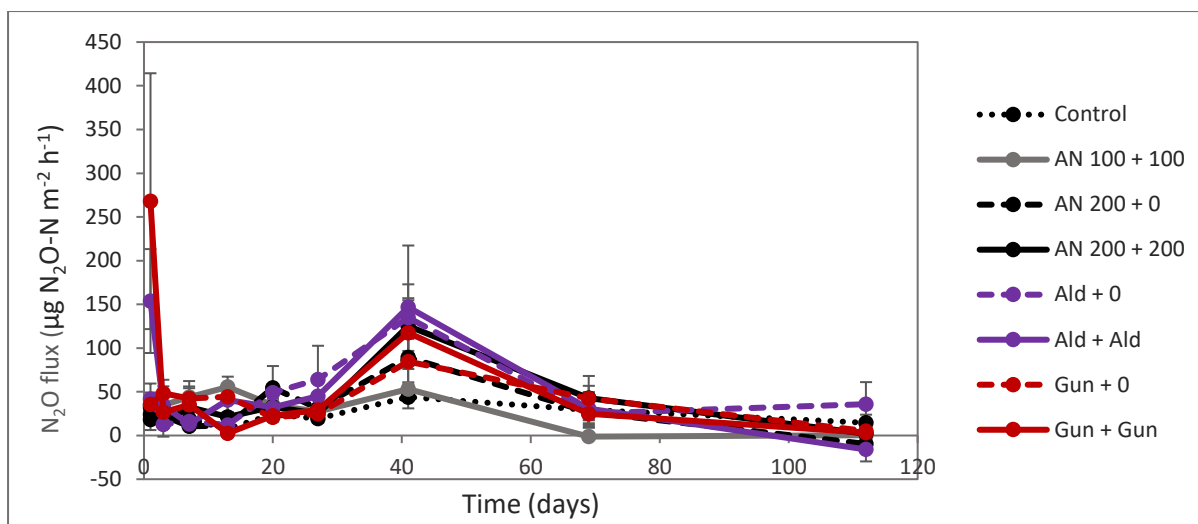


Fig. 6.14. N₂O fluxes from soil. Means \pm SEM ($n = 4$).

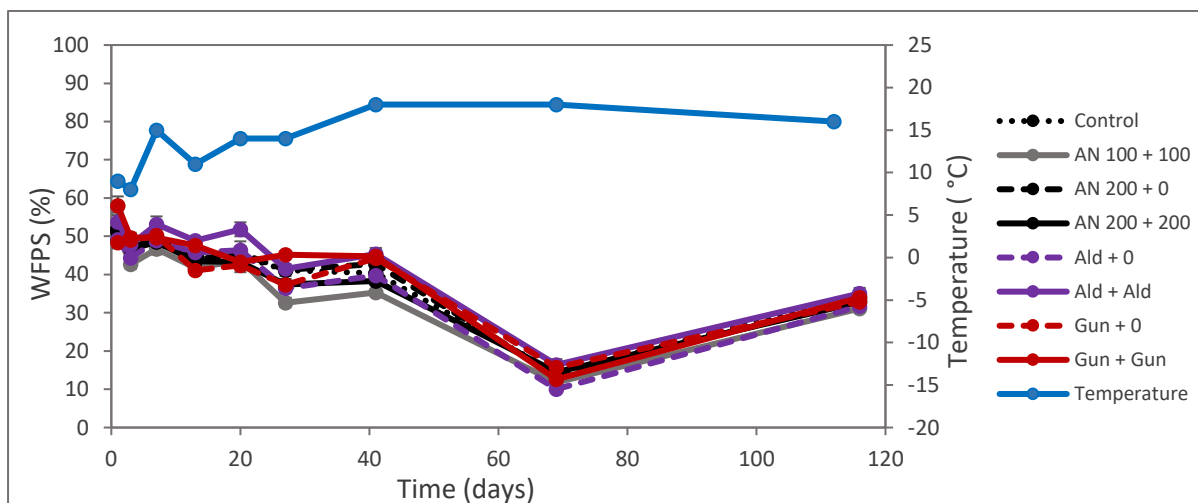


Fig. 6.15. Percent WFPS by treatment (means \pm SEM, $n = 4$), and air temperature for each gas sampling point over the duration of the experiment.

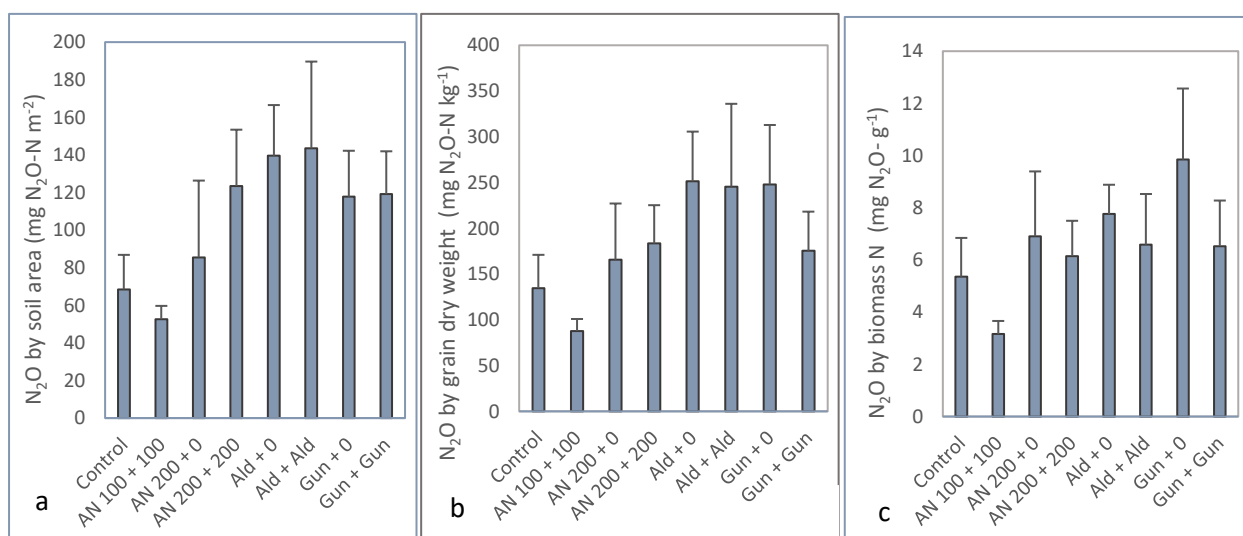


Fig 6.16. Total cumulative N₂O emissions over the growing period by **a)** on an area basis (mg N₂O-N m⁻²), **b)** grain yield intensity (mg N₂O-N emitted kg⁻¹ dry biomass), **c)** by plant N content. No differences between treatments.

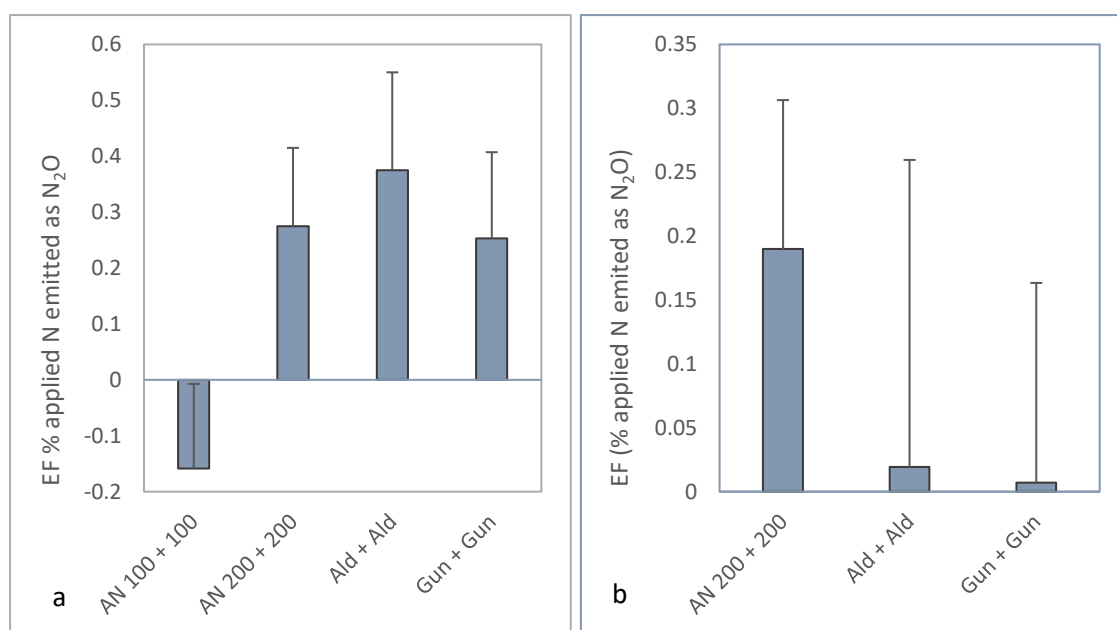


Fig. 6.17. Emission Factors (EFs) (percent of added N emitted as N₂O-N) (Means \pm SEM, $n = 4$). No significant differences. **a)** Two year EFs of N₂O emitted during year 1 + year 2 by treated soil after subtraction of that emitted by the control soil in the same period. **b)** 1 year EFs of N₂O emitted during the second year only by soil which had received repeated treatments in year 2 after subtraction of that emitted during the same period by soil treated in year 1 only. See section 6.2.6 for calculations.

6.4 Discussion

6.4.1 Wheat yield and growing conditions

The grain yields which ranged between 5.1 and 7.8 tonnes ha⁻¹, were below or equal to the average UK wheat yield of 7.8 tonnes ha⁻¹ in 2018 (Defra 2019). This is within the expected range, considering the varied levels of N inputs and that the crop was harvested before full maturity to prevent pest damage. The time from sowing to harvest of 111 days, still amounted to 1475 cumulative day degrees (CDDs), which was an increase on 1279 CDDs in year 1. The average grain yield on an adjacent experimental site under various treatments in 2017 was similar at 7.55 ± 0.44 tonnes ha⁻¹ (Sánchez-Rodríguez et al. 2018). As in the first year of this field experiment, the growing period was atypically dry, with only 83.5 mm of rain falling between sowing and harvest, and none between days 55 and 76. The yearly rainfall average for the site is 1,060 mm and monthly averages in nearby Bangor are 63 mm within each month of June and July and 83 mm within august (Sánchez-Rodríguez et al. 2018, Climate Data 2020). Exceptionally warm and dry weather occurred UK wide (Met office 2018), and this is likely to have reduced yields (Dodd et al. 2011, Webber et al. 2018).

6.4.2 N uptake

The plant N uptake which varied between 110 to 200 kg ha⁻¹, was lower in all treatments than the recommended target of 280 kg ha⁻¹ (ADHB 2016). Limitation of growth by drought, and the early harvesting may have reduced N uptake of that available to the plants, and therefore limited the differential effects of the treatments. If pest damage had not prevented a later harvest, this may have resulted in more significant differences in N uptake, both due to increased uptake of available N, and more time for release of N from PMGMs. The low response of yield and N uptake to the treatments also may be partly due to the soil being relatively rich in N before additions, as shown by the fairly high yield from the control soil. In addition, a slightly suboptimal pH, at a starting point of 6.1 ± 2 (table 4, appendix 3), may have limited biomass yield. Measurements at the end of the growing period show that the addition of Calcifert did not raise pH to optimum levels in all plots, in fact the pH of AN 200 + 200 fell to 5.7 ± 0.1. However, the pH of gunnera treated plots rose to 6.7 ± 0.2 and 6.9 ± 0.2 of Gun + 0 and Gun + Gun respectively and this may have increased the N utilisation of the wheat in the gunnera treated soil in comparison to AN 200 treated soil.

The small size of the crop sampling areas, which was restricted by the sizes of the divided plots combined with the need to avoid edge effects, may also have contributed to the lack of significant differences as variability between replicates is likely to be higher with smaller sampling areas. The leaf chlorophyll (SPAD readings) of Gun + Gun treated plants which were lower than those of the control

plants on day 26 indicate that the gunnera treatment reduced plant N uptake, suggesting immobilisation of N by the gunnera during this time. The delayed N uptake shown by the leaf chlorophyll by Gun + Gun treated plants compared to other treatments repeated the pattern of later N release from the gunnera treatment found in the pot experiment and in year 1 of the field experiment.

6.4.3 Effect of successional treatments

The Ald + 0, and Gun + 0 treatments did not result in higher N uptake than the control, suggesting that there was not a useful amount of plant available N remaining from the year 1 treatments. However, the Gun + Gun FE over both years of 70 ± 22 was higher than the FE of the Gunnera treatment in year 1 alone of 14 ± 5 (at the 0.1 level), indicating higher N uptake from the Gun + Gun treatments than from a single application of gunnera treatment in year one. The trend is also seen when comparing the NREs from both field experiments and the pot experiment after a similar number of CDDs. The year 2 NRE of the Gun + Gun treatment after 1475 CDDs was 36 %, compared to the NRE of gunnera in year 1 of 10 % after 1279 CDDs, and 15.7 % from the single gunnera application in the pot experiment after 1651 CDDs. The higher N uptake in year 2 is despite a higher C:N ratio of gunnera leaves than in year 1 and drier soil in comparison to the pot experiment, both of which would be expected to reduce decomposition speed and N release.

The change in crop species from *Phacelia* to wheat may have affected N uptake. If wheat is better able than *Phacelia* to uptake organic N, this could increase the proportion of N taken up by plants to that taken up by microbes (Ganeteg et al. 2017, Ma et al. 2018). Wheat has been shown to uptake up to 21 % of organic N supplied as glycine (Ma et al. 2018). As far as we are aware there is no research on uptake of organic N by *Phacelia*. Another explanation is that a higher CDD total during year 2 promoted more decomposition of gunnera leading to more plant available N. However, the lower NRE from the pot experiment gunnera treatment after 1651 CDDs suggest this is not the case. Neither of the above theories account for the increase in N uptake from the repeated gunnera additions over that which occurred by repeated alder additions. This therefore suggests an interaction effect of the two gunnera additions that did not occur or did not occur to the same extent with repeated alder treatments.

A disadvantage of difference studies, as used here, is that we cannot distinguish the origin of the N taken up into the plants. Therefore, it is unknown whether the additional N uptake from the Gun + Gun treatment is due to a higher proportion of uptake of the current year's addition, further uptake of the N supplied in year 1, or uptake of N supplied from priming of soil organic matter. The incubation experiment (chapter 4) indicated immediate priming of SOM due to the gunnera treatment in the first week of the experiment, but the low plant N content indicated by the leaf chlorophyll measurements

on day 26 suggests that the Gun + Gun treated plants were not gaining a useful amount of soil derived N due to priming of pre-existing SOM, in this period. Gain of N due to priming of SOM cannot be ruled out, especially later in the growing season, but did not appear to make a significant contribution to N uptake in the gunnera treated plants during the first 26 days.

6.4.4 Effect of year 1 treatments on N uptake from year 2 treatments

It is possible that the PMGMs added in year 2 were decomposed faster due to the presence of the PMGMs added in year 1. An increased speed of decomposition of added material to soil which has received the same material in the past is known as a “home field advantage” effect (van der Putten et al. 2013, Austin et al. 2014, Palozzi and Lindo 2018). Home field advantage can occur when leaf litter enters soil in which the same species of plant as that of the litter is growing. It is also known in soil which has previously received leaf litter of the same species, and this is referred to as a legacy effect (Carrillo et al. 2012, Marschner et al. 2015, Palozzi and Lindo 2018). Previous additions can alter the soil microbiome including crop symbionts, and the soil chemistry, and therefore influence future soil processes (Carrillo et al. 2012, van der Putten et al. 2013).

The timing of N uptake in gunnera treated plants in both the pot and field experiments suggested that an unknown factor of the leaf chemistry affected its decomposition rate, and it is possible, that the leaf chemistry is also contributing to a home field advantage effect. It has been observed that vegetation with a high level of secondary metabolites may degrade faster when in soil where the plants grew than if it is transferred to another environment (Chomel et al., 2016, Paul, 2016). It follows that previous additions of leaf litter containing an uncommon chemistry may cause a home field advantage effect, and microbes are known to specialise in decomposing specific litter types (Li et al. 2020). Therefore, if the effective microbial population was increased due to the gunnera added in year 1, this would promote faster decomposition of year 2 additions. The previous gunnera addition may also affect decomposition due to altering soil chemistry. The higher pH in gunnera treated soil, than AN 200 treated soil found at the beginning and end of year 2, (table 4, appendix 3) could have facilitated increased decomposition of plant litter (Xu et al. 2006, Wang et al. 2017).

6.4.5 Effect of year 2 treatments on N uptake from year 1 treatment remains

Alternatively, it is possible that the year 2 gunnera treatment stimulated further decomposition of the year 1 gunnera remains or priming of gunnera-derived organic matter. This is in keeping with the “like primes like” priming theory described in chapter 4, where an addition with similar chemistry to existing organic matter promotes decomposition of SOM (Fontaine et al. 2003, Liang et al. 2017, Di Lonardo et al. 2017, van der Wal and de Boer 2017). Results of the laboratory incubation experiment

(chapter 4) predicted that over 90 % of alder and gunnera leaf C would be respired within one year. These predictions may not hold true in the field to the same extent, but if a large proportion of the PMGM material added in year 1 was decomposed before the beginning of the year 2 experiment, then N from previously applied PMGM may have been present as PMGM-derived organic N instead of within undecomposed litter remnants. N added to soil within residues has been found to quickly stabilise within soil (Mitchell et al. 2018, Jilling et al. 2020). For example, Hu et al. (2020) found that maize residue N was incorporated into soil organic matter faster than fertiliser N, with 3.5 to 6.7 times more N from maize residues than from fertiliser incorporated into amino sugars in microbial biomass over 5 years. Though the priming experiment showed alder to result in more priming of SOC than gunnera, the treatments may have a very different effect on organic matter derived from their own material than on pre-existing SOM.

Though in this study we can only speculate on the source of the N taken up by plants, a recent meta-analysis by Yan et al. (2020) of N uptake into cereal crops concluded that additions of N in the current year may contribute less to crop N uptake than previously thought. They found that calculations of NREs using the difference method (as used here), showed a higher proportion of N uptake of the current year's applied N, than when the NRE's were measured by isotope studies. For uptake of NH_4NO_3 N, 156 studies by the difference method gave an average of over 50 % of supplied N being incorporated into the crop in the current year, compared to an average of 40 % uptake of the NH_4NO_3 N from 124 isotope studies. The average across the isotope studies found that 29 % of organic N and 42 % of all types of mineral N supplied was taken up into cereal crops within the same year as they were added, and 43 % and 34 % of organic and mineral N respectively was retained in the soil after crop growth. It is possible that a large proportion of N taken up by crops is from stabilised N in soil which originated from previous N additions (Sebilo et al. 2013). In a recent three year study of various crop rotations and organic N inputs Pugesgaard et al. (2017) concluded that applied N which was not taken up by crops was not necessarily lost but contributed to maintaining soil N stocks which later contributed to crop N. Dessureault-Rompere et al. (2013) found that though the amount of plant available N in soil decreased during the growth of a crop, the decline was not large enough to account for the crop N uptake. However, the stocks of readily mineralisable N did not alter greatly, and they suggest that this readily mineralisable N pool is likely to be constantly supplying the pool of plant available N, while continually being replenished itself, for example by residue decomposition. That the readily mineralisable N at the end of our experiment did not differ between treatments is in agreement with Dessureault-Rompere et al. (2013) and therefore the size of this pool may be maintained to some extent regardless of the quantity of available inputs and outputs. The higher variability of the readily mineralisable N pools in the AN 200 + 200 and Ald + 0 treated soils suggests

that these pools were varying either spatially or temporally, which could be due to spatially uneven distribution of the mineralisable alder-derived N, or uneven microbial uptake of the AN 200 over time and/or space. Therefore, soil N may be more important than previously thought for plant N uptake in both fertiliser and organic systems, and Yan et al. (2020) propose a new model in which applications of N are considered as supplies to top up stable N levels, from which the N stocks fertilise crops in future years.

6.4.6 Gaseous emissions

Potential ammonia volatilisation

Losses of N by potential ammonia volatilisation in the first 26 days were low from all treatments at < 0.01 % of N added, and those from the PMGMs were lower than those from AN 200 and AN 100 treated soil. NH_3 emissions from plant material left on the soil surface are usually highest in the first few weeks after addition (Janzen and McGinn 1991, de Ruijter et al. 2010, Nett et al. 2016). However, studies on volatilisation from plant material are few and we cannot discount the possibility that emissions occurred later in the experiment. As the PMGMs have been shown to have a delayed release of plant N, volatilisation may have occurred later when organic N was mineralised to NH_4^+ , and emissions from plant material have been shown to rise after irrigation or rainfall (Janzen and McGinn 1991). Therefore, emissions could have risen if increased NH_4^+ levels occurred to coincide with the rainfall which occurred after day 77. This is a factor to be aware of when adding PMGMs in areas and at times of higher rainfall. Volatilisation is also affected by soil type, as shown by Nett et al. (2016) who recorded high emissions from mulched cauliflower residues in a loamy sand, but not in a silty loam or sandy clay loam. The soil of this experiment is also a sandy clay loam (Sánchez-Rodríguez et al. 2018), and therefore in lighter soils NH_3 loss may be higher. Research to test PMGMs for volatilisation in a variety of soil types, moisture levels and temperatures and over a longer time period would be valuable. In situations where high emissions are a risk, incorporation of the PMGMs is an option which is likely to mitigate N volatilisation (Sommer and Hutchings 2001, Sommer et al. 2004, Rochette et al. 2013).

6.4.7 N_2O emissions

N_2O emissions between the end of the year 1 sampling and the beginning of year 2 sampling were assumed to be low, as in the later weeks of the year 1 experiment, and as has been found in previous experiments in temperate conditions (Bell et al. 2015, Pugesgaard et al. 2017). As in year 1, emissions during the growing period were low, both by soil area and by crop production, and appeared to be influenced more by environmental conditions than by the treatments. The low soil moisture

prevented measurement of N₂O emissions due to PMGMs under high moisture conditions which was also lacking in year 1. WFPS was the most significant factor affecting N₂O emissions, followed by temperature, a reverse of the significance of these factors in year one, which may be due to the generally cooler, drier conditions in year 2. However, temperature and WFPS only accounted for < 7 % of the variability in N₂O emissions, and therefore had a small effect compared to background variability.

Though the Gun + Gun treated soil had higher DOC levels than all other treatments except Ald + Ald, this did not result in higher N₂O emissions. As in year 1 it is likely that the dry conditions prevented a large amount of N₂O production by denitrification. In conditions of high rainfall, however, or where irrigation is necessary for crops which require high soil moisture, raised DOC levels may enable N₂O production. Higher DOC concentrations in soil not only increases the risk of N₂O emissions by acting as an electron donor for denitrification, but can also increase anaerobic conditions due to the provision of C for microbial respiration which can lower O₂ concentrations (Flessa et al. 2002, Mitchell et al. 2013). Therefore, in wetter soils if DOC concentrations are raised by organic amendments, NO₃⁻ levels may become the most important limiting factor for N₂O production, increasing the importance of synchrony between supply and demand of N. Here, DOC concentrations were increased by repeated additions of PMGMs, but not by an addition in one year alone. Therefore, a legacy effect of increased DOC due to PMGM additions, could be a risk factor for N₂O in future years. This was found by Pugesgaard et al. (2017) in a comparison of N₂O emissions resulting from various levels of green manure and fertiliser N additions. They found that the current year's N input had no effect, but N₂O emissions were positively correlated with organic N which had been previously incorporated as crop residues or catch crops, indicating that a legacy of C input was a driver of N₂O emissions.

A challenge is to use organic inputs to increase SOC concentrations for the beneficial effects on soil functioning and climate change mitigation, without increasing N₂O emissions. Fast stabilisation of added C into recalcitrant SOC, if achievable, may serve to sequester C without the risk of higher N₂O. However, much of the role of SOC in soil functioning is due to C turnover by micro- and meso-fauna (Janzen 2015) and so the balance of C turnover and stabilisation needs to be maintained. It is possible that in the long term, repeated additions of organic matter may alter soil conditions so that they are less favourable to N₂O production, by improvement of soil structure and therefore aeration (Graham et al. 2017, Hansen et al. 2019). In one investigation of greenhouse gasses emitted in systems which had been under organic and conventional systems for 34 years, Skinner et al. (2019) found that N₂O emissions were 40 % lower per unit area in organic systems than those under conventional management. In assessing the drivers of N₂O, they found a negative correlation between N₂O and both SOC and microbial biomass C, which were higher in the organically managed systems. Unlike in

studies over a shorter time period, the emissions by crop yield were similar between the organic and non organic systems. Therefore, the risk of N₂O emissions after use of PMGMs may decline over time as the long term addition of organic materials promotes increased aeration which mitigate N₂O production. However, for climate change mitigation, reduction of GHGs in very near future is vital, so consideration of the short term effects of organic matter additions is necessary, even if emissions may become less of a factor in the future.

6.4.8 Strategic use of PMGMs for maximum NRE and mitigation of GHG emissions

With so many inter-relating factors which affect N release and N₂O emissions, prediction of the effects of PMGMs is challenging, however, it may be possible to strategically manipulate some factors to increase the benefits of PMGMs. Greater understanding of the legacy effects of PMGMs would help enable this. For example, increased decomposition of organic matter due to an interaction effect does not always apply to the same type of litter only, and an addition may also speed up decomposition of other plant residues (Carrillo et al. 2012, Barel et al. 2019). Therefore, a possible legacy effect of gunnera may be to increase decomposition rates of other materials, and this could be used to manipulate N release to crops. The type of crop grown also interacts with organic additions to soil. Decomposition of residues has been shown to be affected by the cropping history (McDaniel et al. 2016), as well as the present crops (Wedin and Pastor 1993). Crop type has also been shown to affect N₂O emission (Myrghiotis et al. 2019), so risks of high N₂O emissions could be managed by careful combination of crops and amendments with different risk factors.

Another factor which could be used strategically is the selection of appropriate C:N ratios of the specific PMGMs. C and N contents of plant parts vary over the year's growth due to formation of structural material and mobilisation of nutrients (Gilson et al. 2014, Villar-Salvador et al. 2015). The C:N ratios of the alder and gunnera treatments collected in late summer had higher C:N ratios (16.60 ± 0.04 and 22.22 ± 0.12 respectively used in this experiment, and 15.1 ± 0.01 and 20.5 ± 0.33 respectively used in the pot experiment) than those collected in early summer for year 1 of the field experiment (12.0 ± 0.1 and 15.2 ± 0.2 for dried material). Therefore, choosing the timing of PMGM harvesting could give control over the C:N ratio and therefore N release rates. Use of materials with a lower C:N ratio would also reduce the bulk of materials needed to supply N, increasing practicability of storage and transport.

6.4.9 Conclusions and further research

These results indicate that repeated additions of gunnera and alder PMGMs are unlikely to result in high N₂O pollution when used in dry conditions. However, emissions under wetter conditions in the

field are still unknown and require investigation. Further measurements of NH_3 volatilisation are necessary to generate data on emissions over the whole growing season, and would benefit from more appropriate methods such as the use of wind tunnels (Sommer and Misselbrook 2016). Assessment of gaseous N losses, and potential N fertilisation value need to be considered alongside the effect on soil C, and CO_2 emissions due to priming of soil organic matter.

The increased FE of the repeated gunnera addition is a promising result which could be advantageous in the long term. As this was not quite significant at the $P=0.05$ level ($P = 0.065$), further experimental validation would be useful. Even with small effects on N fertilisation, the accumulated effect of N supplied to a crop from many previous years might make an important contribution to crop nourishment. A difficulty in quantifying effects on soil N stocks by amendments is that N taken up by crops is a small quantity compared to the total N in soil and relatively small changes in N dynamics can have large effects on N availability to plants. To increase N efficiency we need greater understanding of the N dynamics and knowledge of the sources of N supplied to crops (Yan et al. 2020) .

Long term studies using a range of potential PMGM species would be valuable to assess the effects of repeated additions over many years. Isotope studies could elucidate the fates of N from different sources, and the interactions between the additions. For example, measuring the ^{14}C efflux from an addition of labelled gunnera followed by a subsequent addition of unlabelled gunnera, compared to those from unlabelled gunnera followed by labelled gunnera, may elucidate whether there is increased degradation of the first or second additions. Tracing of labelled N could identify the level of uptake of PMGM N compared to soil N uptake, and replenishment of soil N stocks.

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Chapter 7. Synthesis, Conclusions and Outlook

Contributions: CW wrote the chapter with advice and edits from PWH and DRC.

7.1 Aims and experimental work

The core aim of this thesis was to assess the use of PMGMs for agricultural climate change mitigation as set out in Chapter 1. Chapter 2 reviewed the current literature on N and C cycling in agriculture and assessed the environmental benefits and disadvantages green of manure use in comparison with the supply of N by the Haber Bosch process. Both forms of N supply are problematic in various ways, and the unprecedented amounts of reactive N in agricultural systems, causes environmental problems whether the N was originally fixed industrially or by BNF (Smil 2011, Fowler et al. 2013, Battye et al. 2017). Green manures offer the advantages that BNF does not emit CO₂, and that of the input of organic matter to soil. However, these are counteracted by land requirement for BNF and the difficulty in synchronising N supply with demand, which can lead to lower NUEs and N pollution (Crews and Peoples 2005, Chen et al. 2014, Smith et al. 2018).

The supply of N via plant matter harvested from PMGMs grown in permanent areas and added to cropland, could offer an alternative which supplies fixed N without incurring CO₂ emissions, while increasing land use efficiency, and potentially achieving greater synchrony of N supply to crop demand. To evaluate the potential of PMGMs, we assessed their N supply to plants, their effect on N₂O emissions from soil, underlying soil N dynamics, and effect on soil C stocks. Three perennial N fixing plants were chosen for assessment, *Alnus glutinosa* (Alder), *Gunnera manicata* (Gunnera), and *Ulex europaeus* (Gorse).

The pot experiment (chapter 3), assessed the effect of alder, gunnera and gorse plant material containing the equivalent of 200 kg ha⁻¹ N, compared to the same amount of N supplied as the traditional green manure red clover (*Trifolium pratense*) and a range of NH₄NO₃ levels. We undertook measurements of crop growth, N fertilisation, N and C in soil solution and N₂O emissions over 364 days. Evaluation of crop growth, N uptake and N₂O emissions was continued in a field experiment (Chapter 5) over a shorter period of 105 Days. This also assessed the effects of drying and mulching the PMGM material to allow for greater flexibility of application. The second year of the field experiment (Chapter 6) assessed the effect of two successive annual applications of the PMGMs, as well as measuring N loss via NH₃ emissions. To assess the potential effects of PMGMs on SOC, a six week incubation was conducted (chapter 4). Using ¹⁴C isotopes, and a respirometer, this quantified the leaf, soil and total C loss from soils treated with the PMGMs compared to soils treated with clover, and NH₄NO₃, and the C losses over one year were predicted by extrapolating the data. The results

across the experiments on N fertilisation, and potential for climate change mitigation are discussed below.

7.2 N supply by PMGMs

Results from the pot experiment showed effective N fertilisation by PMGMs. Plants grown in gunnera treated soil produced a higher weight of dry biomass than plants fertilised by clover, and those grown in alder and gorse treated soil resulted in equal dry biomass weights to clover fed plants. The NREs of 48 % of the gunnera N and 35 % of the alder N into the plant biomass are higher than typical rates of N recovery from green manures, which are often in the range of 25 to 30% and at the higher end of measured NREs from fertiliser use of 30 to 50 % (Crews and Peoples 2005). It must be noted that most NREs reported in the literature will be measured by crop uptake over a period of months, rather than the year-long growing period of the pot experiment. However, the FEs (Table 7.1) demonstrate the relative value of the PMGMs to fertiliser application and with gunnera contributing the equivalent N supply of 129 kg ha⁻¹ of NH₄NO₃ N, this is a considerable contribution to N fertilisation. Another effect of the gunnera treatment in the pot experiment was that of higher root:shoot ratio over that of plants in NH₄NO₃ or clover fertilised soil. Though the cause of this is unknown, if higher root:shoot ratios were found to commonly occur with application of gunnera, this trait could be taken advantage of, e.g. for root crops, or to increase soil scavenging ability.

Table 7.1 Fertiliser equivalents (FEs) in the pot and field experiments (kg ha⁻¹ NH₄NO₃ N), representing the amount of NH₄NO₃ N required to produce the same crop N uptake. *n* = 5 (pot), *n* = 4 (field). Letters denote significant differences between treatments at *P* < 0.05. There were no significant differences between treatments in the field experiment FEs.

Experiment	Growing period (days)	Clover	Alder	Gunnera	Gorse
Pot (whole plant biomass)	364	144 ± 9 b	95 ± 8 a	129 ± 9 b	90 ± 7 a
Field year 1 (above ground biomass)	105	-	29 ± 6	14 ± 5	20 ± 6
Field year 1 + 2 (above ground biomass)	105 + 111	-	51 ± 13	70 ± 22	-

The PMGM treatments in the pot experiment resulted in a more sustained plant N uptake compared to that from clover or NH₄NO₃ treated soil, and less variability in mineral soil concentration, with peak concentrations of NO₃⁻ of below 25 mg N l⁻¹ compared to over 130 mg NO₃⁻ N l⁻¹ resulting from clover and NH₄NO₃ treatments. These lower NO₃⁻ concentrations represent a lower risk of NO₃⁻ leaching, and reduced substrate availability for N₂O production by denitrification. The rates of N uptake from PMGM

treated soil in the first year of the field experiment, were similar to those during the initial period of the pot experiment. Therefore, the lower FEs in the first year of the field experiment shown in Table 7.1 are to be expected, and over a longer period of time PMGMs could be effective as fertiliser in field soil. Two successional applications of the gunnera treatment resulted in a higher FE over the two years ($70 \text{ kg NH}_4\text{NO}_3 \text{ N ha}^{-1}$) than one application during one year ($14 \text{ kg NH}_4\text{NO}_3 \text{ N ha}^{-1}$) ($P = 0.065$). This suggests that there may be an interaction effect between the two additions causing more release of N from either or both of the gunnera applications.

The method of application of alder as either fresh or dry, mulched or incorporated material in the first year of the field experiment did not show an effect on N uptake or plant growth, and therefore only dried, mulched PMGMs were used in the second field experiment, allowing greater ease and flexibility in application. Measurements of NH_3 volatilisation were not successful in the first year of the field experiment, but during the first 26 days of the second year, they were found to be low from all treatments at $< 0.01 \%$ of N supplied. Results from across the experiments suggested that the gunnera treatment had an immobilisation effect on N. In particular, the chlorophyll meter readings in the second year of the field experiment, indicated lower N uptake by plants in gunnera treated soil on day 26, and in the incubation experiment, a lower TN content was measured in gunnera treated soil than the control after the 42 days ($P < 0.05$) (Chapter 4).

Results from across the experiments indicated a possible allelopathic effect of the gorse treatment. The N uptake from gorse treated soil in the pot experiment was lower than from alder and gunnera treated soil, despite, equal or higher soil NO_3^- concentrations, and in the field experiment (year 1), gorse was the only PMGM treatment not to result in a higher N uptake than the control in the latter part. In the incubation experiment gorse treated soil resulted in lower soil $^{14}\text{CO}_2$ efflux than the other green manures and lower leaf-derived microbial biomass, despite C and N being present in adequate quantities in soil solution. Limited N uptake indicated a possible allelopathic effect on plant functioning and/or microbial action (Pardo-Muras et al. 2018). Due to this, and higher N_2O emissions from the gorse treated soil (see below) it was concluded that gorse was less suitable as a PMGM, and hence this treatment was not used in the second year of the field experiment.

An unknown from the pot and field experiments is how much of the N uptake from treated soil was of pre-existing soil N, which may have been made available for plant uptake due to priming of organic matter stimulated by the additions (Liang et al. 2017, Shahbaz et al. 2018, Kuzyakov et al. 2000). For example, in the alder treated soil of the incubation experiment the predicted amount of C which would be mineralised over the duration of one year from stable SOC was 2.8 g C kg^{-1} dry weight soil, and with a soil C:N ratio of 8.5, this would have mineralised 329 mg N kg^{-1} dry soil. If these levels of priming had

occurred in the alder treated soil of the pot experiment, it would have amounted to N mineralisation of 286 mg N, an amount which is considerably higher than the 169 mg N which was taken up by the alder treated grass over one year. As noted below these levels of priming, which were predicted, not measured, may not occur in soil which contains living plants, but the results highlight the importance of further understanding both the C and N dynamics of plant matter decomposition and plant N uptake.

7.2.1 Matching of N supply by PMGMs to appropriate crops

The slow rates at which N becomes available to plants from treatments of gunnera and alder could be of benefit to vegetable crops which have a long growing season but low initial N demand. This the case for many annual vegetables, and Hartz (2007) estimated that 75 % of N uptake by for cool season vegetables in California is in the last half of the cropping period. As an example, leeks grown in the UK, are not able to uptake large amounts of N until the roots are adequately developed, and it is recommended that only a small proportion of the N required is added to the seedbed (AHDB 2018). The majority of the N uptake by leeks which were sown in April or planted as seedlings in July was found to be between August and October, as shown in Fig 7.1 (Rayn et al 2012). Therefore, supplying a leek crop with green manure N requires a technique which provides most of the N later in the season.

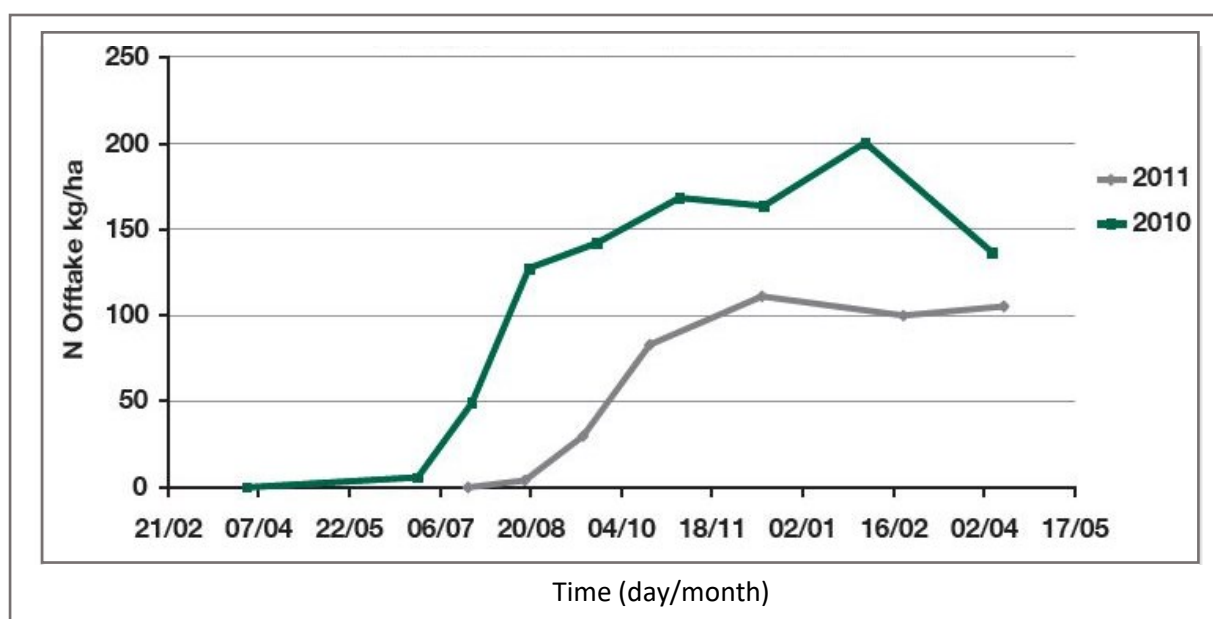


Fig 7.1. N offtake in leeks in temperature agriculture (Wellesbourne, England), measured by the N content of the biomass. Declines in N are due to loss of N from biomass as the crop matures. Taken from Rahn (2012).

The uptake of N from alder and gunnera treated soil in the pot experiment, shows a better correlation with the N offtake of leek crops, than that supplied by the clover (Fig 7.2). Therefore, a possible

method of optimal N supply for leeks could be an application of gunnera or alder before sowing or planting, with additional strategic applications of fertiliser N or clover N (added as a PMGM) at times of especially high N demand.

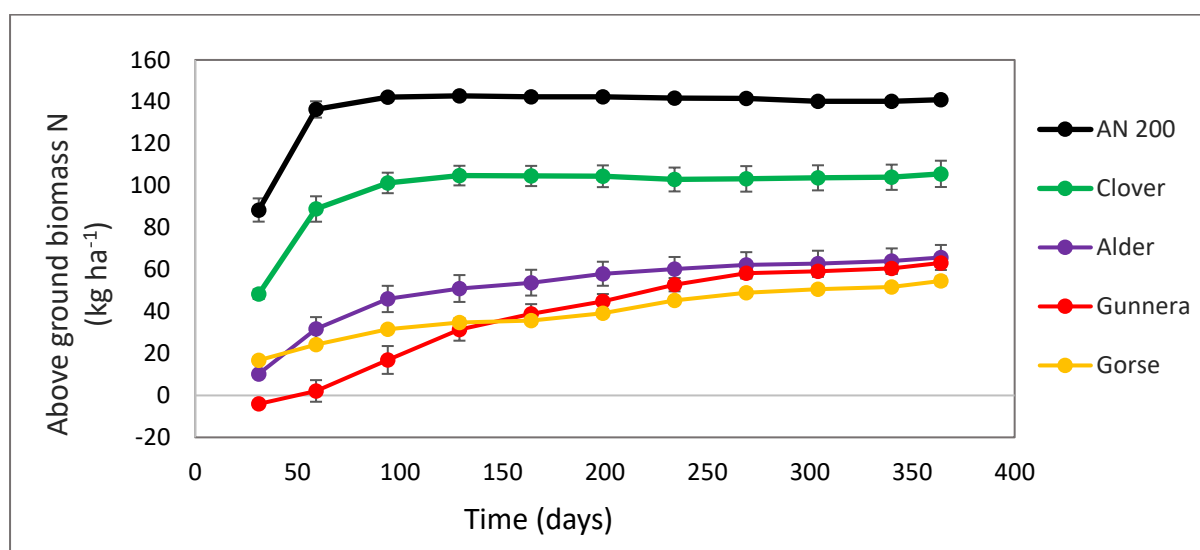


Fig 7.2. Uptake of N added in treatments by grass shoots in the pot experiment. Calculated by cumulative shoot N offtake of treated plants– control shoot N offtake Means \pm SEM ($n = 5$).

In addition to the timescale of N uptake, crop species and cultivars vary in their preference for the form of N they uptake and taking account of this could make N uptake more efficient (Swain et al. 2014, Zhang et al. 2018). Agricultural efficiency may be increased if a higher proportion of N is taken up in organic form due to a C bonus to the plant and lower processing costs of organic N (Schmidt et al. 2013, Franklin et al. 2017). After many years of crops being selected for breeding in an environment high in mineral N, it may be that many crop plants have reduced capacity for organic N uptake compared to their wild relatives (Finlay and Wilkinson 1963, Hirel et al. 2011),

7.3 N₂O emissions

In the pot experiment the PMGM treated soil produced very low N₂O emissions in comparison to those from clover and NH₄NO₃ treated soils (Table 7.2). The EF of 5.3 % measured from the clover treated soil, although not unheard of, is very high in comparison to those previously found after addition of plant material. The EFs used by the IPCC for crop residues in wet soil are 0.6 % with an uncertainty range of 0.1 to 1.1 % (Hergoualc'h et al. 2019), However, many crop residues will be of a higher C:N ratio than the clover amendment and higher emissions have been recorded from high N vegetable residues for example of 6 % by Velthof et al. (2002). These very high EFs from the clover treatment highlight the importance of careful green manure management in warm wet soils, which could have a large climate change impact if large areas of cropland were to have such high N₂O. With increasingly warm summers in the UK, this is a factor to consider for vegetable horticulture especially in irrigated

soil. By contrast the very low EFs from the gunnera and alder treated soil, even in warm wet conditions demonstrate the advantages of reducing excess mineral N in soil, and with an EF of 0.3 %, the application of gunnera is a promising way to provide a sustained supply of N with very low N₂O emissions.

There was a positive correlation between N₂O emissions and soil NO₃⁻ concentrations. However, at the peak time for emissions from clover treated soil (day 13), there was a positive correlation of N₂O with DOC concentrations. The PMGMs, unlike the clover treatment, did not result in high NO₃⁻ and DOC concentrations at the same time, so not providing the necessary factors for high emissions by denitrification (Butterbach-Bahl et al. 2013, Mitchell et al. 2013). In both years of the field experiment the N₂O emissions were low from all treatments. It is likely that low WFPS and therefore high aeration prevented high emissions by denitrification. However, the N₂O emissions which could occur by PMGMs after high rainfall in field soil are unknown. DOC concentrations were higher in field soil after repeated applications of PMGMs than those from one application in year 1. Therefore, soil receiving repeated additions of PMGMs could be at risk of high N₂O emissions at times of higher rainfall. This further emphasises the importance of synchronising N supply with demand to reduce the risks of high NO₃⁻ concentrations coinciding with high rainfall in DOC rich soil (Velthof et al. 2002, Shan and Yan 2013, Charles et al. 2017, Guardia et al. 2019, Hansen et al. 2019).

Table 7.2. N₂O Emission Factors from the pot and field experiments. (% of applied N) Means ± SEMs. *n* = 5 (pot), *n* = 4 (field), Letters denote significant differences between the EFs of the pot experiment. There were no significant differences between treatments in the EFs of the field experiments.

Experiment	Clover	Alder	Gunnera	Gorse	AN70	AN200
Pot	5.3 ± 0.5 c	0.6 ± 0.1 ab	0.3 ± 0.1 a	1.2 ± 0.1 ab	3.6 ± 0.8 c	-
Field year 1	-	0.23 ± 0.26	0.09 ± 0.03	0.14 ± 0.19	-	0.24 ± 0.15
Field year 1 + 2	-	0.37 ± 0.18	0.26 ± 0.15	-	-	0.27 ± 0.14

7.4 Scope for strategic use of PMGMs to improve N synchrony

Higher synchrony of N supply with crop demand both reduces the amount of N required, and the potential for leaching and N₂O emissions due to lower mineral N concentrations in soil. Strategic use of PMGMs taking into account the qualities of the material in combination with the crops grown could contribute to both these aims. To enable maximum synchrony it would be beneficial to use a wide variety of PMGM species with varying leaf properties, such as C:N ratios and lability. Plants currently used as traditional green manures could also be used as PMGMs, for example red clover could also be grown in permanent stands and used as a PMGM, as suggested above for the fertilisation of leek crops.

PMGMs could also be used in combination with mineral fertilisers, with mineral N applied when crop N demand is high, but avoiding times when environmental factors risk high N₂O emissions such as when rainfall is heavy resulting in high soil WFPS. Two potential techniques are explored further below, that of combining different species to influence N release, and that of using PMGMs to immobilise N when supply is higher than demand.

7.4.1 Combining PMGMs for increased NRE

The varying rates of N uptake from soil treated with different PMGMs, gives rise to the possibility of strategically combining different green manures, to give an N release rate tailored to a specific crop (Gunnarsson and Marstorp 2002, Kaewpradit et al. 2008, Truong and Marschner 2018). Tests of N release rates, for example by incubation studies, can be used to identify suitable combinations of PMGMs for specific crops. This approach was used by Handayanto et al., (1997) who researched the potential for maximising the synchrony of nitrogen supplied by addition of tree prunings, with crop demand, by mixing material from two tree species with different N and polyphenol contents: *Gliricidia sepium* and *Peltophorum dasyrrachis*. When the prunings were mixed, the protein binding capacity of the mixture increased sharply when *P. dasyrrachis* made up more than 50%. Therefore, the NRE of use of *Gliricidia sepium* prunings could be improved by mixing with *Peltophorum dasyrrachis*, to strategically immobilise N and reduce N loss. Similarly, Rothé et al (2019) screened various organic materials in the laboratory for N mineralisation rates suitable for pineapple production. The N mineralisation rates from the laboratory study were used to identify suitable combinations for a field experiment in which the combination of the legume *Mucuna puriens* followed by poultry feathers, bloodmeal, and composted manures gave a pineapple crop of yield and quality comparable to mineral fertilisers.

7.4.2 Strategic use of N immobilisation to improve NUE

Incorporation of an N rich, labile green manure before a slow growing crop, risks leaching of NO₃⁻ and N₂O emissions. Addition of gunnera at the same time as the low C:N green manure may serve to immobilise N in soil, with a release later in the season better aligned to crop demand. This approach may also be useful in situations where large amounts of fertiliser N are added to the seedbed due to problems with access later in the season e.g. with tall crops such as maize. Similarly, If N rich crop residues are incorporated into soil in autumn, leaching is a risk. Quantities of N from within crop residues can be in the region of 150 to 200 kg N ha⁻¹ (Neeteson and Carton 2001, Araya et al. 2010). Though risk of leaching can be reduced by the sowing of a cover crop, if temperatures are low and rainfall high, N may be at risk of leaching before the cover crop is established. Incorporating gunnera or another PMGM with an immobilising effect along with the residues could reduce N pollution and

retaining N for future crop uptake. This technique was used by (De Neve et al., 2004) in applying straw with C:N ratio of 40 to leek residues, resulting in immobilising 26 mg N kg⁻¹ soil, with remineralisation beginning after 50 days.

7.5 Soil Carbon Stocks

Results from the incubation experiment (chapter 4) suggest that use of more recalcitrant green manures could result in the most loss of C. Although the clover treatment initially resulted in faster total loss of C, than that from the PMGM treated soil, the rates of C loss changed over time so that during the last week of the incubation (week 6) the total soil C loss was higher from PMGM treated soil than from clover treated soil. The results predicted that after one year, soil treated with alder and gunnera would lose the highest amounts of total C, and soil C of over 8 years old. This would result in total C soil stocks in alder and gunnera treated soil which were lower than that of the control despite the C added within the added leaf matter.

These data cannot be used to estimate the amount of priming which would occur in the field, as many inter-related factors not present in the incubation affect the soil C stocks, e.g. crop roots and exudates, root-associated microbial activity, soil meso and macro fauna, soil disturbance, and plant nutrient uptake, temperature fluctuations and precipitation or irrigation (Kuzyakov et al. 2007, Zang et al. 2016, Bailey et al. 2019). However, these data highlight an important consideration for the use of PMGMs, and further experiments are necessary to ascertain the effect of PMGMs with plants present. Higher C losses due to the gunnera treatment may be offset by production of higher crop root biomass, which was seen in the pot experiment.

It has been suggested that the addition to soil of more labile plant material results in a higher proportion of the added C being retained in SOM due to the lower energetic costs of processing than for more recalcitrant materials (Manzoni et al. 2012, Cotrufo et al. 2013). The results of this experiment concur with this, and if it is the case that materials of lower lability have a detrimental priming effect, it may be necessary to use more labile PMGMs. This would necessarily change the rate of N release to crops, and therefore, higher lability green manures would likely need adding in smaller quantities, but at higher frequency to synchronise with N demand.

7.6 Integrating PMGM use with other agro ecosystem needs

Since work began on this thesis, there have been developments across many areas of agro ecological research which have implications for the use of PMGMs.

7.6.1 Nitrogen use efficiency

There continue to be a large number of studies on increasing nitrogen use efficiency (NUE) in agriculture, (Abalos et al. 2014, Sharma and Bali 2018, Rees et al. 2020, Dimkpa et al. 2020). Though techniques such as precision agriculture can improve NUE, preventing N losses is becoming more challenging due to a more unpredictable climate and therefore less predictable crop N uptake, and more variable precipitation which can increase leaching (Feria et al. 2019). Recently, Bowles et al. (2018) noted that the potential to improve NUE by improving synchrony has been researched intensively for many years and suggested that the potential for improvements of NUE may be limited in an unpredictable climate. Instead they suggest that there needs to be greater focus on the resilience of agroecosystems to N losses, one technique for which is the inclusion of more perennials, for example in buffer strips to intercept N losses. Other authors have also concluded that more perennials are needed in the agricultural landscape to improve resilience to N loss (Lin et al. 2016, Diederich et al. 2019).

It may, therefore, be diligent to accept the need to “mop up” excess N from some areas of agricultural landscapes and return it to cropping land. Perennial plants can take up excess N, as commonly used in buffer strips of unploughed, vegetated land, often near watercourses which serve to protect from leaching of NO_3^- (Di and Cameron 2002, Bowles et al. 2018). Therefore, non N fixing PMGMs could be grown in such areas as N lifters, to redistribute N to where crop demand is high. In other areas a mix of N fixers and lifters could provide the dual functions of mopping and fixing as used in winter cover cropping.

7.6.2 C sequestration

Recent authors have pointed out a potential limitation of the 4 per mille campaign to increase soil C sequestration, in that there is limited availability of the necessary organic matter to add to agricultural land (Poulton et al. 2018, Duran-Lara et al. 2020). In addition, for C be sequestered, additional N is required to balance the C content of organic matter (van Groenigen et al. 2017, Hunt et al. 2020). It has been suggested that the N required could be supplied by BNF or by redistribution of excess N in the agricultural system (Soussana et al. 2017). Use of PMGMs, could therefore contribute to C sequestration in provision of both C and N for organic matter formation.

7.6.3 Land sparing for ecosystem services

The preservation of biodiversity is necessary for agricultural functioning (Pilling et al. 2020), and high yielding farming which reduces the agricultural land area required is increasingly advocated to “spare” land to be preserved or restored as habitats to provide ecosystem services (Lamb et al. 2016, Balmford et al. 2018). The use of PMGMs, instead of temporary green manures, could be valuable as dual

purpose N fixing and biodiversity reserve areas. Another increasingly important ecosystem service provided by trees in the landscape is that of flood prevention due to the slowing of water flow by tree roots (Marshall et al. 2014). Though large areas of habitat are important for environmental stability, smaller areas of high species diversity are also highly beneficial when distributed into the agricultural landscape, such as the current use of “beetle banks”, which contribute ecosystems services to production such as improving pest-predator balance, (Collins et al. 2002, Thomas et al. 2002, Tschumi et al. 2015). Areas of PMGMs could contribute to this if interspersed among arable fields as to provide the dual services of biodiversity reserves and fixing of N.

7.6.4 Reduction of ruminant farming for climate change mitigation

There is now an increasing appreciation of the environmental need to reduce the number of ruminants in agricultural systems due to methane emissions and an inefficiency of conversion from plant to animal protein, a shift towards plant based diets and policy recommendations to that end (Loboguerrero et al. 2019, Willett et al. 2019, Ripple et al. 2019). Growing N fixing short rotation coppice PMGMs in these areas could fulfil the need to reduce methane emissions, increase C sequestration, and provide biodiverse habitats. This plant N, as an addition to agricultural land could help counteract the loss of production of animal protein, by increasing yields of arable crops. A reduction in the number of grazing animals, will also result in a reduction of supplies of manure, used by many growers as a mobile organic source of N, and therefore the supply of N via PMGMs, bypassing the ingestion and excretion by animals could serve to replace manure use.

7.7 Inclusion of PMGMs into the agricultural landscape

It is envisioned that multi-purpose areas of PMGMs would be included within the agricultural landscape to fix and redistribute N to crops, improve pest-predator balance, and sequester C, as represented in Fig 7.3. We refer to these as bio-service areas or bio-efficiency strips which would contain a variety of N fixing and lifting species of PMGMs chosen for the climatic and soil conditions. Tree or shrub PMGMs would be coppiced or pollarded and herbaceous plants mown to harvest the PMGM material.

As described in the introduction, PMGMs could be grown on land unsuitable for arable production, such as steeper slopes and floodplains, and temporary green manure leys could be replaced with permanent bio-service areas. Due to potential invasiveness, it may not be wise to plant large areas of gunnera, but in smaller scale horticulture, patches of gunnera, grown on wet land, could be harvested by hand, dried, and stored for use when appropriate. In addition, it may be advantageous to convert some areas presently used for the grazing of ruminants to bio-service areas. PMGMs grown on flood plains and buffer zones would have the primary purpose of mopping up and redistributing leached N

and as such would contain a higher ratio of N lifters to N fixers than other bio-service areas. Provision of ecosystem services could be increased by distributing PMGMs for maximum proximity to croplands, and could for example include restored or widened hedgerows.

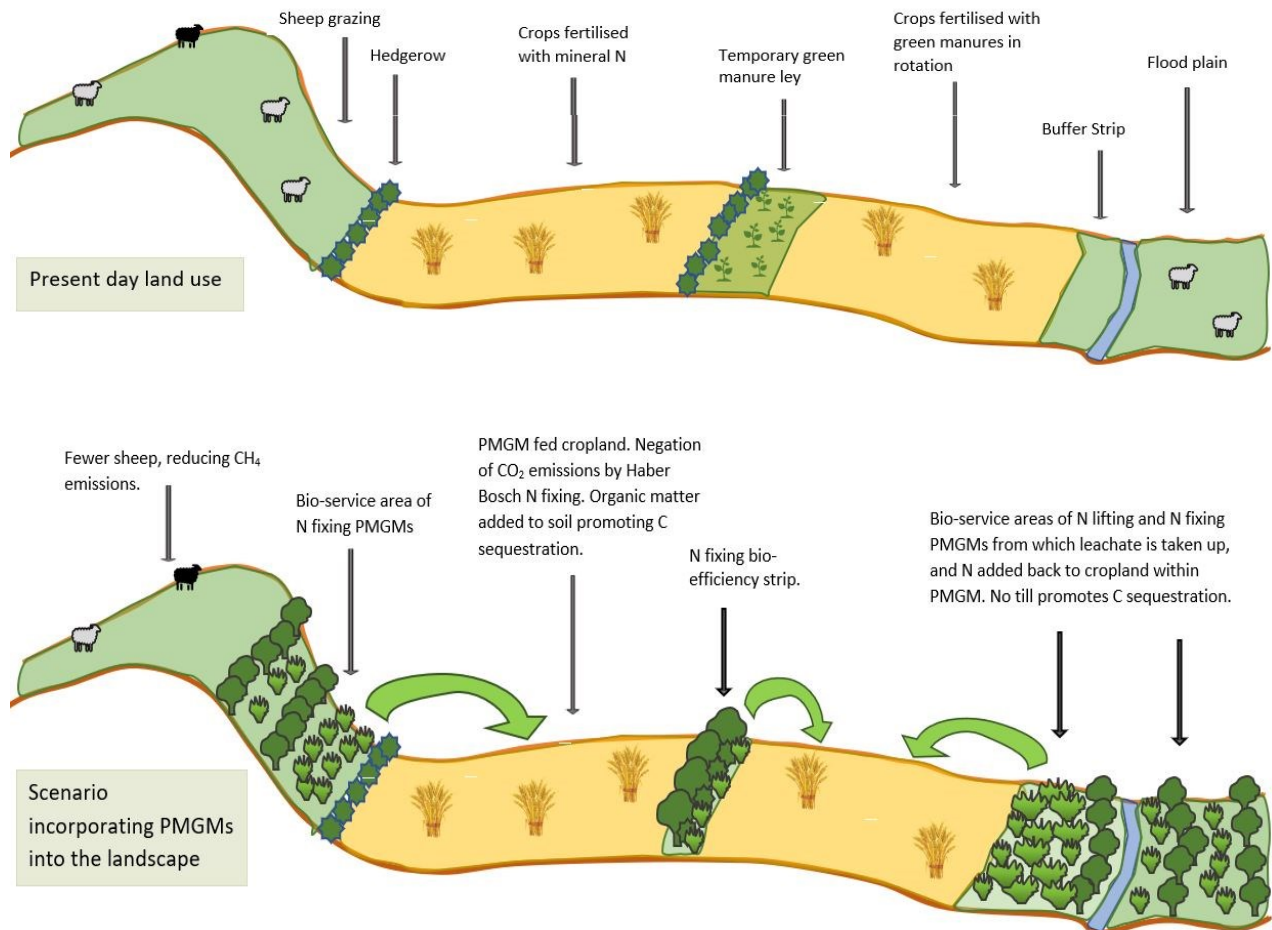


Fig 7.3 Scenario of the present and possible future agricultural landscape, showing possible locations for bio-service areas and their contributions to provision of N to crops and climate change mitigation. The area retained for arable cropping remains the same in the future scenario.

7.8 Economics and upscaling

The introduction of such an unconventional land use into rural economies in temperate agriculture may be challenging, when farmers associate their practices with long held traditions. However, as outlined in section 7.6.4, there are large areas of farmland in the UK devoted to grassland for ruminants, for which a more sustainable land use is required. Changes to subsidy systems to increase the economic rewards offered to farmers for ecosystem services could make other land uses more attractive. Recently O'Neill et al. (2020) assessed the economic case for replacing grazing land with forests, and advocated policy for instigation of C farming by reforestation on land presently used for

sheep farming in the UK. In addition, there is currently an interest among environmental and forestry organisations in the harvesting of non timber forest products, as an economic and social incentive for woodland management which promotes biodiversity and social cohesion (Emery et al. 2006, Ludvig et al. 2016), and it is possible that PMGMs could be grown as one such product.

For the growing of PMGMs to be environmentally beneficial, over that of purely habitat restoration of native forests, the quantity of N produced needs to be sufficient to make a worthwhile contribution to N supply. This is difficult to estimate, as N fixing rate from various species, in different environments are very variable. However, some very broad estimates can be made. Assuming an N fixing rate similar to traditional green manures of approximately 150 kg N ha⁻¹ per year (Rosenfeld and Rayns 2011, Lupwayi and Soon 2015, Carr et al. 2020), and rates of N addition which are typical for the UK at a mean average 137 kg N ha⁻¹ applied to arable crops (Defra 2020), 1 ha of PMGMs may supply enough N for 1 ha of arable land, (though more than that if NUE is increased). In the UK there are approximately 5 million ha of arable cropland (half of which are fed to farm animals, and half used for human consumption), and 11.5 million ha of grassland for ruminant livestock (CAT 2020). Therefore, by very broad calculations, it could be feasible to supply the UKs arable cropland with PMGMs using half of the land which is now presently producing grass for ruminants.

However, a more realistic scenario is that of PMGMs making a contribution alongside other N sources such as crop residues, mineral N, and recycled waste products such as digestates from food waste (Tampio et al. 2016, Sánchez-Rodríguez et al. 2018). Land currently used for grazing of ruminants is of variable ecological value, and highly biodiverse areas need to be retained as grassland (Hopkins and Holz 2005). Other areas may be too inaccessible for PMGM use for the practicalities of harvesting or too far from the receiving agricultural land to make transport feasible. More diverse areas with a mix of hillier and flatter areas are more likely to be suitable, compared to those made up of high quality flat agricultural land or very exposed hilly land with no nearby arable cropping.

Implementing large changes in the landscape for a new and unproven technique, would be a large risk. However, PMGM production could be combined with the production of other resources for agro ecosystems such as coppice for biochar for C sequestration (Shackley et al. 2011, Lal 2020), tree fodder as a supplementary feed for livestock (Smith et al. 2014). By planting areas to supply a mix of these materials, the proportionate uses could change with time depending on which strategies are shown to be most useful.

7.9 Further Research

The sections above point to areas of research into PMGM use which would be of benefit. First it is important to ascertain the effect of PMGM use on C stocks, so further research into the priming effects

of alder and gunnera is recommended, both to ensure that any negative effect of priming does not outweigh the positive effects of greater NUE which may be achieved, but also to identify any strategies which may reduce soil C priming and increase sequestration. The results of the second year of the field experiment suggested that N uptake from gunnera may increase with repeated additions. Further studies over a longer duration (five years or more) would elucidate this effect further and if combined with soil C studies may shed light on the mechanisms causing both the increased soil N uptake and the C dynamics. As noted in section 7.2.1, plant species vary in their preferred N source, and there may be scope for breeding new cultivars which have a higher preference for organic N uptake. Breeding crops with a higher affinity for uptake of organic N could increase NRE in conjunction with use of organic additions (Paungfoo-Lonhienne et al. 2012).

7.9.1 Comparison of PMGMs to a wider range of soil amendments

This thesis assessed PMGMs in comparison to the other sources of N which are produced directly by N fixation, namely traditional green manure N produced by the cultivation of legumes, and mineral fertiliser N produced by industrial N fixation. Other materials which are added to cropland contain N which was originally derived from one or other of these methods of N fixation. These include sewage sludge, animal manures and composted food and garden waste (Defra 2019). Composted materials contain more stable organic matter such as humus (Favoino and Hogg 2008; Yang et al. 2021) and are therefore likely to sequester more C than the addition of the unprocessed plant material of PMGMs. The use of PMGMs would not directly replace these materials, which as the products of various waste streams are likely to be available for use in agriculture regardless of the balance of methods used for N fixation. However, an assessment of the effects on soil C and N dynamics of PMGMs in comparison to these materials or used in conjunction with them would be valuable.

7.9.2 Expanding the range of PMGM species

Knowledge of the qualities of a wide range of PMGM candidate species would increase the variety of PMGMs which could be used. The species researched here were chosen for the reasons outlined in section 2.6.3, however a range of species, with a variety of properties of leaf material, but also a variety of environments in which they are suited to growing would increase possibilities. Candidate species include other shrubby legumes such as tree lupins (*Lupinus arboreus*), laburnum (*Laburnum anagyroides*), and the UK native Broom (*Cytisus scoparios*). Non leguminous shrubs and trees which form associations with N fixing actinobacteria include sea buckthorn (*Hippophae rhamnoides*), and a range of plants in the *Elaeagnus* genus (Santi et al 2013). Perennial clovers and vetches which are currently grown in temporary ley systems, could also be used as PMGMs. In addition, it would be useful to assess some non N fixing plants such as willow species (*Salix* spp.) or comfrey (*Symphytum*

spp.) which could be grown as N lifters in multi species plantations along with N fixers as described in section 7.6.2. A useful first step, therefore, would be to test a wide range of potential plant species for C and N contents, phenolic content and possible allelopathic effects by analyses of leaf tissue and short term pot experiments. This could lead to the drawing up of an organic resource database, such as that which exists for plants in tropical systems and described by Palm et al. (2001).

7.9.3 Practicalities of PMGM harvesting and processing

To use leaf matter from woody species such as alder, an efficient method is needed to separate leaves from branches. Therefore, a future research aim is to trial methods of processing the harvested branches. For example, if the material is first chipped, it may be possible to separate the light leaf fragments from the heavier wood fragments by use of a threshing machine, similar to the method for separating harvested grains from chaff. It would be an advantage if this could be achieved using machinery which many farms already possess, even if some adaptations to the machinery are required. Another strategy deserving of further research is to chip small branches and leaves together to produce a high N ramial wood, which may be used directly or composted before application.

7.9.4 Impact assessment of PMGMs.

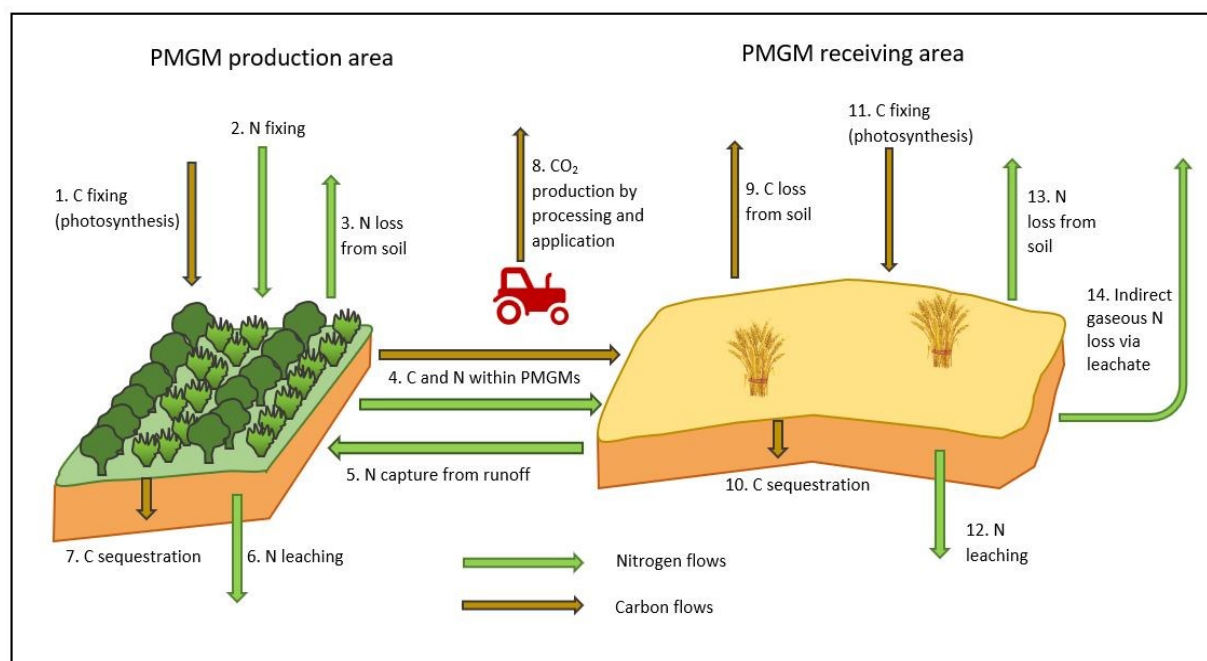
To assess whether the replacement of traditional green manures or fertiliser N by PMGMs is beneficial for climate change mitigation, the total overall impacts need to be quantified. This is a difficult task due to the many varied and inter-related impacts. Impacts within the cropping area, as researched in this thesis, are principally N₂O emissions (both direct and indirect), and soil C loss or gain. Impacts in the PMGM producing area include the reduction in emissions of CO₂ by replacement of the Haber Bosch process by BNF, and changes in soil C stocks in areas converted from temporary leys to permanent areas of PMGMs.

7.9.4.1 Combined assessment of PMGM producing and receiving areas

If the PMGM producing and receiving areas are in close proximity there will be interactions between the two sites. These include the capture and uptake of NO₃⁻ which may be leached from arable fields into the PMGM production area, and possibly benefits of increased biodiversity for pest control and pollination. A PMGM production site may also produce other materials, such as woody material for biochar production. Other impacts to assess include C emissions from processing and transport of PMGM material, and that of a possible increase in N₂O emissions which can occur from soil where N fixing plants are grown, including *Alnus* species (Parkin et al. 2016, Buhlmann et al. 2017).

Fig. 7.4 shows the C and N flows into, between and out of an area of PMGM production and an adjacent cropping area in receipt of the PMGMs. Knowledge gaps due to unknown impacts of these

flows and possible research methods are listed along with suggested methods for addressing them. Approximate quantifications for some unknowns can be made by use of existing knowledge and literature, such as the expected C sequestration of converting arable land to permanent plantings. Others could be addressed by measurements made in existing stands of candidate PMGM species. Further areas of research would require field trials of PMGM plantings, for example to identify the optimum mix of species and planting patterns to maximise N fixing while minimizing N₂O emissions or N leaching.



C or N flow	Unknowns	Possible research methods
1, 2, 4	N and C fixing rates	Measurement of total N and C production by periodic harvesting of existing stands of candidate PMGM species.
3	N loss from soil in PMGM production (largest impact of which is the GHG N ₂ O)	Measurement of N ₂ O emissions from existing stands of candidate species growing in a range of environments.
2,3,4,6	Balance of N fixers to N lifters required to maximise efficiency	Long term trial plots of various mixtures of candidate species with measurements of N fixing, N ₂ O emission and N leaching.
5	Prevention of leaching	Use of existing literature to identify appropriate siting of bio-service areas for leachate capture
7	C sequestration rates in PMGM soil	Use of literature on sequestration rates for similar plantings and soil types.
8	C emissions from processing and transport	Trials of methods of processing. Measurement of required fuel use.
9 – 14	N and C flows within PMGM receiving area	Continuation of research of this thesis.

Fig 7.4 Flows of N and C within a PMGM fertilised arable system, showing the unknown impacts of both the production and use of PMGMs, and where more knowledge is necessary for the design of the lowest impact PMGM systems.

7.9.4.2 Life Cycle Analyses

Though complex, a life cycle analyses (LCA) of PMGM use, giving a broad estimate of the likely overall impacts in comparison to traditional green manures or mineral fertiliser would be useful. Effects of both the production site and crop site need to be assessed against annual green manures and Haber Bosch N e.g. the biodiversity and C sequestration benefits of a stand of perennials compared to a temporary green manure ley, and the energy costs of processing the materials against the production and application of Haber Bosch N. An LCA should compare each method conducted in an efficient, but realistic way, e.g. mineral fertiliser added in stages for maximum efficiency, and PMGMs added at such a time to allow plant available N to best be taken up by a crop.

7.10 Conclusions

This thesis concludes that the PMGMs tested were viable in supplying N to crops and can potentially be used as a climate change mitigation strategy due to their slower, more sustained release of N which resulted in lower soil mineral N concentrations and therefore lower pollution by N_2O or risk of leaching of NO_3^- . Counteracting this was the potential net lowering of soil C stocks, following alder and gunnera treatments, which requires further investigation, to assess C dynamics with plants present. The crop N uptake from two successional applications of gunnera indicated that N uptake from repeated PMGM use may increase with time. Addition of PMGMs can also result in higher DOC concentrations highlighting the need to avoid high NO_3^- levels which combined with DOC would provide the factors required for denitrification. There is potential for improving the NUE by increased synchrony of N supply with crop demand by techniques such as mixing PMGMs of various leaf chemistry, or supplementing PMGMs with mineral fertiliser, and application to appropriate crops. There is much scope to develop these methods, for example by using a wider range of species as PMGMs, and designing the most beneficial layouts of systems at the landscape scale. All of the above requires more research, and long term studies are needed to assess the effect of PMGMs on N and C dynamics and crop yields over many years.

It may appear to be too late to be undertaking long term research for climate change mitigation, when faced with the current emergency. The quote below is taken from a statement signed by 11,258 scientists from 153 countries.

“Scientists have a moral obligation to clearly warn humanity of any catastrophic threat and to “tell it like it is.” On the basis of this obligation and the graphical indicators presented below, we declare, with more than 11,000 scientist signatories from around the world, clearly and unequivocally that planet Earth is facing a climate emergency.” (Ripple et al. 2020)

Clearly, strategies with known benefits which can be applied more immediately, such as the reduction of food waste, and the number of ruminant animals must take priority (IPCC 2019). However, the use of PMGMs as an N supply to crops is a solution which requires an infrastructure (of biodiverse areas of mainly perennial plants) that is known to be necessary for climate change mitigation and the resilience of agro-ecosystems (IPCC 2019, FAO 2019, Pilling et al. 2020). Therefore, the consideration of the potential use of PMGMs when re-instating biodiverse areas, for example, by considering the choice of plants and access for harvesting, could create a future resource of PMGMs to be used if or when they are proved effective. We conclude that the use of PMGMs offers a promising contribution to climate change mitigation and deserves further research and consideration by those involved in decision making in agriculture.

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Appendix 1

Chapter 3 Supporting data

Table 1. % N in shoot and root tissue Means \pm SEM, ($n = 5$)

Experiment	Day	31	59	94	129	164	199	234	269	304	340	364	Root
Main	Control	3.5 \pm 0.12	2.5 \pm 0.06	2.0 \pm 0.04	1.7 \pm 0.04	1.7 \pm 0.04	1.7 \pm 0.04	1.7 \pm 0.09	1.2 \pm 0.03	1.2 \pm 0.03	1.2 \pm 0.03	0.9 \pm 0.03	1.0 \pm 0.03
Additional	Control	2.6 \pm 0.06	1.8 \pm 0.08	1.7 \pm 0.04	1.4 \pm 0.03	1.7 \pm 0.09	1.2 \pm 0.04	1.5 \pm 0.08	1.1 \pm 0.03	1.1 \pm 0.03	1.3 \pm 0.03	0.8 \pm 0.05	1.0 \pm 0.05
Main	AN 35	4.8 \pm 0.05	2.6 \pm 0.07	1.8 \pm 0.02	1.7 \pm 0.04	1.7 \pm 0.04	1.7 \pm 0.04	1.8 \pm 0.07	1.2 \pm 0.04	1.2 \pm 0.04	1.2 \pm 0.04	0.9 \pm 0.03	0.9 \pm 0.04
Main	AN 70	5.3 \pm 0.12	3.6 \pm 0.56	1.6 \pm 0.00	1.6 \pm 0.03	1.6 \pm 0.03	1.6 \pm 0.03	1.7 \pm 0.06	1.2 \pm 0.02	1.2 \pm 0.02	1.2 \pm 0.02	1.0 \pm 0.04	0.9 \pm 0.05
Additional	AN 70	4.0 \pm 0.24	1.8 \pm 0.09	1.6 \pm 0.01	1.3 \pm 0.04	1.7 \pm 0.08	1.3 \pm 0.04	1.4 \pm 0.05	1.1 \pm 0.01	1.2 \pm 0.04	1.4 \pm 0.05	0.9 \pm 0.07	0.9 \pm 0.05
Additional	AN140	5.3 \pm 0.19	2.0 \pm 0.21	1.4 \pm 0.05	1.3 \pm 0.03	1.5 \pm 0.06	1.4 \pm 0.07	1.6 \pm 0.04	1.1 \pm 0.03	1.2 \pm 0.08	1.4 \pm 0.06	0.9 \pm 0.01	1.0 \pm 0.05
Additional	AN 200	5.6 \pm 0.07	2.6 \pm 0.18	1.4 \pm 0.03	1.3 \pm 0.03	1.6 \pm 0.09	1.4 \pm 0.03	1.6 \pm 0.07	1.1 \pm 0.03	1.1 \pm 0.06	1.4 \pm 0.07	0.9 \pm 0.04	1.0 \pm 0.03
Main	Clover	5.7 \pm 0.05	4.5 \pm 0.23	1.8 \pm 0.03	1.6 \pm 0.02	1.6 \pm 0.02	1.6 \pm 0.02	1.7 \pm 0.09	1.2 \pm 0.04	1.2 \pm 0.04	1.2 \pm 0.04	0.9 \pm 0.05	0.9 \pm 0.05
Main	Alder	4.4 \pm 0.11	3.4 \pm 0.20	2.0 \pm 0.08	1.6 \pm 0.03	1.6 \pm 0.03	1.6 \pm 0.03	1.7 \pm 0.03	1.3 \pm 0.04	1.3 \pm 0.04	1.3 \pm 0.04	0.9 \pm 0.04	1.0 \pm 0.07
Main	Gunnera	3.3 \pm 0.06	3.0 \pm 0.40	2.2 \pm 0.12	1.6 \pm 0.03	1.6 \pm 0.03	1.6 \pm 0.03	1.8 \pm 0.10	1.2 \pm 0.02	1.2 \pm 0.02	1.2 \pm 0.02	0.7 \pm 0.04	1.1 \pm 0.07
Main	Gorse	4.5 \pm 0.10	2.6 \pm 0.04	1.9 \pm 0.05	1.7 \pm 0.03	1.7 \pm 0.03	1.7 \pm 0.03	1.8 \pm 0.09	1.3 \pm 0.04	1.3 \pm 0.04	1.3 \pm 0.04	0.9 \pm 0.02	1.0 \pm 0.04

Table 2. Mean shoot biomass N uptake per cumulative day degree (CDD) (g N m⁻²)

day	31	59	94	129	164	199	234	269	304	340	364
CDD of period	387	316.5	396.5	551.5	514.5	553	832.5	764	779.5	622.5	
CDD	387	703.5	1100	1651.5	2166	2719	3551.5	4315.5	5095	5717.5	
Control	2.2	3.3	4.1	4.7	5.4	6.2	7.6	8.8	9.4	9.8	10.6
AN 35	4.9	6.2	7.2	7.8	8.4	9.2	10.5	11.7	12.3	12.6	13.6
AN 70	6.1	9.0	10.0	10.6	11.3	12.0	13.3	14.5	15.1	15.4	16.3
Clover	7.0	12.2	14.2	15.2	15.9	16.6	17.9	19.2	19.8	20.2	21.2
Alder	3.2	6.5	8.7	9.8	10.8	12.0	13.6	15.1	15.7	16.2	17.2
Gunnera	1.8	3.5	5.8	7.8	9.3	10.7	12.8	14.7	15.4	15.8	17.0
Gorse	3.9	5.7	7.3	8.1	9.0	10.1	12.1	13.7	14.5	15.0	16.1

Table 3. Fertiliser Equivalents (FEs) of shoot N uptake during the initial 1100 cumulative degree days (CDDs). Means \pm SEM, ($n = 5$)

Day	31	59	94
CDDS	387	704	1100
Alder	12.8 \pm 0.9	39.2 \pm 7.0	49.8 \pm 6.8
Gorse	20.8 \pm 1.7	30.0 \pm 2.0	34.1 \pm 1.4
Gunnera	-5.0 \pm 0.8	2.6 \pm 6.4	18.3 \pm 7.2

Table 4. Additional experiment total N in soil solution (mg L⁻¹). Means \pm SEM ($n = 5$).

Time (days)	1	5	18	39	95
Control	21.1 \pm 3.9	31.1 \pm 10.9	1.8 \pm 0.7	1.0 \pm 0.2	2.9 \pm 0.4
AN 70	332.3 \pm 95.2	191.5 \pm 28.0	201.9 \pm 18.4	2.8 \pm 0.7	3.0 \pm 0.4
AN 140	489.2 \pm 98.3	393.0 \pm 99.0	504.3 \pm 47.1	31.4 \pm 20.0	2.8 \pm 0.4
AN 200	837.0 \pm 97.9	435.5 \pm 64.3	623.5 \pm 90.1	212.9 \pm 37.8	2.6 \pm 0.5

Table 5. Additional experiment NO₃⁻ in soil solution (mg L⁻¹). Means \pm SEM ($n = 5$).

day	1	5	18	25	39	67	95
Control	17.9 \pm 4.0	30.4 \pm 11.8	0.1 \pm 0.1	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.08 \pm 0.03
AN 70	270.2 \pm 71.5	140.8 \pm 17.9	177.1 \pm 18.1	26.1 \pm 24.3	0.6 \pm 0.3	0.2 \pm 0.2	0.4 \pm 0.3
AN 140	396.9 \pm 65.4	290.6 \pm 69.5	434.2 \pm 30.1	312.3 \pm 96.2	27.3 \pm 20.1	0.5 \pm 0.2	0.07 \pm 0.03
AN 200	628.8 \pm 73.5	352.3 \pm 55.6	568.7 \pm 82.8	525.7 \pm 122.4	245.3 \pm 50.5	4.1 \pm 1.4	0.5 \pm 0.1

Table 6. Total NH₄⁺ in soil solution (mg L⁻¹). Means ± SEM (n = 5).

Day		1	5	18	25	39	67	95
Exp								
Main	Control	0.04 ± 0.03	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.01	0.08 ± 0.02	0.03 ± 0.01
Additional	Control	0.09 ± 0.06	0.11 ± 0.07	0.02 ± 0.01	0.06 ± 0.04	0.03 ± 0.02	0.04 ± 0.02	0.07 ± 0.04
Main	Clover	1.63 ± 0.99	3.69 ± 1.26	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.01	0.04 ± 0.02	0.09 ± 0.01
Main	Alder	0.01 ± 0.004	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.03 ± 0.02	0.05 ± 0.02	0.06 ± 0.02
Main	Gunnera	0.02 ± 0.02	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.05 ± 0.01
Main	Gorse	0.05 ± 0.03	0.28 ± 0.12	0.05 ± 0.03	0.00 ± 0.00	0.02 ± 0.01	0.10 ± 0.02	0.10 ± 0.03
Main	AM 35	3.42 ± 0.77	0.31 ± 0.19	0.00 ± 0.00	0.05 ± 0.05	0.03 ± 0.01	0.10 ± 0.01	0.03 ± 0.02
Main	AM 70	6.39 ± 0.76	4.76 ± 1.12	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.06 ± 0.02
Additional	AN 70	62.28 ± 21.91	17.50 ± 6.86	13.08 ± 9.69	0.01 ± 0.01	0.04 ± 0.03	0.03 ± 0.02	0.04 ± 0.02
Additional	AN 140	112.25 ± 34.68	69.59 ± 27.34	11.47 ± 5.20	0.79 ± 0.39	0.02 ± 0.02	0.08 ± 0.05	0.26 ± 0.15
Additional	AN 200	213.32 ± 63.49	103.41 ± 27.81	56.69 ± 6.32	10.51 ± 2.46	0.08 ± 0.07	0.10 ± 0.06	0.06 ± 0.03

Table 7. Amino Acids in soil solution (µg L⁻¹). (means ± SEM, n = 5)

Time (days)	1	3	5	9	13	18	39	53	67	95	124	151	180
Control	12.7 ± 3.5	0.3 ± 0.3	0.4 ± 0.4	1.3 ± 1.3	6.0 ± 2.2	23.0 ± 20.9	26.2 ± 18.0	8.6 ± 6.4	3.4 ± 1.3	13.3 ± 3.1	11.5 ± 1.7	27.6 ± 8.2	11.5 ± 3.2
AN 35	10.1 ± 10.1	13.6 ± 11.2	19.2 ± 5.3	2.9 ± 2.4	3.5 ± 1.8	1.6 ± 1.2	26.2 ± 20.4	0.0 ± 0.0	0.8 ± 0.5	12.8 ± 1.5	7.6 ± 1.5	8.0 ± 1.9	4.7 ± 1.1
AN 70	535.7 ± 137.4	290.0 ± 102.3	22.5 ± 21.2	21.6 ± 11.5	1.3 ± 0.6	0.0 ± 0.0	7.8 ± 2.3	1.4 ± 1.4	8.6 ± 1.9	16.4 ± 2.4	10.1 ± 3.0	11.0 ± 2.2	8.7 ± 1.5
Clover	211.2 ± 124.9	46.1 ± 32.7	21.2 ± 17.9	115.3 ± 61.1	0.0 ± 0.0	19.1 ± 6.3	18.1 ± 2.7	1.6 ± 0.7	15.3 ± 5.9	19.5 ± 4.0	6.8 ± 3.2	15.6 ± 1.9	9.3 ± 1.2
Alder	28.0 ± 10.8	35.4 ± 18.9	24.4 ± 9.4	23.4 ± 13.4	18.5 ± 6.0	9.8 ± 3.2	10.9 ± 3.2	7.9 ± 2.6	13.6 ± 6.4	21.3 ± 3.7	22.3 ± 5.6	29.8 ± 17.0	8.9 ± 2.1
Gorse	24.5 ± 8.3	3.4 ± 2.1	7.4 ± 5.0	10.9 ± 4.4	16.7 ± 8.8	10.8 ± 4.5	6.2 ± 5.8	3.2 ± 6.1	3.7 ± 3.3	22.5 ± 2.6	27.5 ± 1.5	20.9 ± 37.4	21.4 ± 4.1
Gunnera	16.2 ± 17.1	4.1 ± 1.1	7.2 ± 2.1	8.2 ± 6.6	12.6 ± 5.8	7.8 ± 4.5	27.3 ± 2.0	31.3 ± 2.4	15.4 ± 2.3	30.7 ± 1.9	31.3 ± 4.9	94.8 ± 1.1	25.3 ± 4.1

Table 8. Microbial biomass (by Carbon) Using $k_{ec} = 0.45$ Means \pm SEM ($n = 5$).

Treatment	mg g ⁻¹ dry soil
Control	0.34 \pm 0.02
Clover	0.44 \pm 0.02
Alder	0.55 \pm 0.03
Gorse	0.56 \pm 0.01
Gunnera	0.60 \pm 0.02
AN 35	0.43 \pm 0.07
AN 70	0.46 \pm 0.02

Table 9. Mean average temperature during each grass growth period.

Days	0 to 31	32 to 59	60 to 94	95 to 129	130 to 164	165 to 199	200 to 234	235 to 269	270 to 304	305 to 339
Main experiment	17.5	16.3	16.3	20.8	19.7	20.8	28.8	26.8	27.3	22.8

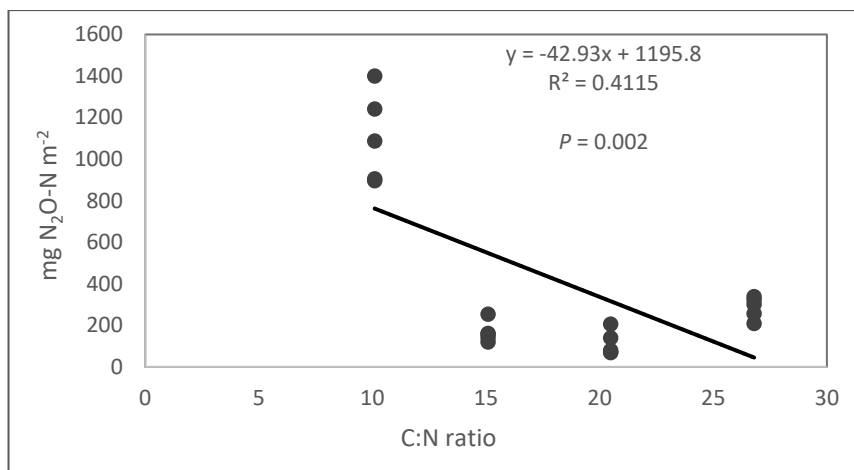


Fig 1 Relationship between total cumulative N₂O and C:N ratio of green manures.

Appendix 2 Supporting data Chapter 5

Table 1. Above ground biomass (g m^{-2}). Means \pm SEM ($n = 4$)

	Cut 1	Cut 2 Phacelia	Cut 2 weeds
Control	403 \pm 13	94 \pm 22	46 \pm 21
AN25	530 \pm 65	162 \pm 17	21 \pm 10
AN50	521 \pm 24	165 \pm 29	31 \pm 6
AN100	567 \pm 32	185 \pm 16	22 \pm 7
AN200	621 \pm 42	238 \pm 73	51 \pm 24
Ald F Inc	364 \pm 56	185 \pm 22	98 \pm 25
Ald F M	382 \pm 36	227 \pm 24	42 \pm 11
Ald D Inc	407 \pm 57	151 \pm 17	145 \pm 18
Ald D M	448 \pm 23	178 \pm 27	57 \pm 13
Gorse D M	464 \pm 30	100 \pm 17	69 \pm 9
Gun D M	368 \pm 16	168 \pm 23	83 \pm 21

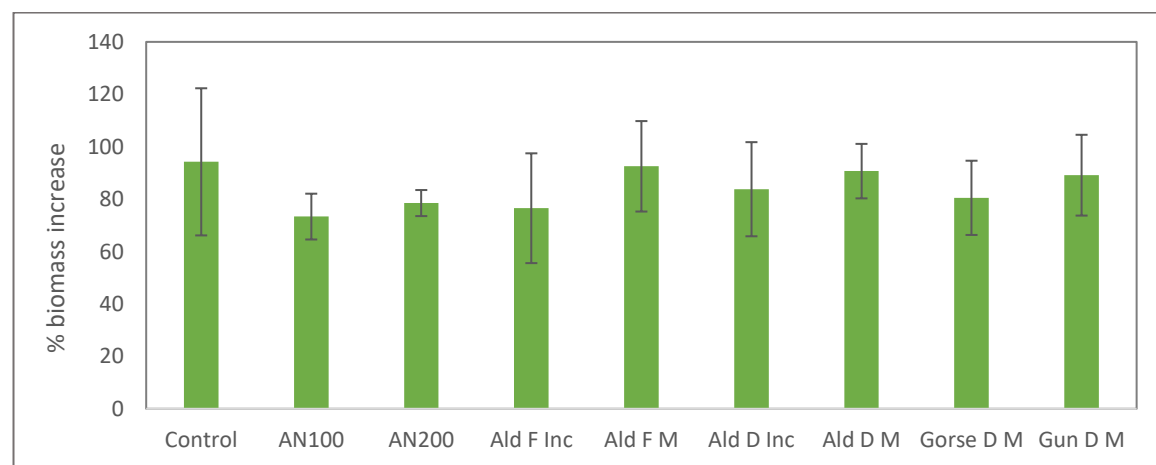


Fig 1. % Increase in biomass dry weight sampled inside the chambers over that sampled outside the chambers.

Table 2. % N in above ground biomass. Means \pm SEM ($n = 4$)

	Crop 1	Cut 2 Phacelia	Cut 2 weeds
Control	1.59 \pm 0.14	1.26 \pm 0.12	2.33 \pm 0.12
AN25	2.07 \pm 0.03	1.32 \pm 0.08	2.83 \pm 0.16
AN50	2.23 \pm 0.05	1.37 \pm 0.04	2.61 \pm 0.09
AN100	2.60 \pm 0.11	1.42 \pm 0.07	2.84 \pm 0.02
AN200	3.57 \pm 0.59	1.31 \pm 0.16	2.59 \pm 0.19
Alder F Inc	2.81 \pm 0.82	1.34 \pm 0.07	2.59 \pm 0.04
Alder F M	1.98 \pm 0.25	1.72 \pm 0.16	2.93 \pm 0.18
Alder D Inc	2.06 \pm 0.12	1.23 \pm 0.05	2.52 \pm 0.12
Alder D M	1.90 \pm 0.10	1.46 \pm 0.05	2.80 \pm 0.15
Gorse D M	1.73 \pm 0.08	1.52 \pm 0.08	2.81 \pm 0.16
Gunnera D M	1.68 \pm 0.17	1.54 \pm 0.21	2.61 \pm 0.22

Table 3. Nitrogen Recovery Efficiency (NRE) of crops 1 and 2. Means \pm SEM ($n = 4$)

	Crop 1 (Kg ha ⁻¹)	Crop 2 (Kg ha ⁻¹)
AN25	182.6 \pm 76.4	16.3 \pm 15.4
AN50	103.1 \pm 11.5	15.9 \pm 8.8
AN100	83.7 \pm 11.5	9.7 \pm 1.7
AN200	81.8 \pm 24.0	10.3 \pm 1.8
Ald F Inc	14.3 \pm 10.1	10.1 \pm 3.8
Ald F M	5.7 \pm 4.0	14.4 \pm 1.2
Ald D Inc	10.5 \pm 5.5	16.0 \pm 1.9
Ald D M	10.2 \pm 6.0	9.5 \pm 1.0
Gorse D M	9.3 \pm 4.3	4.3 \pm 1.4
Gun D M	1.2 \pm 3.3	8.8 \pm 3.9

Table 4. TN ($\mu\text{g g}^{-1}$ dry soil). Means \pm SEM ($n = 4$) Letters denote significant differences between treatments. There were no significant difference from day 55 onwards.

Day	1	3	7	13	27	41	55	69	97	209 (0 -15cm)	209 (15 - 30cm)
Control	30.3 \pm 3.0 a	34.1 \pm 1.9 a	36.5 \pm 1.7	30.6 \pm 2.3 a	25.9 \pm 1.5 a	16.3 \pm 1.9 a	18.8 \pm 0.9	14.1 \pm 1.5	18.3 \pm 0.7	32.6 \pm 0.3	30.3 \pm 1.9
AN100	112.2 \pm 34.5 b	81.9 \pm 7.5 a	104.3 \pm 16.2	105.9 \pm 23.9 b	34.6 \pm 3.6 a	18.5 \pm 0.7 a	21.5 \pm 1.4	15.8 \pm 0.8	17.5 \pm 1.9	30.6 \pm 0.8	31.5 \pm 3.6
AN200	198.8 \pm 31.4 c	210.1 \pm 63.9 b	293.3 \pm 42.4	172.8 \pm 3.8 c	73.1 \pm 21.7 b	20.6 \pm 1.4 a	20.2 \pm 2.9	14.8 \pm 1.9	25.6 \pm 8.0	30.9 \pm 2.2	33.3 \pm 4.4
Ald F Inc	34.2 \pm 3.2 ab	32.8 \pm 2.7 a	33.6 \pm 3.0	33.7 \pm 1.9 a	34.6 \pm 3.2 a	18.8 \pm 1.3 a	20.9 \pm 1.5	17.5 \pm 0.9	20.5 \pm 1.6	30.3 \pm 1.8	31.5 \pm 0.3
Ald F M	34.5 \pm 3.8 ab	34.8 \pm 2.8 a	35.7 \pm 3.7	29.5 \pm 2.4 a	26.6 \pm 1.2 a	19.6 \pm 2.3 a	21.5 \pm 1.9	21.5 \pm 3.0	18.6 \pm 1.2	32.7 \pm 2.7	32.3 \pm 1.7
Ald D Inc	32.2 \pm 13.2 a	35.2 \pm 8.2 a	45.2 \pm 7.5	39.5 \pm 4.5 a	28.0 \pm 2.4 a	34.5 \pm 3.9 b	25.8 \pm 4.0	19.3 \pm 0.5	22.6 \pm 1.7	29.8 \pm 2.4	29.8 \pm 1.8
Ald D M	29.4 \pm 11.5 a	44.5 \pm 6.2 a	45.1 \pm 6.7	44.1 \pm 4.0 a	24.6 \pm 1.4 a	25.5 \pm 3.1 b	22.2 \pm 1.6	18.3 \pm 3.0	22.6 \pm 1.0	29.5 \pm 1.0	26.7 \pm 4.7
Gorse D M	35.8 \pm 2.1 ab	41.9 \pm 3.7 a	30.5 \pm 3.5	37.9 \pm 4.2 a	28.7 \pm 4.1 a	N/A	22.8 \pm 2.8	17.9 \pm 1.3	19.2 \pm 0.8	26.7 \pm 1.6	32.8 \pm 1.5
Gun D M	43.5 \pm 6.7 ab	34.6 \pm 1.2 a	27.8 \pm 2.31	28.2 \pm 2.1 a	24.1 \pm 1.2 a	22.2 \pm 3.13 a	13.5 \pm 4.56	14.7 \pm 2.2	19.3 \pm 1.4	28.6 \pm 1.9	29.7 \pm 1.4

Table 5. NO_3^- ($\mu\text{g g}^{-1}$ dry soil). Means \pm SEM ($n = 4$) Letters denote significant differences between treatments. There were no significant difference from day 27 onwards.

Day	1	3	7	13	27	41	55	69	97
Control	16.5 \pm 3.0 a	17.7 \pm 1.2 a	2.9 \pm 0.2 a	2.9 \pm 0.5 ab	4.3 \pm 0.5	1.3 \pm 0.2	2.9 \pm 0.3	1.8 \pm 0.2	2.6 \pm 0.3
AN100	64.1 \pm 18.8 ab	41.3 \pm 5.7 a	7.7 \pm 1.4 b	8.0 \pm 0.5 c	5.4 \pm 0.6	1.5 \pm 0.4	4.0 \pm 0.7	1.9 \pm 0.2	2.2 \pm 0.2
AN200	197.4 \pm 87.7 b	139.0 \pm 48.9 b	18.1 \pm 2.2 c	15.0 \pm 0.5 d	38.6 \pm 23.3	1.3 \pm 0.3	4.6 \pm 0.7	2.0 \pm 0.4	3.1 \pm 0.8
Ald F Inc	11.6 \pm 1.5 a	13.2 \pm 3.3 a	2.2 \pm 0.3 a	2.6 \pm 0.3 ab	7.1 \pm 0.7	1.5 \pm 0.3	5.6 \pm 1.0	2.3 \pm 0.2	2.2 \pm 0.1
Ald F M	14.9 \pm 2.0 a	11.9 \pm 2.1 a	2.6 \pm 0.3 a	2.3 \pm 0.3 ab	4.4 \pm 0.6	1.3 \pm 0.2	4.5 \pm 1.1	3.2 \pm 0.4	2.5 \pm 0.2
Ald D Inc	14.7 \pm 3.0 a	12.0 \pm 1.1 a	2.7 \pm 0.4 a	2.7 \pm 0.1 ab	5.1 \pm 1.1	1.1 \pm 0.1	6.6 \pm 1.8	2.7 \pm 0.3	3.2 \pm 0.2
Ald D M	13.9 \pm 0.4 a	19.9 \pm 4.3 a	3.2 \pm 0.4 a	3.9 \pm 0.4 b	5.4 \pm 0.8	1.6 \pm 0.2	4.3 \pm 1.4	2.9 \pm 0.9	2.3 \pm 0.2
Gorse D M	16.0 \pm 1.9 a	18.9 \pm 0.8 a	2.5 \pm 0.4 a	2.9 \pm 0.2 ab	5.1 \pm 0.8	1.3 \pm 0.2	2.5 \pm 0.1	2.3 \pm 0.2	2.5 \pm 0.2
Gun D M	13.6 \pm 0.7 a	11.6 \pm 0.8 a	2.5 \pm 0.1 a	2.2 \pm 0.1 a	3.9 \pm 0.9	1.4 \pm 0.1	4.1 \pm 1.1	3.1 \pm 0.2	2.9 \pm 0.3

Table 6. DOC ($\mu\text{g g}^{-1}$ dry soil). Means \pm SEM ($n = 4$)

Day	1	3	7	13	27	41	55	69	97
Control	135.3 \pm 7.0	146.2 \pm 10.0	151.2 \pm 16.4	138.2 \pm 1.6	133.8 \pm 9.6	101.8 \pm 4.8	107.6 \pm 2.5	89.1 \pm 7.4	130.7 \pm 2.5
AN100	147.9 \pm 9.4	157.3 \pm 7.5	162.6 \pm 3.5	155.8 \pm 9.1	124.2 \pm 5.7	106.9 \pm 3.1	111.4 \pm 3.7	105.1 \pm 2.8	125.1 \pm 13.9
AN200	134.7 \pm 7.2	199.2 \pm 24.8	175.8 \pm 13.2	158.0 \pm 14.6	129.1 \pm 11.5	127.3 \pm 4.7	94.0 \pm 13.5	110.8 \pm 9.6	159.3 \pm 35.6
Ald F Inc	171.5 \pm 7.6	165.0 \pm 3.0	176.2 \pm 12.3	158.5 \pm 11.7	164.9 \pm 10.5	111.4 \pm 4.2	110.6 \pm 3.0	100.8 \pm 6.7	148.9 \pm 7.9
Ald F M	162.4 \pm 10.1	189.1 \pm 11.1	153.6 \pm 7.3	155.9 \pm 11.2	144.8 \pm 6.1	123.0 \pm 11.9	115.3 \pm 8.2	119.2 \pm 10.9	147.9 \pm 9.4
Ald D Inc	164.4 \pm 65.8	206.1 \pm 60.9	197.6 \pm 13.9	154.3 \pm 7.1	132.0 \pm 9.0	205.8 \pm 17.3	229.8 \pm 145.7	111.5 \pm 5.6	168.8 \pm 12.8
Ald D M	139.7 \pm 53.9	201.3 \pm 10.1	194.5 \pm 11.5	186.6 \pm 16.0	118.0 \pm 6.3	145.0 \pm 16.9	113.6 \pm 4.8	103.3 \pm 10.7	164.2 \pm 7.6
Gorse D M	175.2 \pm 7.8	180.8 \pm 17.0	167.1 \pm 12.2	146.2 \pm 11.0	131.7 \pm 12.4	N/A	107.7 \pm 7.5	107.1 \pm 9.0	138.4 \pm 6.2
Gun D M	219.6 \pm 34.1	193.8 \pm 6.5	166.1 \pm 5.8	157.4 \pm 7.8	122.7 \pm 2.0	129.3 \pm 9.9	108.6 \pm 14.4	91.5 \pm 11.6	145.8 \pm 14.1

Table 7. Soil water content ($\text{g H}_2\text{O g}^{-1}$ dry soil). Means \pm SEM ($n = 4$)

Day	1	3	7	13	20	27	34	41	55	69	97
Control	0.22 \pm 0.01	0.22 \pm 0.00	0.28 \pm 0.01	0.26 \pm 0.01	0.27 \pm 0.01	0.20 \pm 0.01	0.22 \pm 0.01	0.23 \pm 0.01	0.26 \pm 0.01	0.24 \pm 0.01	0.31 \pm 0.01
AN100	0.21 \pm 0.01	0.24 \pm 0.01	0.28 \pm 0.01	0.24 \pm 0.02	0.29 \pm 0.01	0.16 \pm 0.01	0.21 \pm 0.01	0.23 \pm 0.02	0.27 \pm 0.02	0.23 \pm 0.01	0.21 \pm 0.07
AN200	0.22 \pm 0.01	0.23 \pm 0.00	0.29 \pm 0.02	0.25 \pm 0.02	0.25 \pm 0.01	0.16 \pm 0.01	0.21 \pm 0.00	0.23 \pm 0.02	0.26 \pm 0.01	0.28 \pm 0.08	0.31 \pm 0.01
Ald F Inc	0.23 \pm 0.01	0.24 \pm 0.01	0.28 \pm 0.01	0.26 \pm 0.01	0.26 \pm 0.01	0.19 \pm 0.00	0.24 \pm 0.01	0.27 \pm 0.01	0.26 \pm 0.01	0.24 \pm 0.02	0.31 \pm 0.02
Ald F M	0.23 \pm 0.02	0.26 \pm 0.01	0.28 \pm 0.01	0.27 \pm 0.00	0.27 \pm 0.01	0.18 \pm 0.01	0.24 \pm 0.01	0.27 \pm 0.01	0.29 \pm 0.01	0.26 \pm 0.01	0.32 \pm 0.01
Ald D Inc	0.21 \pm 0.01	0.23 \pm 0.01	0.29 \pm 0.01	0.25 \pm 0.02	0.26 \pm 0.01	0.20 \pm 0.01	0.23 \pm 0.01	0.26 \pm 0.01	0.28 \pm 0.01	0.25 \pm 0.00	0.35 \pm 0.03
Ald D M	0.23 \pm 0.00	0.23 \pm 0.01	0.28 \pm 0.02	0.26 \pm 0.01	0.23 \pm 0.01	0.19 \pm 0.01	0.24 \pm 0.00	0.28 \pm 0.02	0.28 \pm 0.02	0.26 \pm 0.01	0.30 \pm 0.01
Gorse D M	0.22 \pm 0.02	0.24 \pm 0.01	0.27 \pm 0.01	0.25 \pm 0.01	0.24 \pm 0.02	0.19 \pm 0.01	0.23 \pm 0.01	0.28 \pm 0.00	0.28 \pm 0.01	0.27 \pm 0.01	0.28 \pm 0.01
Gun D M	0.23 \pm 0.01	0.25 \pm 0.01	0.27 \pm 0.01	0.26 \pm 0.01	0.24 \pm 0.01	0.20 \pm 0.01	0.25 \pm 0.00	0.23 \pm 0.01	0.28 \pm 0.01	0.25 \pm 0.02	0.29 \pm 0.01

Table 8. Microbial biomass (mg C g⁻¹ dry soil) Means ± SEM (*n* = 4)

Day	27	98
Control	0.69 ± 0.03	0.62 ± 0.03
AN100	0.62 ± 0.04	0.54 ± 0.05
AN200	0.59 ± 0.05	0.59 ± 0.05
Ald F Inc	0.91 ± 0.12	0.66 ± 0.05
Ald F M	0.70 ± 0.05	0.48 ± 0.12
Ald D Inc	0.73 ± 0.18	0.63 ± 0.07
Ald D M	0.72 ± 0.05	0.56 ± 0.06
Gorse D M	0.70 ± 0.04	0.71 ± 0.05
Gun D M	0.75 ± 0.05	0.57 ± 0.07

Table 9. Multivariate regressions with N₂O as the dependent variable and temperature, soil WFPS, NO₃⁻ and DOC as the independent variables. Unstandardised coefficients represent an increase of 1 µg N₂O-N m⁻² h⁻¹ for each additional unit of the variable.

Variable	Unstandardised Coefficient	Significance (<i>P</i> value)
Temperature (°C)	8.33	< 0.01
WFPS (%)	1.225	0.048
DOC (mg g ⁻¹)	218.82	0.052
NO ₃ ⁻ (mg g ⁻¹)	-108.15	0.376

Table 10. Daily minimum, maximum and mean ground temperatures (°C)

days from start of trial	Date	Min	Max	Mean
0	21/06/2017	17.23	32.01	24.62
1	22/06/2017	15.2	23.14	19.17
2	23/06/2017	14.51	20.01	17.26
3	24/06/2017	14.49	24	19.245
4	25/06/2017	14.57	21.19	17.88
5	26/06/2017	13.99	26.23	20.11
6	27/06/2017	15.34	22.78	19.06
7	28/06/2017	14.82	18.64	16.73
8	29/06/2017	13.84	15	14.42
9	30/06/2017	14.02	15.29	14.655
10	01/07/2017	13.88	23.15	18.515
11	02/07/2017	13.92	23.67	18.795
12	03/07/2017	14.6	20.42	17.51
13	04/07/2017	15.77	22.6	19.185
14	05/07/2017	16.47	24.37	20.42
15	06/07/2017	15.93	27.27	21.6
16	07/07/2017	15.58	23.81	19.695
17	08/07/2017	14.56	26.17	20.365
18	09/07/2017	14.79	23.24	19.015
19	10/07/2017	16.1	20.45	18.275
20	11/07/2017	15.73	20.45	18.09
21	12/07/2017	14.31	25.24	19.775
22	13/07/2017	13.95	23.82	18.885
23	14/07/2017	15.51	21.65	18.58
24	15/07/2017	15.81	23.72	19.765
25	16/07/2017	16.28	25.46	20.87
26	17/07/2017	15.3	26.25	20.775
27	18/07/2017	14.72	27.49	21.105

28	19/07/2017	17.73	26.77	22.25
29	20/07/2017	15.3	21.69	18.495
30	21/07/2017	15.61	18.68	17.145
31	22/07/2017	14.46	22.25	18.355
32	23/07/2017	14.68	23.72	19.2
33	24/07/2017	16.58	24.94	20.76
34	25/07/2017	15.68	23.7	19.69
35	26/07/2017	14.75	20.34	17.545
36	27/07/2017	14.47	19.28	16.875
37	28/07/2017	14.4	18.89	16.645
38	29/07/2017	14.63	19.83	17.23
39	30/07/2017	14.66	18.95	16.805
40	31/07/2017	14.38	17.73	16.055

days from start of trial	Date	Min	Max	Mean
41	01/08/2017	14.57	18.49	16.53
42	02/08/2017	14.73	23.02	18.875
43	03/08/2017	15.35	20.24	17.795
44	04/08/2017	15.59	22.27	18.93
45	05/08/2017	14.38	22.06	18.22
46	06/08/2017	14.42	19.35	16.885
47	07/08/2017	14.67	19.96	17.315
48	08/08/2017	14.59	20.52	17.555
49	09/08/2017	15	20.11	17.555
50	10/08/2017	15.07	21.35	18.21
51	11/08/2017	14.98	17.99	16.485
52	12/08/2017	15.42	18.97	17.195
53	13/08/2017	14.09	19.3	16.695
54	14/08/2017	15.53	19.11	17.32

55	15/08/2017	14.79	18.93	16.86
56	16/08/2017	14.44	20.21	17.325
57	17/08/2017	15.79	21.38	18.585
58	18/08/2017	15.06	19.35	17.205
59	19/08/2017	14.56	18.03	16.295
60	20/08/2017	13.98	20.12	17.05
61	21/08/2017	14.35	20.57	17.46
62	22/08/2017	16.22	21.91	19.065
63	23/08/2017	15.83	20.3	18.065
64	24/08/2017	15.55	19.57	17.56
65	25/08/2017	15.12	18.82	16.97
66	26/08/2017	15.06	22.16	18.61
67	27/08/2017	14.84	21.4	18.12
68	28/08/2017	15.12	21.44	18.28
69	29/08/2017	14.85	19.15	17
70	30/08/2017	14.37	19.19	16.78
71	31/08/2017	14.09	17.28	15.685
72	01/09/2017	13.51	17.91	15.71
73	02/09/2017	12.97	18.83	15.9
74	03/09/2017	14.47	17.37	15.92
75	04/09/2017	15.6	18.32	16.96
76	05/09/2017	15.03	17.08	16.055
77	06/09/2017	14.38	16.92	15.65
78	07/09/2017	14.44	16.9	15.67
79	08/09/2017	13.73	17.16	15.445
80	09/09/2017	13.49	17.33	15.41
81	10/09/2017	12.83	16.83	14.83
82	11/09/2017	12.71	15.41	14.06
83	12/09/2017	12.31	16.75	14.53
84	13/09/2017	11.93	15.9	13.915

85	14/09/2017	11.46	15.73	13.595
86	15/09/2017	12.16	15.7	13.93
87	16/09/2017	11.69	15.65	13.67
88	17/09/2017	10.59	15.94	13.265
89	18/09/2017	11.72	16.68	14.2
90	19/09/2017	11.2	16.94	14.07
91	20/09/2017	13.02	18.06	15.54
92	21/09/2017	11.9	16.44	14.17
93	22/09/2017	11.11	15.66	13.385
94	23/09/2017	11.15	19.28	15.215
95	24/09/2017	13.2	16.79	14.995
96	25/09/2017	13.11	18.14	15.625
97	26/09/2017	12.09	18.46	15.275
98	27/09/2017	13.38	15.83	14.605
99	28/09/2017	12.12	17.49	14.805
100	29/09/2017	11.93	14.93	13.43
101	30/09/2017	11.53	15.81	13.67
102	01/10/2017	12.98	16.69	14.835
103	02/10/2017	11.7	13.86	12.78
104	03/10/2017	10.84	13.55	12.195
105	04/10/2017	10.96	13.28	12.12

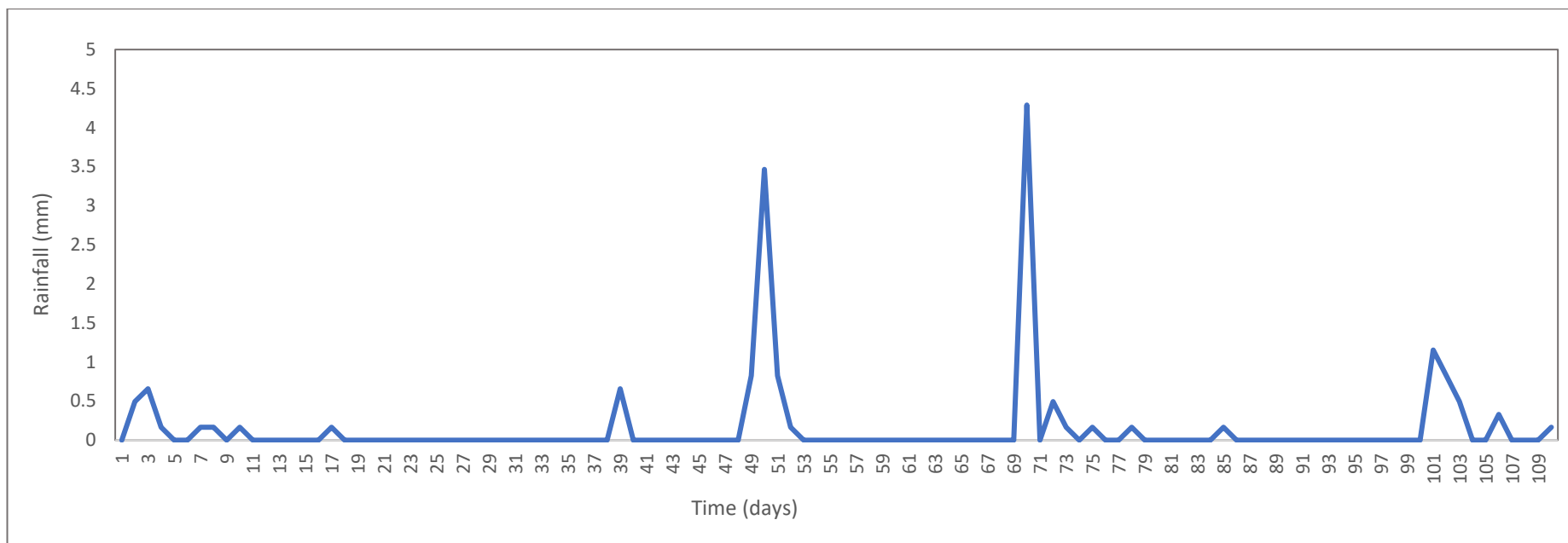


Fig 2. Rainfall during the duration of the field experimentl (mm). Total rainfall for the period = 16.34 mm

Appendix 3. Supporting data chapter 6

Table 1 Root biomass and root:shoot ratio. Means \pm SEM ($n = 4$).

Treatment	Root dry weight (g m ²)	Root: Shoot ratio
Control	131 \pm 40	0.14 \pm 0.02
AN 100 + 100	135 \pm 18	0.12 \pm 0.02
AN 200 + 0	118 \pm 21	0.13 \pm 0.01
AN 200 + 200	103 \pm 15	0.09 \pm 0.02
Ald + 0	141 \pm 20	0.14 \pm 0.03
Ald + Ald	232 \pm 73	0.22 \pm 0.06
Gun + 0	168 \pm 42	0.20 \pm 0.05
Gun + Gun	212 \pm 33	0.17 \pm 0.01

Table 2 N content Wheat (%) Means \pm SEM ($n = 4$).

	Root	Stems and chaff	Grain
0 + 0	0.70 \pm 0.09	0.47 \pm 0.03	2.01 \pm 0.04
AN 25 + 25	N/A	0.44 \pm 0.05	2.00 \pm 0.04
AN 50 + 50	N/A	0.49 \pm 0.03	1.94 \pm 0.04
AN 100 + 100	0.95 \pm 0.06	0.61 \pm 0.06	2.04 \pm 0.03
AN 200 + 0	0.71 \pm 0.02	0.42 \pm 0.04	1.87 \pm 0.08
AN 200 + 200	1.00 \pm 0.07	0.75 \pm 0.06	2.18 \pm 0.16
Alder + 0	0.69 \pm 0.06	0.50 \pm 0.06	2.03 \pm 0.15
Alder + Alder	0.70 \pm 0.07	0.50 \pm 0.04	2.00 \pm 0.01
Gunnera + 0	0.62 \pm 0.07	0.47 \pm 0.04	1.97 \pm 0.09
Gunnera + Gunnera	0.68 \pm 0.10	0.56 \pm 0.04	2.13 \pm 0.07

Table 3 Grain Quality. Means \pm SEM ($n = 4$).

Treatment	Grain dry weight (t ha ⁻¹)	Grain TGW (g)	Grain % N
Control	5.1 \pm 0.9	41.7 \pm 1.0	2.01 \pm 0.04
AN 25 + 25	5.7 \pm 1.1	41.0 \pm 0.6	2.00 \pm 0.04
AN 50 + 50	7.8 \pm 1.3	39.8 \pm 1.3	1.94 \pm 0.04
AN 100 + 100	6.1 \pm 0.6	37.0 \pm 1.1	2.04 \pm 0.03
AN 200 + 0	5.2 \pm 0.8	41.6 \pm 0.9	1.87 \pm 0.08
AN 200 + 200	6.9 \pm 0.6	37.2 \pm 1.5	2.18 \pm 0.16
Ald + 0	5.7 \pm 0.8	39.9 \pm 2.0	2.03 \pm 0.15
Ald + Ald	6.0 \pm 0.8	42.9 \pm 0.8	2.00 \pm 0.01
Gun + 0	5.1 \pm 0.7	42.5 \pm 1.0	1.97 \pm 0.09
Gun + Gun	7.4 \pm 0.8	43.1 \pm 1.2	2.13 \pm 0.07

Table 4 pH. Pre-year 2 experiment (March) given as single values from NRM laboratories. Post-year 2 (September) as means \pm SE, $n = 4$). Letters denote difference at $P < 0.05$.

Treatment	Pre-experiment	Post-experiment
Control	6.0	6.1 \pm 0.1 ab
AN 100 + 100	5.9	6.5 \pm 0.2 ab
AN 200 + 0	5.9	6.0 \pm 0.3 ab
AN 200 + 200	5.9	5.7 \pm 0.1 a
Ald + 0	6.0	6.1 \pm 0.1 ab
Ald + Ald	6.0	6.3 \pm 0.1 ab
Gun + 0	6.3	6.8 \pm 0.2 b
Gun + Gun	6.3	6.7 \pm 0.2 b

Table 5 NH₃ emissions. Means \pm SEM ($n = 4$).

Treatment	NH ₃ emitted (mg NH ₃ N m ⁻²)
Control	0.04 \pm 0.03 a
AN 100 + 100	0.43 \pm 0.12 b
AN 200 + 0	0.05 \pm 0.03 a
AN 200 + 200	0.46 \pm 0.10 b
Ald + 0	0.08 \pm 0.02 a
Ald + Ald	0.03 \pm 0.01 a
Gun + 0	0.03 \pm 0.01 a
Gun + Gun	0.12 \pm 0.07 a

Table 6 NH₃ Emission Factors. % of Treatment N emitted as NH₃, paired by block. Means \pm SEM ($n = 4$).

Treatment	EF (%)
AN200 + 200	0.004 \pm 0.001
Ald + Ald	-0.001 \pm 0.000
Gun + Gun	0.001 \pm 0.001

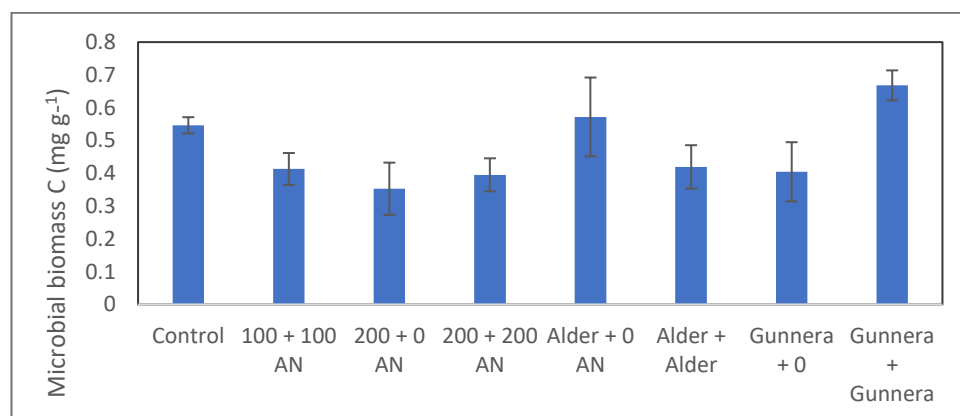
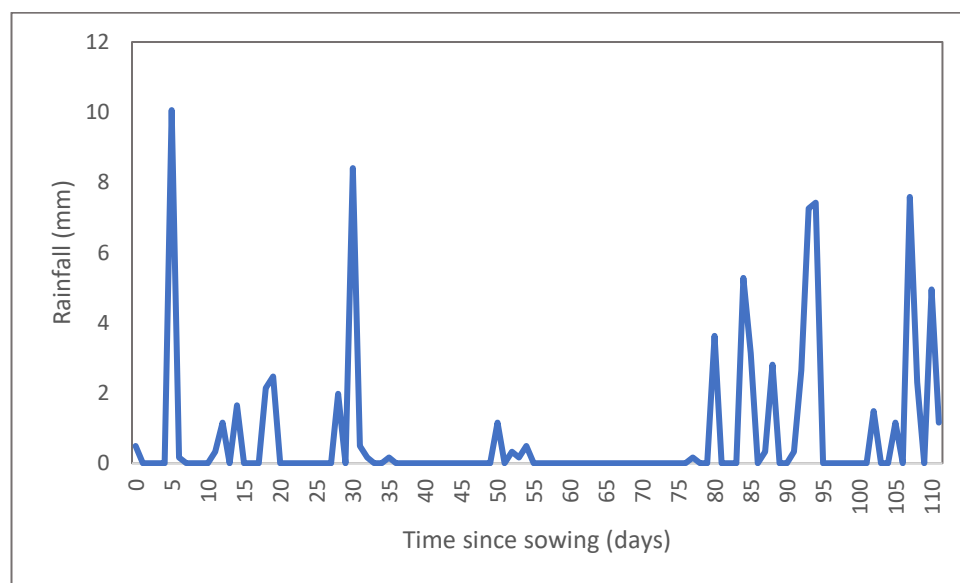


Fig 1. Microbial biomass C day 125. Means \pm SEM ($n = 4$).

Table 7 Output of multi linear regression for N₂O

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
1	(Constant)	-76.971	40.106		
	WFPS	1.570	.522	.258	3.009
	DOC	81.941	100.042	.062	.819
	NO3	-170.841	156.268	-.079	-1.093
	Temp	4.074	1.615	.215	2.523

a. Dependent Variable: N2O_flux

**Fig 2.** Rainfall during the course of the field experiment (year 2).