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Ecosystem carbon dynamics - as influenced by tree species and mixture in temperate deciduous woodland

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ABSTRACT

Enhancing carbon sequestration in woodland ecosystems through new planting is a recognized measure to mitigate anthropogenic emission of CO_2. However, species specific tree effects on biomass allocation (above and belowground), leaf decomposition, storage and stability of soil organic matter (SOM) under single species and mixtures are largely unclear. We investigated the ecosystem C pools and processes in response to species traits in single stands and the interactive effects in mixed stands of birch (*Betula pendula*), alder (*Alnus glutinosa*) and beech (*Fagus sylvatica*).

To estimate standing aboveground biomass, species specific allometric models were developed, and DBH and basal diameter were found as the best predictors of plant woody biomass. Significantly higher aboveground woody biomass was observed in the single stand of birch than alder and beech. In estimates of belowground biomass and turnover, fine root (< 2 mm) biomass was higher in alder whilst the root turnover rate was higher in birch. At the stand level, clear additive mixture effects on above ground biomass was observed, however at the tree level, birch tended to lower biomass in mixture presumably due to suppression by the faster growing alder. Significantly lower biomass of beech was observed in mixture compare to monoculture. A similar pattern was observed with fine root biomass production.

In investigations of leaf litter decomposition, in laboratory incubation experiment significantly higher level of water soluble phenolics was found in the soil solution with birch and beech leaf litter. An *in situ* litterbag experiment also showed a faster relative decay rate of alder leaf litter compare to birch and beech. This is suggested to be due to higher litter quality of alder, and the higher secondary metabolites in birch and beech. The absolute decay rates and the mass loss revealed a two phase decay pattern. Clear mixture effects were observed with a slower decay during initial stage and higher decay rate at latter stage, suggesting possible antagonistic effects of species specific compounds in the mixed litter.

After 4 years of afforestation, 7.3 and 8.0 kg m^{-2} C stocks in the top metre of soils planted with trees and grassland were estimated, respectively. Up to 40% of total the SOC stock was found in subsoil layers (30-100 cm) suggesting significant contribution of deep soil C pools to sequestration. No clear effect of tree species or mixture on C pool size was observed. Over all, our studies revealed that in addition to species specific effects, C storage in soil is largely controlled by soil conditions. Fractionation of SOM into easy degradable labile and recalcitrant pools revealed that species identity and composition did not affect relative proportions of these fractions in the top 2 soils layers, however; in deep layers differences were highly statistically significant. The absolute recalcitrant pool in top meter soil increased following the order: grassland < beech < alder < birch < mixed soil. Of the total storage, 30-51 % C was recalcitrant. Litter quality particularly root litter and subsequent decomposition- translocation interactions might be the cause of the high labile C in the deep soil layers.

Overall C dynamics in different plant species showed that birch stands have the highest aboveground woody biomass. In addition, higher root turnover rate and slower leaf litter decomposition were found in birch stands than alder, suggesting favourable traits for long term storage of C in soil. However, the antagonistic effects on leaf litter decomposition, relatively higher fine root production and turnover, together with the highest recalcitrant SOC pool suggests that, tree mixtures might be the best option in plantations.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
DBH	Diameter at Breast Height
DEFRA	Department of Environment, Foor and Rural Affairs
DOC	Dissolved Organic Carbon
DIN	Dissolved Inorganic Nitrogen
DOM	Dissolved Organic Matter
DON	Dissolved Organic Nitrogen
EC	Electrical Conductivity
ECCP	European Climate Change Programme
FACE	Free Air Carbon Enrichment
FAO	Food and Agriculture Organization
HSD	Honestly Significant Difference
IPCC	Intergovernmental Panel for Climate Change
LULUCF	Land Use, Land Use Change and Forestry
MRT	Mean Residence Time
NSI	National Soil Inventory
RMSE	Root Mean Square of Error
SEM	Standard Error of Mean
SCOPE	Scientific Committee On Problems of Environment
SOC	Soil Organic Carbon
SOM	Soil Orgain Matter
SPSS	Statistical Package for the Social Science
TXRF	Total Reflaction X-Ray Fluorescence

1. General Introduction

1.1 Introduction

1.1.1 Carbon in a changing world

The frequently asked question "Are the increases in atmospheric carbon dioxide and other greenhouse gasses during the industrial era caused by human activities?" has been answered yes with a high degree of certainty by the IPCC (IPCC, 2007). Approximately 650 thousand year ago, during the glacial-interglacial period, the atmospheric concentration of CO_2 was between 180 ppm (glacial maxima) to 300 ppm (warm interglacial maxima) (Siegenthaler *et al.* 2005). Before the industrial revolution (1750) it was relatively constant at 280 ±10 ppm for several thousand years (IPCC, 2001). After the 1750, the CO_2 concentration has been increasing gradually, and in July 2011 it was 393.7 ppm (*CO_2 Now.org*, 2011) and the predicted concentration in 2100 would be 540 to 970 ppm (SCOPE, 2006). There is a common consensus among the scientists that global warming and consequent climate change are influenced largely by the increasing atmospheric concentration of CO_2 (Beedlow *et al.* 2004). Thus the most basic building material of life in the earth has become the civilization's greatest threat (Roston, 2008). The concern over the huge impacts of elevated CO_2 on earth's climate change and subsequently on different ecosystems and its functions has received much attention in the recent decades (IPCC, 2007).

The two major human activities that contribute to elevation of atmospheric CO_2 are use of fossil fuel and change in land use, especially deforestation and agricultural development which accounted for 65 and 35 % of CO_2 increment during the last 255 years respectively (IPCC, 2007). Deforestation is the single major process responsible for nearly 90 % of the estimated CO_2 release originated from land use change since 1850 (IPCC, 2001). On the other hand afforestation and formation of new woodland has been recognized as a potential mitigation measure (IPCC, 2007). Thus the potential of forest management for reduction of both further emission and existing concentration of atmospheric CO_2, is leading to many research efforts world wide at local, regional and global scales.

1.1.2 Temperate forest ecosystem and C dynamics

Temperate forest includes climate with moderate winter frost and regular precipitation. Globally the largest C sink is in the northern hemisphere (Houghton, 2003) and Goodale *et al.* (2002) estimated a net sink of 0.6 to 0.7 Pg of C y^{-1} in the temperate forest of the northern hemisphere. However, under the background of global change it is uncertain whether the current C sequestration rate can be sustained (Goodale *et al.* 2002). In temperate woodland, the plants traits such as growth rate, litter quality and quantity, enhance the soil C cycling faster compared with boreal forests (De Deyn *et al.* 2008). Adequate knowledge about forest C cycle is necessary for a proper understanding of ecosystem responses to future climate change.

From biomass to humus, C travels through many pools and transfer from one pool to another through particular processes in forest ecosystems (Beedlow *et al.* 2004). The major pools of C in forest ecosystems are biomass C (above and belowground), forest litter, soil organic matter, microbial biomass and humus. Among different processes,

photosynthesis, canopy respiration, litter flux, litter decomposition, soil respiration, SOC sequestration etc. play an important roles in C dynamics. Forest ecosystems are intrinsically dynamic and continuously influenced by the climate and climate changes (Rabindranath and Ostwald, 2008). Therefore the C pools and processes also change in response to changing time and other climatic variables. To quantify the rate of changes is necessary to assess such natural dynamic entities. Ecosystem C inventory is essential for C mitigation and greenhouse gas inventory, forest conservation and land development programme. To improve the estimation of C storage at ecosystem level it is essential to assess the C dynamics in different pools and processes in an integrated approach.

1.1.3 Carbon dynamics - in relation to single and mixed woodland

Net primary production (NPP) is the annual plant biomass that remains in the woodland ecosystem after release of CO_2 as autotrophic respiration. Part of this NPP is subjected to another two processes viz. decomposition and heterotrophic respiration when biomass transfered to the forest floor as litter. These ecosystem processes are generally controlled by the plant's functional traits in a species specific way (De Deyn *et al.* 2008). Litter quality from broadleaved trees is generally higher than that from needle leaf (Silver and Miya, 2001) but differences between broadleaved species and even between genotypes was also observed in temperate forest. Most studies on the role of species traits in C cycling have focused on above ground biomass while only recently the importance of belowground litter for soil C cycling in temperate forest has become apparent (Pollierer *et al.* 2007, Matamala *et al.* 2003). Species specific root turn over rate contributes to ecosystem C cycling in association with ecto- and arbuscular mycorrhizal fungi in broadleaved temperate forests (Cornelissen *et al.* 2001; Read and Perez-Moreno, 2003).

It has been long debated whether mono-species woodlands or mixed-species forests can fulfil the requirements of sustainability (Knoke *et al.* 2008). In many countries of Central and northern Europe it is currently a major objective of forest management and policy to convert coniferous mono-species forest into deciduous or mixed species forest justified mainly by expected ecological advantages (Baumgarten and von Tueffel, 2005; Fritz, 2006). Growth, wood quality and management-simplicity are the principal advantages of monoculture (Kelty, 2006). On the other hand, resisitance against biotic and abiotic disturbances, ecological stability and stand level productivity are the factors that favour the mixed species woodland (Kelty, 2006; Konke *et al.* 2008). The environmental benefits of tree mixtures and their effects on C storage have recently been focused. In the present study the effects of three broadleaved tree species and their mixture on C dynamics have been investigated to elucidate the responses of tree mixture on C transformation and storage in woodland ecosystems.

1.1.4 Native broadleaved tree species

The broadleaf species of trees are particularly important for its higher contribution to carbon sequestration than the narrow leaf coniferous plants. The forestry policy of UK supports the extension of native broadleaf species in connection with removal of carbon from the atmosphere. The efficiency of CO_2 uptake is higher in broadleaved species than coniferous because of the low leaf area of coniferous species (Baldocchi and Vogel, 1996). Planting of local provenances of native species is also important due to adaptation to local conditions and to maintain biodiversity and a native genetic base (Ennos *et al.* 2000).

Forest plantations have been advocated as a measure to sequestrate C from the atmosphere and to mitigate future climate change (Winjum and Schroeder, 1997). However, it is uncertain whether the plantations are net sink for C at an ecosystem level

and this depends on a various factors including stand type, land use history, climate and geographic conditions (Liao *et al.* 2010). The responses of species diversity on ecosystem C dynamics have been studied between broadleaved and coniferous species in many previous studies. However, few studies addressed the comparison among the broadleaved species. For common native broadleaved species it is important to elucidate C pools and processes in birch, alder and beech stands to examine species interactions for better understanding of species specific responses to ecosystem C dynamics.

1.1.5 Study on species specific effects – common garden approach

To study the ecosystem C dynamics and to evaluate the species specific effects on different C pools and processes, identical pedo-climatic conditions is prerequisite because in addition to soil and climate factors, tree species may be one of the possible factors that influence the ecosystem C flow (Vesterdal *et al.* 2008). Most of the studies evaluate the comparative response of tree species growing under different locations and site conditions and consequently the differences in soil properties such as parent materials, hydrology, management, land use etc. can influence the assessment of species effects (Binkley, 1995; Vesterdal *et al.* 2008). Although limited common garden experiments were conducted, most of those suffered from lack of replications (Hobbie *et al.* 2006; Oostra *et al.* 2006). Therefore it is imperative to follow strict common garden design with sufficient replicates to explore the effects of different tree species on ecosystem biogeochemical processes.

1. 2 Objectives and hypotheses

1.2.1 Aim and Objectives:

The main aim of the research project is to study the major C pools and transformation processes in the woodland ecosystem of single and mixed plantations of birch (*Betula pendula*), alder *(Alnus glutinosa*) and beech (*Fagus sylvatica*). The following objectives have been set for this study:

1. To study the influence of plant species and mixtures on above and belowground biomass, root biomass production and turnover.
2. To developed allometric models for birch, alder and beech plants to predict the aboveground woody biomass.
3. To study the of leaf litter decomposition dynamics in mono and mixed culture stand of birch, alder and beech.
4. To estimate organic C storage in soils in relation to single and mixed species tree plantings.
5. To fractionate storage C in single and mixed stands of birch, alder and beech into labile and recalcitrant pools.

1.2.1 Hypotheses

The following four hypotheses will be tested during the studies

1. Mixed species stands of birch, alder and beech have higher aboveground and fine root standing biomass, fine root biomass production compared with monoculture stand under similar pedo-climatic conditions.
2. In mixed stands of birch, alder and beech, the decomposition rate of mixed leaf litter is faster compared with decomposition rate of pure leaf litter in their respective stands.
3. Soil organic carbon storage in deciduous tree stand is higher than the adjacent grassland; and in mixed stands of birch, alder and beech, the C stock is higher than single stands of component species.
4. Mixed species stands of birch, alder and beech have higher recalcitrant C pool in soil compare to mono species stand.

2. Review of literature

2.1 Above and belowground biomass

2.1.1 Plant biomass – definition

Generally, the biomass of forest stands can be defined as the quantity of dry materials or sometimes expressed as the amount of carbon contained in woody plants (trees and shrubs) and under story vegetation per unit area (gm^{-2}). According to FAO (2004) biomass is "organic material both above-ground and belowground, and both living and dead, e.g., trees, crops, grasses, stem, stump, branches, bark, seeds, and foliage". Above ground biomass is the total amount of biological material present above the soil surface over a specified area (Drake *et al.* 2003). In a forest stand, tree biomass is usually the major fraction of standing biomass. Tree biomass is generally divided into different components such as foliage, branches, stem, stump etc. on the basis of physiological functions. As atmospheric CO_2 sequestrates in the plant biomass through photosynthesis processes, the quantification of the vegetative biomass becomes essential in forest ecosystem studies in order to estimate carbon pools at multiple scales (Losi *et al.* 2003).

Another part of tree biomass is below-ground biomass or root biomass which includes all structural coarse roots, mycorrhizal fine roots and the mycorrhizal hyphal mycelium (Lukac and Godbold, 2011). The contribution of coarse roots is mainly as support organs and as long-distance transport pathways, and the fine roots in association with mycorrhizal fungi, facilitates nutrient and water uptake, and the uptake of nutrients often involves secretion of root exudates (Smith and Read, 1997). Most of the previous biomass assessment studies conducted focus on above-ground forest biomass (Aboal *et al.*

2005; Brown, 1997; Losi *et al.* 2003) because generally it accounts for the majority of the total accumulated biomass in the forest ecosystem. However, recently research studies on the functions and ecological role of root biomass has received more attention (Millikin and Bledose, 1999), realising the fact that root production contributes about half of the C being cycled annually in many forest ecosystem (Vogot *et al.* 1996), and 33% of the global annual net primary production (Jackson *et al.* 1997).

2.1.2 Importance of biomass

Estimation of biomass is important for many purposes (Zheng *et al.* 2004). At a national or regional level, when biomass is considered as a raw materials or energy source, it is necessary for planner and policy maker to know how much timber or fuel wood is available for national consumption. From an environmental management point of view, biomass quantification is important to assess the productivity and sustainability of the forest. Biomass is also an important indicator of carbon sequestration, as forest biomass absorbs C from the atmosphere and stores it in the plant tissue (Matthews *et al.* 2000). To study the sequestration potential it is necessary to estimate forest biomass. To fulfil the requirements of the Kyoto protocol it is necessary to estimate the removal and accumulation of C in forest biomass.

2.2.3 Forest biomass and climate change

Carbon dioxide (CO_2) is one of the major greenhouse gases (approximately 72% of the total anthropogenic greenhouse gases) and considered as a primary agent of global warming (IPCC, 2007). It has been estimated that CO_2 is responsible for about 9-26% of the global greenhouse effect (Kiehl and Trenberth, 1997). IPCC (2007) reported that the amount of carbon dioxide in the atmosphere has increased from 280 ppm of the pre-

industrial era (1750) to 379 ppm in 2005, and is increasing by 1.5 ppm per year. The dramatic rise of CO_2 concentration is attributed largely to human activities. Deforestation is the human induced conversion of forest to non-forest land use and causes immediate emission of huge forest carbon stocks through land clearing (IPCC, 2007). Due to deforestation, C is released from both plant biomass and emission of soil C due to disturbance. Forest degradation especially non sustainable harvesting, anthropogenic disturbance and collection of fuel wood etc. cause substantial reduction in forest C stock (IPCC, 2007). Thus, the destruction of forest biomass has raised concerns over global warming and climate changes at a global scale. Conversely, sustainable forest management measures and preventing deforestation can play key role in mitigation of climate change (IPCC, 2001). As the most widely distributed terrestrial ecosystcem on earth, forests produce 70 percent of the annual net global carbon accumulation which results in the uptake of atmospheric carbon and the conversion of green house gases to biomass (Wulder, 1998). Therefore forests play a significant role in the carbon cycle, through both CO_2 uptake and emission and thus regulating global climate change.

2.1.4 Plant biomass and carbon sequestration

Forest biomass maintains a potential role in the uptake and reabsorbion of some of the excess CO_2, which emitted due to burning of fossil fuel and deforestation. Sequestration of carbon in plant biomass and in the soil is an important environmental benefit of afforestation of agricultural lands (Uri *et al.* 2007). The forest ecosystem plays a very important role in the global carbon cycle. It stores about 80% of all above-ground and 40% of all below-ground terrestrial organic carbon (IPCC, 2001). Losi *et al.* (2003) and Phat *et al.* (2004) suggested that during the productive season, CO_2 from the atmosphere is taken up by vegetation and stored as plant biomass. For this reason, the UNFCC and its

Kyoto Protocol recognized the role of forests in carbon sequestration especifically, Article 3.3 and 3.4 of the Kyoto Protocol pointed out forest as potential carbon sequester (Brown, 2002). It was reported that a hectare of actively growing forest can sequester 2-5 t of carbon per year (Brown, 1996).

2.1.5 Plant biomass in single and mixed culture plantings

The key concept regarding the higher biomass production in mixtures is that the species in mixture should differ in characteristics such as shade tolerance, height growth rate, crown structure, foliar phenology and root depth etc. so that they can use resources more efficiently in producing biomass, resulting a greater total stand biomass production than would occur in monocultures of the component species (Kelty, 2006). Montagnini (2000) pointed out that if planned with consideration for each species' response to mixed condition; mixed design can be more productive than monoculture systems. Binkley *et al.* (1997) and Folster and Khanna (1997) suggested that the component species with different nutrient requirements and different nutrient recycling properties may be overall less demanding on site nutrients than pure stands. Many investigations have been designed to develop the facilitative and complementary interactions in the mixture. For example, incorporation of N fixing species with a non N fixing valuable timber species showed substantial growth responses due to increased N availability (Kelty, 2006). However, these types of interactions were not always successful because of competitive effects between the companion species in mixture. Parrotta *et al.* (1999) reported no significant difference in total biomass production between mixture and monoculture plantations in an experiment using *Eucalyptus robusta* and N fixing *Casuarina equisetifolia.* In this case no stratified canopy was formed and the mean height of the both species did not differ greatly and N fixing species was a strong competitor to eucalyptus.

1.1.6 Methods of biomass estimation (Aboveground biomass)

Many approaches have been developed world wide to estimate the aboveground biomass at the stand level. These can be categorized in three broad divisions: field measurement, remote sensing, and GIS-based approach (Lu, 2006). The field measurement is considered to be accurate but proves to be very costly and time consuming (de Gier, 2003). In any of these approaches, ground data is essential for validation.

Field measurement: The conventional method of biomass estimation is based on field measurements. However this approach is time consuming, labour intensive, and difficult to implement in remote areas (de Gier, 2003; Lu, 2004). For small scale studies, the conventional method may be appropriate; but for studying the area of wider spatial scale or the issue of studying carbon sequestration, the use of the field measurement approach is much more challenging. The most common approach to estimate the above ground biomass includes harvesting and measuring the dry mass of sample trees (Rana *et al.* 1988, Parresol, 1999) and use of allometric regression functions (Pajtíka *et al.* 2008). Allometric functions established in one area are often expected to be applicable to areas with a similar climate and other conditions, e.g. site conditions, silvicultural measures (Kärkkäinen, 2005).

Two methods of measuring model tree biomass are generally followed: (1) destructive and (2) non-destructive. The commonly used destructive method includes the felling of the model trees, recording different biometric measurements and carefully separating different parts of trees such as leaves, stem, branches, twigs, fruits and flowers, roots etc. and then weighing the fresh mass of each component. Direct weighing can only be done for small trees, but for larger trees, big portions can be cut into small parts and carefully bagged and labelled. In cases where the tree is large, volume of the stem is

measured. Sub-samples are collected from each section, and its fresh and oven-dry weight and volume are measured. The dry weight of the tree (biomass) is calculated based from the ratio of fresh weight (or volume) to the dry weight. However some authors mentioned that the procedure requires considerable amount of labour and cost (Ketterings *et al.* 2001).

The second stage of the studies consists of the development of allometric models from the obtained data, and finally uses the derived equations to assess the standing biomass of the study sites. De Gier (2003) suggested that the protocol for the forest biomass assessment based on allometric relationship, involves four steps: (1) selecting a suitable mathematical function for the allometric equations; (2) parameter estimation in the equation; (3) measurements of tree variables such as diameter at field level and (4) using the allometric equation to obtain area based data. Most of the studies use diameter at breast height (DBH) as the independent variable, and develop an allometric relationship between DBH and component biomass (Gower *et al.* 1999). Some studies proposed to include tree height (H) as the second predictor and develop DBH–H combined equation to improve the precision of biomass estimates (Ketterings *et al.* 2001). Other variables such as basal area, basal diameter has also been found to be appropriate (Alamgir and Al-Amin, 2008). Some equations are species-specific whilst others are generalized models having a great potential for large-scale carbon budgets derived from inventory data (Pastor *et al.* 1984). Ketterings *et al.* (2001) suggested that when estimating the above ground biomass of a forest, the use of species-specific equations is preferred because trees of different species may differ greatly in tree architecture and wood density.

The non-destructive approach includes biometric measurement of the whole tree by climbing the tree, measuring its various parts and computing the total volume. Tree density data can be used to convert the measured volume into biomass (Aboal *et al.* 2005). However, this procedure sometimes can be more time consuming and costly. Montes *et al.*

(2000) proposed photographic techniques to assess tree biomass. In this method two photographs of the tree at orthogonal angles are used to calculate each tree components (stem, branch, foliage) from photographic scale. Density of the different tree components is calculated and used to convert the volumes into biomass which is then validated against the model tree harvesting data.

Remote sensing: The role of remote sensing technologies for forest biomass assessment has also been recognized (Patenaude, 2005) and several studies had been conducted for this purpose (Chen *et al.* 2004; 2003; Lu *et al.* 2004; Rahman, 2005). Forest attribute information including species, crown closure, age, height and volume has usually been acquired through aerial photo interpretation (Leckie *et al.* 2003). In this technique multiple regression models are developed based on integration of satellite images and vegetation inventory data and thus provide a method for biomass estimation. The combination of GIS data and modelling techniques can improve the model performance (Lu *et al.* 2002). Lidar (light detection and ranging) technology is an active remote sensing tool that provides three dimensional vertical measurement of ground target, and thus can be quantified certain forest attributes such as mean stand height, horizontal and vertical crown dimensions etc. Using such attribute data, forest characteristics like stem diameter, basal area, above ground biomass can be calculated from allometric relationships (Lim *et al.* 2003). Although remote sensing techniques provides information on stand related parameters, Franklin and McDermid (1993) pointed out that most of the orbital sensors are inadequate to fully capture forest stand parameters with high level of confidence.

1.1.7 Methods of biomass estimation (Root biomass)

The relationship between global climate change and plant growth and the role of forests as C sequester have encouraged the refinement of the estimates of root biomass and production (Vogt *et al.* 1998). In comparison to above-ground biomass, the estimation of below-ground biomass is more complicated and laborious. Consequently fewer case studies have been conducted to investigate tree root biomass at a stand level, and more uncertainties exist in below-ground biomass estimation on a large-scale (Cannell, 1982; Gower *et al.* 2001).

Different procedures and techniques have been followed to study the fine root biomass and turnover in the field. However, so far no one technique has been recognized as the best universally (Vogt *et al.* 1998). The direct approaches are sequential soil coring, ingrowth cores, minirhizotrons and root mesh, whilst the indirect methods include carbon fluxes or nitrogen budget approaches and correlations of root biomass or production to pools or fluxes of limiting abiotic resources (Vogt *et al.* 1998, Godbold *et al.* 2003, 2007).

Sequential soil coring: The sequential coring method is the most common approach to estimate fine root biomass and fine root turnover in the field (Vogt and Persson, 1991), and thus belowground NPP. The coring depth depends on the age and type of forest species but typically 0-30 cm depth is employed for estimating root biomass (Finer *et al.* 2011), as coring to this depth has been shown to capture a high percentage of the total fine biomass (Finer *et al.* 2011). The difference between biomass estimates at each sampling date is used to estimate the fine root production. Among the different approaches of data analysis, estimating fine root NPP is the most commonly used approach where the differences in biomass between the maximum and minimum fine root biomass measured during a year (Vogt *et al.* 1986). A second approach introduced by Santantonio and Grace (1987), called

a Compartmental Flow model or Decision Matrix method, and incorporates changes in live and dead root biomass and losses from dead root biomass due to decomposition. The third approach was introduced by Persson (1978) where all positive differences in root biomass between each sequence of sampling dates were summed. If the intervals between the root sampling are too long the intervening variation can be lost (Makkonen and Helmisaari, 1999). Vogt *et al.* (1996) suggested that since a mean fine root biomass value is obtained by integrating all sampling intervals during the year, the error is less in this method compared to other methods measuring production.

Ingrowth cores: The ingrowth core technique involves the replacement of a mesh bag filled with root free soil into a cored or augured hole and after a period of time when new roots grow into the core the whole mesh bag is removed from the hole by complete excavation (Milchunas, 2009). The ingrowth core method has been used alone or in association with the sequential core method to estimate fine root production (Makkonen and Helmisaari, 1999). An over estimation of root productivity due to high proliferation of new root growth into the competition free spaces is the major disadvantage of ingrowth core method. The artificial repacking of the soil may alter bulk density (Milchunas, 2009). However, it allows the direct calculation of fine root production and is thus especially suitable for comparison of fine root production between sites or treatments.

Minirhizotrons: The minirhizotron uses a clear transparent tube with a miniature camera, which are inserted into the ground. The camera, fitted inside the tube can capture photographic images of fine-root growth at different depths outside of the tube surface (Hendrick and Pregitzer, 1993; Smucker *et al.* 1987). The minirhizotron technique allows spatial sampling by the placement of multiple observation tubes in the ground. Within the

last decade the use of minirhizotrons has become a favourite method of many researchers (Majdi and Andersson, 2005; Borja *et al.* 2008; Gaudinski *et al.* 2010). The minirhizotron technique can be used to obtain (1) quantitative information on root length, rooting density, root dynamics, lateral root spread and the depth of rooting, and separation of roots into structural/functional diameters (McMichael and Taylor, 1987), and (2) qualitative information on root colour, branching characteristics, patterns of senescence and observations of parasitism and symbiosis (Hendrick and Pregitzer, 1993; Smucker *et al.* 1987).

Root mesh method: The root mesh method has been proposed as an alternative technique that overcomes the problems associated with the conventional methods for estimating root production (Godbold *et al.* 2003; 2007; Lukac and Godbold, 2007). Using this method, root production is estimated by placing a mesh vertically into forest soil for a specific period of time and then measuring the number and weight of root that grow through the mesh. The procedure is much easier than other methods, requires only simple equipment, and cause minimal soil disturbance (Godbold *et al.* 2007).

2.2 Leaf litter decomposition (incubation and litterbag methods)

Litter decomposition is a key function of all terrestrial ecosystems particularly in forest ecosystems, which results in the formation of soil organic matter in the first instance, and eventually provides other ecosystem services like supply of nutrients for primary producers (Karberg *et al.* 2008). Two major consequences of litter decomposition are plant nutrient flux from litter to soils (Moretto *et al.* 2001; Liski *et al.* 2003) and mineralization of organic carbon and release to atmosphere and storage in soils (Matthew *et al.* 2010).

2.2.1 Nutrient cycling and litter decomposition

Nutrient cycling through decomposition of forest litter has received substantial attention for a long period in relation to soil fertility and plant productivity (Montagnini, 1990), as decomposition of leaf litter and roots is the main process of nutrient transfer from trees to soils. Soil organic matter is generally considered as a slow releasing reservoir of plant nutrients, and during the decomposition process organic macromolecules are broken down into simple plant available inorganic forms (Berg and McClaugherty, 2005). This process is extremely important in moderate to poor fertile forest soils where tree growth is often limited due to low nitrogen and phosphorus availability (Vitousek and Howarth, 1991). Litter decomposition is also important for food webs, as soil macro and micro organisms are depend on litter for their food (Terrell *et al.* 2001). The term 'litter quality' often has been described as a controlling factor of decay rate of litter. However, from nutritional point of view high quality litter can be characterized as providing a high energy and nutrients (especially N and P) supply for both plants and soil organisms (Cotrufo *et al.* 2009).

2.2.2 Greenhouse gases and SOC storage and link to litter decomposition

Recently, environmental aspects of litter decomposition are receiving huge attention and much effort has been devoted to elucidate the responses of global climate change on litter decomposition and vice versa. This is because a considerable amount of CO_2, methane and nitrogenous gases, which are recognized as greenhouse gas, escape to the atmosphere during litter decomposition (Berg and McClaugherty, 2005). In addition, formation of resistant organic materials contributing long term C storage and its stability in soils are gaining substantial attention to predict global atmospheric C budgets (Schlessinger and Andrews, 2000). The possible fates of carbon in litter after decomposition are; immediate escape to the atmosphere through heterotrophic respiration, sequestrated in soils as recalcitrant humic substances, and also physically protected and adsorbed by clay colloids (Cotrufo *et al.* 2009). Heterotrophic soil respiration C, which originates mainly from litter decomposition, is the largest source of terrestrial CO_2 release to the atmosphere (Bhupinderpal-Singh *et al.* 2003; Anderson, 2005). Camila and Adalardo (2008) pointed out that any changes in litter decomposition rate may affect the nutrient availability, organisms and plant growth, and ecosystem carbon balance. Litter decomposition and subsequent humus formation are in fact microbial processes and thus have a profound influence on soil microbial ecology.

2.2.3 General process and factors affecting litter decomposition

Although litter decomposition is a complex biogeochemical process functioning with variations over different ecosystems, it consists of 3 universally recognized processes: i) physical movement of dissolved organic materials into the soil profile by leaching, ii) fragmentation of litter into smaller sizes by biotic (mostly soil macro invertebrates) and abiotic processes, and iii) catabolic activities of soil microorganisms

(bacteria and fungi) which ultimately mineralises C and other nutrient elements (Swift *et al.* 1997). Decomposition of organic materials may be influenced by a large number of physical, chemical and biological factors and their interactions. Berg and McClaugherty (2003) categorized the factors into 3 broad classes: i) environmental conditions, ii) substrate quality (chemical composition and origin) and iii) soil macro and microorganisms (structure and diversity).

At the initial stages, chemical composition of litter changes due to loss of soluble compounds like sugars, low molecular weight phenolics and some nutrients, collectively known as dissolved organic materials (DOM), through dissolution and leaching processes (Berg and McClaugherty, 2005). The catabolic activities of the saprophytic community are also responsible for enzymatic breakdown of intermonomeric bonds, and produce low molecular weight compounds as mentioned above (Mayer, 1993). At this stage the physical breakdown of fresh litter occurs combined with the action of rapidly growing facilitative microorganisms, with subsequent immobilization and mineralization of nutrient elements occurring depending on microbial demand (Berg and Staff, 1981). Next, hemicellulose and somewhat later, degradation of cellulose starts. At the second phase of the process, the break down of recalcitrant compounds like lignin becomes dominant (Aber *et al.* 1984). Although individual processes may dominate a particular stage of decomposition, any or all of the processes may occur to some extent throughout the decay continuum (Berg and McClaugherty, 2005). The formation of humus is the last stage of decomposition process. Polyphenols, derived from either degraded lignin or synthesised by microorganisms, are enzymatically converted to quinones which combine with N containing amino compounds and form the dark colour polymers of humus (Stevenson, 1994).

In addition to the common pattern, some inconsistency such as formation of new derivatives, admixing of compounds with contrasting degradability may happen during

decomposition. Berg *et al.* (2008) reported formation of glucose during cellulose degradation at the later stage of decomposition, and phenolic substances, which are generally found in newly shed litter, can be derived from decomposing lignin. Carbohydrates that exist as an integral part of the fibre structure of lignin decomposed at the same rate during lignin decomposition due to them being more lignified. The inhibitory effects of lignin and nitrogen have been recognized by many investigators. Meentemeyer (1978) and Berg *et al.* (1993) found that increasing lignin level related to decreasing decomposition rate of litter, and the influence of lignin can be so strong that when lignin concentration is high it suppressed the normally dominant strong climatic effects. Similarly Fogel and Cromack (1977) reported that a high concentration of N may have a suppressing effect on the formation of ligninase enzyme and thus retard lignin mass loss.

2.2.4 C: N ratio and decay process

The rate of litter decomposition is related to the substrate quality especially C and N content (Kemp *et al.* 2003; Swift et al 1979). Therefore, in many studies litter nitrogen content and C/N ratio has been used as an indicator of litter quality as decomposer microorganisms utilize them and thus is critical for litter decay rates (Melillo, *et al.* 1988; Camila and Alexandre, 2008). Other factors that regulate the decay rates include lignin content (Gholz, et al, 1985) and lignin: nitrogen ratio (Aerts, 1997; Moore *et al.* 1999). Taylor *et al.* (1989) studied litter decomposition rates using a forest floor microcosm and concluded that over a wide range of lignin contents, the C/N ratio or % N of litter provided better predictions of decomposition rate than lignin: N ratio. However, some authors suggested that in early stages of decomposition C/N ratio may be the best predictor of mass loss, while lignin indexes become the regulating factor in the later stages of decay (Berg, 1986; McClaugherty and Berg , 1987).

2.2.5 Species and mixture effect on decomposition

The effects of plant species identity on litter decomposition have been studied by many authors (Hobbie, 1996; Cornelissen, 1996; Vivanco and Austin, 2006). Plants affect litter decomposition through the production of species-specific litter quality and quantity, changing the forest microenvironment and rhizosphere interactions (Hobbie, 1992). In addition to litter input, plant species can change the abundance and function of microbial decomposer and thus affect the decomposition process (Grayston and Prescott, 2005). Gholz *et al.* (2000) reported considerably slower decomposition of poor-quality conifer litter than high-quality broadleaf litter when experiments were conducted across a broad geographical gradient. Variations in decomposition rates among different single plant species litter under same environmental conditions have been reported by Cornelissen (1996).

In natural forest ecosystems, a variety of different plant species including understory vegetation, grasses, mosses etc. contribute to the formation of litter layers. Litter of several species accumulate on the forest floor and decomposition occurs as a mixed substrate. However, in single species managed forests, the litter layer is generally composed of monospecific litter. Species composition generally affects the litter quality, availability of nutrients in the forest soils and decomposition of litter (Rothe and Binkley, 2001; Berger *et al.* 2002; Prescott, 2002). Chapman *et al.* (1988) suggested that, in mixtures, the interactions of different species affect the decomposition rates of individual leaf types, nutrient availability, as well as structure and activities of the decomposer communities. Leaf litter of different plant species may exhibit synergistic (enhance decomposition) or antagonistic (decrease decomposition) effects when they decomposed in the mix condition compare to the single species condition (Hattenschwiler, 2005). However, many investigators reported no interactive effects in the mixture that these are

purely additive i.e. decomposition rates in mixture can be predicted from the rates of individual single species litter (Hansen and Coleman, 1998; Nilsson *et al.* 1999; Prescott *et al.* 2000). Although the chemical composition of litter influences the overall litter decomposition, the contribution of diversity of litter producing species on the decomposition rate and nutrient release is still not clear and hardly considered in biogeochemical models (Hattenschwiler, 2005).

There are several possible mechanisms of how interactive effects of different species' litter act in mixed conditions. A different abundance of decomposers including litter feeding macrofauna which prefer certain litter types, are very sensitive to small alterations in litter quality, and may change decomposition rates in mixed species litter (Blair *et al.* 1990; Hattenschwiler and Bretscher, 2001). Species-specific litter compounds such as polyphenols may influence the decomposition processes. Schimel *et al.* (1998) observed in Alaskan Taiga forests that phenolic acids from *Populus balsamifera* leaf litter enhanced microbial immobilization of N by providing a microbial growth substrate, while specific tannins inhibit microbial activity. Another mechanism was described by Chapman *et al.* (1988); and Wardle *et al.* (1997), that the synergistic mixture effects might be due to stimulating effects on low-quality litter types by the presence of high-quality litter. High-quality litters are subjected to microbial attack, which results in an increase in the availability of nutrients for transferring to low-quality litter. Subsequently, a rapid utilization of substrate in the low –quality litter and thus the overall decomposition rate in litter mixture becomes faster. Similar mechanisms of nutrients transfer have been observed by Salamanca *et al.* (1998) with increased mass loss of low-quality litter correlating with increasing microbial activity, with an apparent net nitrogen transfer from high to low-quality litter. In contrast, reviewing the recent information Song *et al.* (2010) concluded that litter decay rate may not be accelerated due to mixing of litter types.

Alteration of structure and activities of microbial population may influence the potential changes in the decay process in species mixtures. Chapman (1986) found a significantly higher earthworm population in spruce/pine mixture than estimated from the pure stands; however, amounts in a spruce/alder mixture were not significantly different from those in the pure stands. Kautz and Tipp (1998) reported a greater number of soil fauna (Lumbricidae, Enchytraeidae, Collembola and Oribatidae) in mixed forest than pure coniferous forest. Higher filamentous fungi such as *Pinus massoniana* and *Liquidambar formasana* in mixed forest have been reported by some investigators (Song *et al.* 2004). Although a positive effect of litter mixture on the microbial community has been documented, litter quality has been considered as a dominant factor influencing decomposer diversity and structure (Hooper *et al.* 2005). Considerable fungal-bacterial antagonistic interactions on decomposing mixed litter have been observed by some researchers (Muller *et al.* 1999).

2.2.6 Dissolved organic matter (DOM) and litter decomposition

The soil solution contains dissolved organic compounds of various quality and quantity originating from both fresh and decomposed plant litters, microbial biomass and roots exudates (Kalbitz *et al.* 2000). DOM generally consists of a wide range of substances including sugars, organic acids, dissolved nutrients (C, N, P, and S etc.), amino acids, low molecular phenolics and high molecular humic substances (Kalbitz *et al.* 2000). The flux of DOM from litter layer to lower soil layers plays an important role in the activities of belowground autotrophic and heterotrophic microorganisms (Zsolnay and Steindl, 1991; Qualls *et al.* 1991). Lundquist *et al.* (1999) and Moller *et al.* (1999) suggested that incompletely decomposed litter by fungi might be the most important source of DOM and the microbial metabolites contribute significantly to the amount of DOM released from the

forest floor. In our microcosm experiment we analyzed the following common forms of soluble organic compounds released during decay process.

2.2.7 Release of dissolved organic carbon (DOC) from leaf litter

Mineralization of C during litter decomposition is considered as an index of decay rates and in forest ecosystems a positive correlation between carbon decomposition rate and DOC leaching has been reported by some investigators (Currie and Aber, 1997). McClaugherty *et al.* (1983) reported that 33% of the soluble compounds in sugar maple litter gradually leached to DOC pools. DOC is the major form of C that can be sequestrated by clay particles in the lower soil layers or hydrologically transported from the forest floor to under ground and surface water through the soil profile (Kolka *et al.* 2008). Uselman *et al.* (2007) studied DOC release from leaf litter labelled with ^{14}C and found that 8.2% of total leaf C leached as DOC during a 47 day incubation experiment. At the field level in northern Germany, annual carbon transport from litter layers of alder and beech forest was estimated as 0.8-1.4% of annual gross carbon production (Czech and Kappen, 1997).

2.2.8 Dissolved organic and inorganic N (DON and DIN) dynamics

N plays a critical role in primary production, litter decomposition and plant nutrition and thus provides many ecosystem services. In many forest ecosystems, half or more of the N in soil solution is in organic forms (Sollins and McCorison, 1981; Qualls *et al.* 1991). This dissolved organic nitrogen (DON) is derived from both freshly fallen and partially decomposed plant litter (Casals *et al.* 1995), and considered as an important constituent of DOM. In the soil solution DON and mineralized dissolved inorganic nitrogen (DIN) collectively form total dissolved nitrogen (TDN). DON includes a range of compounds from low molecular weight amino acid, polypeptides and polyamines to high molecular weight protein and humic acids (Jones *et al.* 2005). Recent studies confirmed

that DON plays a significant role in nutrient cycling especially in the direct uptake of organic forms of nitrogen (Jones *et al.* 2004; Chapin *et al.* 1993). Microbial activity and leaching have been regarded as the major processes during initial stages of decomposition (Tietma and Wessel, 1994). Therefore both DON and DIN (NO_3^- and NH_4^+) concentrations in soil solution originating from decomposing litter can be used as an index of decay rate.

2.2.9 Plant secondary metabolites and its ecological significance

Phenolics are plant secondary metabolites chemically characterized as an aromatic ring with one (known as phenols) or more (known as polyphenolics) rings comprising more than 1000,000 compounds in nature (Waterman and Mole, 1994). The phenolics, are often termed as monomers, are low molecular weight compounds that have been recognized as toxins, qualitative or mobile defences. In contrast, the polyphenolics are high molecular weight and have been recognized as digestibility reducing substances, quantitative and immobile defences (Horner *et al.* 1988). Among the secondary metabolites tannins are the most common water soluble polyphenolics in plants. There are two types of tannin, hydrolysable and condensed. Condensed tannins, also known as proanthocyanidins are widespread in woody plants (Gessner and Steiner, 2005).

Ecological functions of plant secondary metabolites have received much attention during last 50 years because of its inhibitory role in enzymatic activities, decomposition and nutrients cycling (Hattenschwiler and Vitousek, 2000). The mechanism, by which water soluble phenolics decrease decomposition rate, may be due to the formation of complex proteins which are unavailable to decomposer organisms (Quested *et al.* 2003; Hattenschwiler and Vitousek, 2000). However, all phenolic compounds do not retard the decay process, as low molecular weight phenolics such as simple phenol, phenolic acids, flavonoids etc. can be decomposed easily, but high molecular weight polyphenols like

tannins inhibit the decomposition (Kraus *et al.* 2003; Fierer *et al.* 2001). Therefore polyphenol content in litter is considered as an important criterion used to define litter quality in relation to litter decomposition (Palm and Rowland, 1997). Phenolic compounds interact in nutrient cycling in two major ways firstly, it can directly affect the abundance and activities of microorganisms, and secondly, it can influence the quality and quantity of nutrient elements in plant litter (Hattenschaeiler and Vitousek, 2000). Soluble polyphenols can inhibit the spore germination and hyphal growth of saprotrophic fungi, growth of mycelium biomass of mycorrhizal fungi; although the opposite effects are also possible depending on the types of polyphenols (Hattenschaeiler and Vitousek, 2000; Leake and Read, 1989). Rice and Pancholy (1973) reported that very small concentrations of polyphenols may inhibit nitrification by Nitrosomonas in soil suspensions incubated with leaf litter.

2.2.10 Decomposition at field level: Litter bag technique

Litter bag approach is the most frequently used method to study *in situ* litter decomposition dynamics for the last 50 years, because of its simple and straightforward approach to accommodate spatial, temporal and ecological variables affecting on the litter during decomposition (Karberg *et al.* 2008). Different mesh sizes are chosen to allow access of macro and meso-fauna and to retain litter fragments inside the bags (Schadler and Brandl, 2005). Large mesh size (>2mm) is generally used to ensure the access of macrofauna but at the same time there is high risk of litter fragment loss. On the other hand, although small mesh (0.5mm) bags prevent the fragmentation loss, they also restrict the entrance of most fauna. Therefore 1-2mm mesh is the most common size used in the litter bag experiments (Robertson and Paul, 1999). The bags are deployed at different depths within the soil profile according to aim of the experiment; however, the bags are

often buried at the litter-mineral interface where decomposition generally takes place (Cotrufu *et al.* 2009). During collection of litterbags, mineral soils often contaminate with decomposed or partial decomposed litter, therefore this should be corrected by measuring the ash content of litter and also organic carbon content of the original soils. The main limitation of the litterbag technique is the possibilities of exclusion of some macro-invertebrates from litterbag due to mesh size. The contamination of soil mineral, especially fine clays with decomposed litter requires correction. Disturbance in soil microclimate may happen during placement of bags into soils (Karberg, 2008).

Mass loss is generally analogous to "decomposition" which was defined by Berg and McClaugherty, (2008) as "sum of CO_2 released, and leaching of C compounds and plant nutrients". Thus the magnitude of decomposition is estimated by the physical disappearance of litter from the system, and mass loss is universally accepted as an index of decomposition rate (Berg and McClaugherty, 2008). Many mathematical models have been used to estimate decay rate from mass loss during decomposition. The following single exponential decay model proposed by Jenny (1949) and Olson (1963) has been used extensively for describing the litter decay rates:

$$X_t = X_0 e^{-kt}$$

Where X_t is the amount of litter remains in the bag at time t, X_0 is the initial amount of litter and k decomposition rate. McClaugherty and Berg (1987) stated that the single exponential model may be suitable for homogeneous substrate and the materials with high resource quality and less complex materials. For long term decomposition experiments with the litter of two different qualities, a double exponential model was proposed by Lousier and Parkinson (1976). This approach is a reasonably better fit than the Olsen's single exponential model (Rovira and Rovira, 2010). However due to its simplicity and good fit at the early stages the Olson model is still being used widely (Gholz *et al.* 2000).

2.3 Organic carbon storage in tree planting and grassland soils

2.3.1 Soil organic C and global warming

Soil organic C is connected to global carbon cycle and consequently related to global warming because it has the potential to both sequestration and release of C to the atmosphere as CO_2, one of the major green house gases. Green house gases allow the solar radiation to reach the earth surface and absorb and re-radiate the longer wavelength (infrared) radiation during its outward flux to space which effectively warms the atmosphere (Luo and Zhou, 2006). Since soil is the second largest reservoir of C in the terrestrial ecosystem, and globally the soil C pool is about four times the atmospheric pool, any changes in the flux of CO_2 from soil to atmosphere has paramount importance in balance of atmospheric CO_2 (Luo and Zhou, 2006). On the other hand, long term sequestration of C in soil has a positive feedback to the atmosphere by locking up plant biomass C, once CO_2 is fixed from atmosphere through photosynthesis. In this case forest soil plays a more important role than other ecosystems as it carries a huge plant biomass. Kauppi *et al.* (2001) suggested that the forest sector has a biophysical mitigation potential for elevation of atmospheric CO_2.

2.3.2 Soil organic C storage global and regional perspectives

During the last couple of decades many research studies have addressed the estimation of the soil organic carbon pools at global, regional and local levels. Yet uncertainties exist in soil C quantification due to inadequate sample numbers used for global scale estimation and assumptions on mean soil depth (Rodeghiero *et al.* 2009). Lal *et al.* (1998) estimated that the global storage of SOC ranged from 1500 to 2000 Pg in the top metre and soil inorganic carbon ranged from 700 to 1000Pg. The amount of SOC is 2-3

times higher than the C contained in terrestrial vegetation (550 ±100) and about the double of atmospheric C pool (800 Pg) (Houghton, 2007).

The diversified landscape and land use pattern in the UK causes spatial variation in the density and pool size of the SOC (Ostle *et al.* 2009). In addition, different parts of the UK have different SOC databases because of differences in assessment approaches (Bradley *et al.* 2005). Most recently Countryside Survey of UK studied the C densities and stocks in the top soils (0-15 cm) across the UK and estimated 69 t ha^{-1} of soil C density (ranging between a mean of 47 t ha^{-1} n the Arable and Horticulture Broad Habitat to a mean of 91 t ha^{-1} in acid grassland) and 1582 Tg of soil C stock across Great Britain in 2007 (Emmett *et al.* 2010). Earlier, Bradley *et al.* (2005) estimated 4562 Tg C (2543 Tg in 0-30cm soil depth) in 1 m soil depth across the UK with an average C density of 18 kg m^{-2}. At local scale, land use is the one of the major attributes that influences the C densities and stocks in soils (Ostle *et al.* 2009). The organic C in the soils of Wales assessed during the last decade under different projects has been presented in Table 2.1.

Table 2.1 Soil organic C densities (kg m^{-2}) in the soils of different land use types in Wales, UK. Data sources are: LULUCF inventory reports published by DEFRA (for Milne *et al.* and Thomson *et al.*), LandIS and National Soil Inventory (NSI) (for Bradley et. al.) and Countryside Survey of 2007 (for Emmett *et al.*).

Sampling Depth (cm)	Forest land	Cropland	Grassland	References
0-100	22.8	9.3	20.0	Milne *et al.* (2001)
0-100	20.0	11.0	14.0	Bradley *et al.* (2005)
0-30	13.7	7.5	11.0	Thomson *et al.*(2007)
0-15	7.8	5.6	7.7	Emmett *et al.* (2010)

2.3.3 Organic C in forest soils

The forest ecosystem plays an important role in global C cycle and posses two major pools of terrestrial C, plant biomass C and soil C (Lal, 2005). The global forest area is 3952 million ha which is approximately 30% world land cover (FAO, 2006). The C stock in living forest biomass was equivalent to 1,036,200 Mt CO_2 in 2005 and is declining due to continuous deforestation (FAO, 2006). In forest soil, diversity in litter quality and quantity, nutrient status, root nutrient uptake and activity , interception of atmospheric deposition, canopy interactions and leaching, and soil biological activity can cause differences in the chemical composition of top soils under various tree species (Hagen-Thorn et al, 2004). C content in soils of three major forest biomes (viz. boreal, temperate and tropical) are about 471 Pg, 100 Pg and 216 Pg (343 Mg ha^{-1}, 96 Mg ha^{-1} and 123 Mg ha^{-1} respectively) (Lal, 2005). In the UK, the area of forest land is about 2,841,000 ha which is about 11.6 % of the total land cover (FAO, 2010), and in Wales is about 286,769 ha or 13.8% of the total area (Forestry Commission, 2003).These forest and woodlands account for 80% of the UK vegetation C stocks (Ostle *et al.* 2009). It has been estimated that total C stock in the UK forest ecosystems in 2010 was 893 Mt, of which forest soil contained 730Mt (around 80%) (FAO, 2010). A major part of soil C reserve moves back to the atmosphere during the course of organic matter decomposition, and the rest sequestrates in soils for longer periods.

2.3.4 Afforestation of broadleaved trees and SOC stocks

According to IPCC (2007), afforestation can be defined as "direct human-induced conversion of land that has not been forested for a period of at least 50 years to forested land, through planting, seeding and/or the human-induced promotion of natural seed sources". In the UK, recent trends of afforestation focus on planting broadleaved tree

species on ex-arable land due to their potential to sequester C, (Cannell and Dewar, 1995; Ross *et al.* 2002; Silver *et al.* 2000). The general trend is that on soil with low organic matter content, due to long term cultivations or other intensive land use such as mining, there is significant accumulation of C on the soil surface and in the mineral layers. Johnston *et al.* (1996) found an average increase in SOC stock at the rate of 0.8 Mg C ha^{-1} $year^{-1}$ in the mineral soil over a 40-year period following afforestation, on degraded arable land in east-central Minnesota. However, soil initially rich in organic C such as grassland soils may decline in SOC due to afforestation (Tate *et al.* 2005). Tree planting on abandoned land may affect the soil C balance in two contrasting processes: a higher rate of soil organic matter decomposition, microbial respiration and drainage due to higher aeration, as well as disruption of soil aggregates during site preparation may ultimately cause C loss from soil; in contrast, aboveground and root biomass and root activities may contribute to accumulation of organic matter in soil at a faster rate than the previous (without planting) conditions (Cannell and Dewar 1995).

In UK woodlands, 49.0 % of the area is stocked by conifer trees, and 32 % is broadleaved species and the rest 18.9 % is mixed woodland, open spece within woodland etc. (Forestry Commission, 2003). New planting of broadleaved and conifers species were 42,000 ha and 9.2,000 ha respectively during 2003-2008, (FAO, 2010). The selection of tree species is important especially with the aim of C sequestration, because different tree species may have different mitigation potential to global climate change (Schulp *et al.* 2008). Generally conifer species (softwood) have a lower net C density than same-aged broadleaf deciduous (hardwood) species, thus affecting the species selection during the afforestation plan (Cannell, 1999; Ostle *et al.* 2009). Milne *et al.* (2001) found that broadleaved species such as beech (*Fagus sylvatica*) increased the net amount of carbon in litter and soils because of the slow degradation of tree products. Thus site and species

selection are the two major factors that affect C storage potential during afforestation. Johnson and Curtis (2001) suggested that C conscious site selection, preparation and harvesting may positively influence soil C stocks through the planting of native hardwood species.

2.3.5 Factors affecting SOC storage

Both natural and human induced factors can influence the concentration and stock of soil C in the forest ecosystem. The natural factors are climate, vegetation, soil quality, soil microbial populations, and forest fire etc., and anthropogenic factors are forest management, afforestation, deforestation etc. (Lal, 2005). Tree species (or species composition) is the single most important factor that influences SOC storage in the plantation ecosystem (details discussed in the next section).

Forest management that affects SOC stocks includes thinning, harvesting and site preparation, maintain continuous canopy cover (Thornley and Cannell, 2000), fertilization and liming (Hoover, 2003). Thinning practice can affect soil C storage negatively in several ways: thinning causes changing the stand microclimate by reducing evapotranspiration and increasing soil temperature, and can stimulate the decomposition of the forest floor resulting in a decreasing soil C pool (Piene and van Cleve, 1978). In addition, litterfall can be lowered in heavily thinned stands and thus decrease SOC stocks (Jandl *et al.* 2007). In contrast, Suni *et al.* (2003) reported enhanced growth of the understory vegetation due to thinning measures in an experimental site in Finland which compensated for the reduction of C from tree biomass.

Harvesting can affect soil C storage positively or negatively. Removal of whole trees reduces seasonal litter inputs and disturbance affects forest floor and mineral soils leading to soil C loss. In addition, harvesting causes decrease in photosynthesis and can turn the forest into a C source (Kowalski *et al.* 2004). Measurement of net ecosystem C

exchange showed that the increased rates of soil respiration due to harvesting continued for at least 14 years after logging (Schulze *et al.* 1999; Yanai *et al.* 2003). However, other research has shown that harvest residues left on the forest floor can also increase C stocks on mineral soils (Jandl *et al.* 2007).

In afforested land, site preparation generally promotes soil C loss due to exposure of the mineral soil by removal or mixing of the organic layer, and consequently soil disturbance and changes in microclimate stimulate the decomposition of SOM (Palmgren, 1984; Johansson, 1994). The effects of site preparation is generally more pronounced in coarse textured soils It has been observed that sandy soils are particularly sensitive to management practices, which result in significant losses of soil C and N (Carlyle, 1993). As clear cut harvesting decreases SOC stocks, continuous-cover forestry may be an effective option for reduction of soil C losses following selective harvesting and thinning operations (ECCP-Working group on forest sinks, 2003). Fire is another major disturbance that can impact soil C stocks in forest ecosystems, and may have a particularly long-term impact on C stock in soils of the boreal regions. The impact of fire on SOC stock depends on fire temperature and duration, SOC stock and its distribution in the soil profile, and change in the decomposition rate of SOC following the fire event (Page-Dumroese *et al.* 2003).

2.3.6 Soil C storage under single and mixed stands

Tree species has enormous effects on soil C storage mainly because of quality and quantity of organic matter that inputs to soils through litterfall and root activities (Binkley and Valentine, 1991; Hagen-Thorn *et al.* 2004; Oostra *et al.* 2006). Plant species influence the soil organic C stocks through different ways : species may differ in various traits such as NPP and production of detritus (Montagnini *et al.* 1993) depth and distribution of roots

(Carvalheiro and Nepstad, 1996), soil invertebrate populations (Warren and Zou, 2002; Hobbie *et al.* 2006) among others. Variations in any trait related to quantity and turnover of soil input can eventually affect SOC in soil (Russell *et al.* 2007). Vesterdal *et al.* (2008) studied the effects of six common European broadleaved tree species on soil C pool using common garden approach and found that slightly more C in 15-30 cm under ash and lime than under spruce and postulated that the studied tree species would develop larger differences in soil C content over a full rotation.

Generally mixed species stands are believed to have advantages in terms of nutrient supply and decomposition of organic matter at the forest floor compared to single species stands. Differences in chemical properties of top soils, particularly the forest floor, which has developed under different species, have also been reported by some investigators (Binkley and Valentine, 1991; Ranlund- Rasmussen and Vejre 1995). In mixed stands of Scots pine and Sitka spruce, several fungi colonized the roots of both tree species, but only *Suillus* Sp. was confined to the root of Scots pine. Roots may be colonised by a greater range of fungi than those found in single species stands (Ryan and Alexander, 1993). The enrichment of soil nitrogen by nitrogen fixing plant species may also influence C sequestration as a higher supply of N to primary producers leads to production of more biomass, therefore nitrogen fixing tree species have larger effects on forest soils than other species, and these effects include consistent increases in soil organic matter and carbon (Binkley, 2005). Thus, fixation, turnover and transformation processes generally differ between soils under single and mixed species stands. However, the effects of plant species on mineral soil are variable Jandl *et al.* (2007). Berger *et al.* (2002) reported a significant increase in total storage of both C and N in pure stands of spruce (*Picea abies*) than in admixture of beech (*Fagus sylvatica*) in acidic soils in Austria.

2.3.7 Sampling depth and vertical distribution of SOC

The top soil contains the highest amount of organic C, and higher turnover of C and is generally confined to the top 45 cm, as maximum soil microbial activity is restricted to this depth (Ravindranath and Ostwald, 2008). Since most of the root activities are also concentrated within the top 30 cm, this sampling depth has been recommended by IPCC for soil C inventories (IPCC, 2004). However for estimating soil C stocks the typical sampling depth is one metre (Bradley, 2005). Most of the previous studies on soil C were limited to the upper 15 to 30 cm of soil because of the difficulties associated with sampling (Conant and Paustian, 2002). However, a recent study has suggested that considerable amounts of soil organic matter is stored in the subsoil layers (below the A horizon), which due to high residence time may be a potentially stable soil C store (Rumpel and Kogel-Knabner, 2010). Strahm *et al.* (2009) reported the translocation of dissolved organic carbon (DOC) through the soil profile contributing to the recalcitrant C pool between the depths of 20 and 100 cm in managed forest sites. However, the controls of the vertical distribution of SOC in to the deep layers still remains poorly understood (Jobbagy and Jackson, 2000). In addition to climatic controls, the composition and stability of subsoil organic carbon may be influenced by other factors such as vegetation and the soil clay content (Jobbagy and Jackson, 2000), soil forming processes (Rumpel *et al.* 2002), root activity and dissolved organic carbon (DOC) (Rumpel and Kogel- Knabner, 2010). Jobbagy and Jackson, (2000) hypothesized that vegetation, through its above and belowground allocation patterns, may be the major determinant of the vertical distribution of SOC in soil profile. However, Paul *et al.* (1997) and Trumbore *et al.* (2000) suggested the influence of clay content on SOC pool in deeper layers due to higher proportion of organic molecules protected by clay coatings. Substantial amounts of SOC originates from belowground plant biomass such as structural coarse roots, mycorrhizal fine roots and the

mycorrhizal hyphal mycelium, and Lukac and Godbold (2011) estimated that root C accounted for about 42% of the total belowground organic carbon in temperate forests. Although a large portion of this C returned to atmosphere through root and rhizomicrobial respiration, parts of root exudates, secretions and root residues stay in soils for long time contributing to the SOC stock (Moyano *et al.* 2009).

2.4. Fractionation of C in soil organic matter

2.4.1 Soil organic carbon and its fractionation

Soil Organic Matter (SOM) is a dynamic and complex heterogeneous mixture of plant and microorganism residues. As C is the building block of all organic substances, dynamics of SOM is analogous to organic carbon dynamics in soil. The dynamic nature of SOM causes release of C and other elements through the decomposition processes. But the decomposition rate and turnover time of different organic compounds vary widely (Davidson and Janssens, 2006). Although a wide range of physico-chemical to biogeochemical and environmental factor affects the process, the chemical nature (quality) of organic compounds is the first regulator of decomposition dynamics (Swift *et al.* 1979). The quality of soil organic compounds in relation to C release refers to biochemical quality reflecting biodegradability (Rovira and Vallejo, 2007). Therefore fractionation of soil organic carbon refers to repartition of SOM into several discrete pools on the basis biodegradability and turnover time. This concept of fractionation is simple and suitable for ecological research. Other approaches of carbon fraction include physical fractionation to quantify free and physically protected organic fractions and fractionation of humus into fulvic acid, humic acid and humin (Stevenson, 1982).

Carbon in SOM has been divided into several pools on the basis of decomposition rate and turn over time (Rovira and Vallejo, 2007). The most common approach is two pools system, in which fractions having rapid turn over time is termed as labile and the slow one as recalcitrant (Kendra *et al.* 2004). Labile pool is further divided into two pools, labile-I and labile-II by others (Rovira and Vallejo, 2002; Asfaw *et al.* 2009). On the other hand Hoosbeck *et al.* (2006) termed the same fractions as labile, refractory and stable pools.

2.4.2. Labile C pool in soil

Labile soil carbon refers to the organic C fraction having a turnover time of less than a few years in contrast with recalcitrant C having turn over time of several thousands years (Coleman *et al.* 1996, Harrison *et al.* 1993). Chemically labile C is largely comprised of carbohydrate, polysaccharides of plants (hemicellulose, starch residues) and microbial origin (chitin) and cellulose (Oades *et al.* 1970). Polysaccharides of both plant and microbial origin (hemi cellulose and starch) termed as labile-I, are hydrolysable with medium concentrated acids. On the other hand labile-II fraction is largely cellulose and rather resistant to decomposition. This fraction is hydrolysable with very high concentration of acid (Rovira and Vallejo, 2007). Karberg *et al.* (2008) suggested that there is a transformation of labile-II to labile-I during decomposition of litter. Cellulose can be quickly cleaved by exoenzymes into simple sugars which are readily metabolized by soil microorganisms. Labile C compounds are generally easily biodegradable; however, some labile C can not be subjected to microbial attack due to protection by clay particle or coated by recalcitrant materials, hence labile C must be chemically degradable and physically accessible to microbes (Baldock and Skjemstad, 2000, Zang *et al.* 2001).

2.4.3 The recalcitrant C pool in soils

The recalcitrant C pool consists of more stable (chemically humified and physically protected) C compounds in soil organic matter with a slow turnover time. It includes humic substances, lignin and related compounds along with fats, waxes, resins and suberins (Silveira *et al.* 2008). These compounds consist of large polymers which can not pass through cell membrane; in addition, the irregular chemical structure and complex bonding cause these substances to be resistant to enzymatic attack (Karberg *et al.* 2008). Using an acid hydrolysis technique, Collins *et al.* (2000) isolated recalcitrant C and found

it comprised 30-50% of total soil organic carbon in US Corn Belt soils and estimated mean residence time (MRS) of 2600 yr for this fraction.

2.4.4 Ecological significance of carbon fractionation in SOM

The dynamics of SOM influences the overall forest ecology through different types of ecosystem services such as release and sequestration of C provide energy and nutrients, affect structure and functions of soil the microbial community etc. SOM comprises heterogeneous mixture of organic compounds with different degradability depending mainly on the susceptibility to microbial attack which influence over all residence time of organic C in soils (Stevenson 1994; Parton *et al.* 1987). Therefore the fractionation of SOM into discrete degradable pools provides information about the following ecosystem processes:

1. Release and sequestration of C: Increasing emissions of carbon dioxide from soil is related to rapid biological decomposition of soil organic matter and thus enhance global warming (Zou *et al.* 2005). The labile fraction of soil organic matter plays a dominant role in the CO_2 efflux process due to its rapid turnover rate (Belay-Tedla *et al.* 2009). The turnover rate of labile C in organic compounds such as soluble sugars, starch, carbohydrates is very rapid, as fast as a few days to a few years (Brady and Weil, 2008). In contrast, the contribution of recalcitrant C pool in long term C storage is enormous. Lignin and some physically protected labile SOM can stay in soils for several thousand years (Zou *et al.* 2005). Therefore, C fractionation provides information about both short- and long-term soil C responses to changes in the soil environment.

2. Provide nutrients and energy to plants and microbes: The labile C pool has potential to provide nutrients to other primary users and thus is associated with ecosystem productivity in the short term (Khanna *et al.* 2001). During initial stage of SOM

decomposition, readily degradable C components, especially carbohydrates, are the major energy sources for microorganisms to synthesise new cells (Cheshire, 1985; Khanna *et al.* 2001). Plant nutrient elements such as nitrogen, phosphorus, potassiun etc. are released from labile portion of SOM within a short time and become readily available to other plants. As forest soils are generally deficient in plant nutrients due to high demands of major nutrients, the labile fraction of SOM plays a vital role in plant nutrient supply (Khanna *et al.* 2001).

3. Structure and functions of microbial community: Fractionation of C into labile and recalcitrant pools is based on microbial degradability of the SOM. These two broad pools further consist of different organic compounds and many microorganisms are involved in the degradation of these compounds (Berg and McClaugherty, 2008). Soil microbial biomass itself is a component of labile SOC pool because of its availability to other decomposers within a short period (Khanna *et al.* 2001), other labile compounds of SOM are generally decomposed by rapidly growing opportunistic microorganisms (Berg and McClaugherty, 2008). Specific groups of fungi and bacteria are involved in enzymatic degradation of more recalcitrant parts of SOM. Cellulose is degraded by hydrolytic and cellulolytic organisms that produce enzymes to breakdown polymers and degradation of lignin is dominated by different types of fungi, especially white-rot, soft-rot and brown rot (Berg and McClaugherty, 2008). Labile and recalcitrant C pools in soil thus influence the size, composition and function of soil microbial community engaged in decomposition of a particular pool.

2.4.5 C fractionation techniques

Various methods have been used to quantify the labile and recalcitrant pools of soil organic matter (Karlen *et al.* 1998). Physical fractionation techniques are based on separation of particles by sieving or floating; chemical fractionation relies on biochemical quality of the substrate, and biological separation based on empirically quantification of mineralizable C. However, up to the present, no single technique has been developed that adequately describes the continuum in the degradability of soil SOC that exists in nature (Paul *et al.* 2006).

Physical fractionation includes separating light and heavy fractions which are considered as labile and recalcitrant pools respectively. To isolate these fractions, Gregorich and Janzen (1996) followed the technique of floating the materials in a dense liquid. Cambardella and Elliott (1992) suggested the dispersion and sieving technique to separate the particulate organic matter (POM) fraction which was considered as labile organic matter pool. Biological fractionation techniques include laboratory incubation experiments of C mineralization under controlled temperature and moisture, and the CO_2 evolved during the initial stage of incubation is used to estimate thex labile pool of organic carbon (Alvarez and Alvarez, 2000). Other investigators (Beck *et. al.* 1997; Paul *et al.* 1999) have used the estimation of soil microbial biomass carbon by fumigation and extraction methods and have considered this to be the pool size of readily decomposable C. Chemical fractionation is carried out by using a number of different techniques. Fractionation of soil organic matter has been carried out by acid hydrolysis (Stout et al. 1981; Paul *et al.* 2001), by digestion with permanganate (Weil *et al.* 2003) and by extraction with hot water (Gregorich *et al.* 2003). Hot water treatment releases mainly microbial biomass carbon, and parts of the polysaccharides and carbohydrates carbon, and as a consequence the pool size estimate is lower than that determined by acid hydrolysis

(Silveira *et al.* 2008). Among the different methods, acid hydrolysis is the most widely used technique to separate resistant and active pools of soil organic matter. Most of the labile portions (carbohydrates and proteins) of soil organic matter are released during the acid hydrolysis, whereas, most recalcitrant organic polymers such as lignin, suberin, resins and waxes are resistance to acid hydrolysis (Rovira and Vallejo, 2002). There is considerable debate about the strength of the acid to be used in acid hydrolysis to remove the labile C. Preston and Schnitzer (1984) reported that 90% (w/w) of carbohydrates in SOM can be potentially removed after treatment with 6M HCl without any significant changes in aliphatic, aromatic and remaining carboxyl groups in the soil extract. More recently Rovira and Vallejo (2002) suggested the use of 26N H_2SO_4 in acid hydrolysis as the best predictor to estimate mineralizable pools of soil organic carbon

A combination of acid hydrolysis and incubation has been proposed by some investigators to determine the size of labile pools (Collins *et al.* 1999; Haile-Mariam *et al.* 2000; and Paul *et al.* 2006). They argued that fractionation techniques should take into account the various controls involved in soil organic carbon dynamics. In addition to acid hydrolysis, long term incubation experiments allow soil enzymes and micro organisms to fractionate SOC into some relevant pools (Paul *et al.* 2006).

3. Background information of study area

3.1 Study sites

The Henfaes Research Centre, the research station of Bangor University is located in the village of Abergwyngregyn, 12 km east of Bangor City, North Wales, UK (Geographic position 53°14 N, 4°01 W and National Grid : SH 653 741 GB). The landscape of the area comprises high mountains with steep slopes, broad valley and flatter land adjacent to the coast which provide diversity in nature and properties of soils mainly due to climate (temperature and precipitation) and topography (drainage). The present plantation area is located on a piedmont plain which further extended to marshy coast land off the Irish Sea (Figure 3.2). The topography of the area includes a shallow slope on a deltaic fan of approximately 1-2° towards northwest, at an altitude of 4-14 m above sea level (Teklehaimanot and Sinclair, 1993). The climate of the area is Hyperoceanic and the seasonal temperature varying between -3 to 10 °C in winter and 12 to 25 °C in summer and the annual rainfall of about 1000 mm (Figure 3.1).

The area was covered by typical mixed oak woodland and was transferred to an agricultural system from Roman times onward (Avery, 1990). The present land use of the farm includes forest and grassland with intensive grazing in the upland and new woody plantation, grassland and arable crop plots at the low-lying areas.

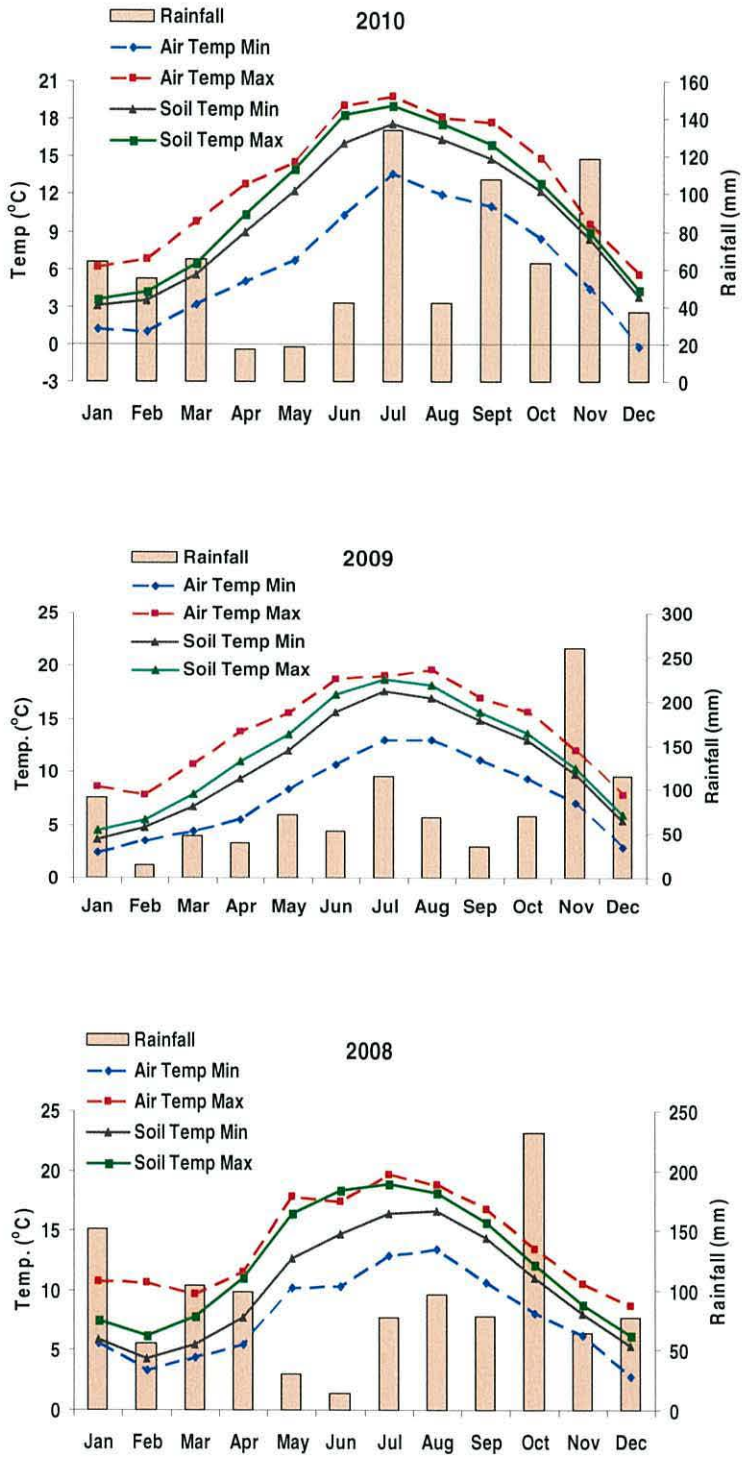


Figure 3.1 Annual temperature (Soil and atmosphere) and rain fall in the experimental site recorded by a Campbell Automatic Weather Station (Campbell Scientific Ltd, Shepshed, UK)

3.2 Soils

The soils developed under more or less well drained conditions, non calcareous and from unconsolidated parent material traditionally grouped in Britain as Brown earth (Clarke, 1940) which are classified as Dystric Cambisols according to the FAO system and recognized as Rheidol series (Teklehaimanot *et al.* 2002). The soil is loamy in texture, brown colour originated from glaciated shales, sandstone and mudstone at the upper portion and glacifluvial deposits of clay, silt, sand and gravels at the lowland areas. The parent material of soils originated from two sources: postglacial alluvial deposits from the Aber River and rhyolitic tuffs and lavas from Snowdonian Mountains with microdiorites and dolerite in the stone fractions and Lower Paleozoic shale in the finer fractions (Teklehaimanot and Sinclair, 1993). The ancient natural broadleaved vegetation of the area, like a typical temperate region, affected the formation of this soil through decomposition of plant residues (Avery, 1980).

To compare the storage and fractionation C between tree plating and grassland soils, we selected four grassland spots adjacent to the four different planting plots. The grass species were identified as a mixture of perennial ryegrass (*Lolium perenne* L), cocksfoot (*Dactylis glomerata L)* and creeping bent *(Agrostis stolonifera)*. These grassland areas have the same previous land use and management history as the plantation blocks.

3.3 Afforestation

The plantation was established on 2.36 hectare of ex-arable lands in March, 2004 with a range of broadleaved tree species to introduce a Continuous Cover Forestry system. Reflecting the forestry policy of UK, the plantation scheme included single and mixture plots of different native broadleaved species (Figure 3.2).The present work has been carried out in the single species plots of birch (*Betula pendula* Roth), alder (*Alnus*

glutinosa L. Gaertner) and beech (*Fagus sylvatica* L.) and the mixture plots of the 3 species with 4 replicate plots for each species types. The trees are planted at 1m spacing. The layout is a block design with 4 block replicates with a minimum of 50 useable trees from each species in each plot excluding the 2 rows of edge trees. The detailed information regarding the planting blocks is given in Table-3.1.

Table 3.1 Plot size and tree measurements in 16 plantation blocks. (Height and diameter at breast height (DBH) data from survey in 2010). (Means, n = 4 plots, for Height and DBH).

Species	Plot ID	Area	Stone volume	Trees per block	Height	DBH
		m x m	(%)		(m)	(mm)
Birch	B(22)	8 X 8	20	81		
(B)	B(37)	8 X 8	9	89	5.68	41.79
	B(56)	8X 6	14	70		
	B(91)	12 X 10	15	119		
Alder	A (4)	12 X 10	16	133		
(A)	A(39)	8 X 8	8	79	6.18	50.77
	A(50)	10 X 8	16	85		
	A(85)	8 X 7	10	69		
Beech	F(7)	9 X 9	7	86	3.19	23.91
	F(40)	8 X 7	12	79		
(F)	F (71)	9X 7	10	83		
	F (79)	8 X 8	10	79		
Mixed	M(16)	13 X 13	7	217	*	*
(M)	M(33)	13 X 13	5	212	5.83 (Birch)	44.76 (Birch)
	M(52)	13 X 12	27	195	6.18 (Alder)	57.61 (Alder)
	M(67)	12 X 12	8	161	2.11 (Beech)	9.50 (Beech)

* Average of 4 mixed plots

3.4 Three native broadleaved tree species

In the present study, three native tree species were used which have distinct physiological and growth characteristics as described below.

Birch (*Betula pendula*), commonly known as silver birch, is a native, fast growing pioneer species and the fourth most common trees (after Sitka spruce, Scots pine, and oak) in the forest of British Isles. It occupies about 6% of the total forest area of Britain (Locke, 1987). They grow fast when young but never grow to large dimensions. In UK, the highest DBH of birch tree is generally 30 cm. Birch roots adapt to difficult condition such as stony soil, roots expand in the upper soil layers but the sinkers penetrate deep into the soil layers (Perala and Alm, 1990). Absence of birch in generally considered as a deficiency symptom of phosphorus in the soil (Savill, 1991).

Alder (*Alnus glutinosa*), also known as black alder, is an indigenous species in all part of Great Britain. It is typically a component of mixed broadleaved forest and characterized by a very high growth rate when young (Thibaut *et al.* 2004). It is one of the the British native nitrogen fixing species associated with *Frankia* Sp. Nodulation occurs best in the pH range 5.5-7.2 and in low nitrogen soil (Claessens *et al.* 2010). It can significantly contribute to the nitrogen content of litter and soils and consequently benefit the growth of companion tree species in mixed plantation (Giardina *et al.* 1995; Vares *et al.* 2004). However, as the early growth of alder is very fast, sometimes it is not suitable as a nurse tree because the companion trees can easily be suppressed.

Beech (*Fagus sylvatica*) is a late successional, slow growing and strongly shade-bearing tree. This UK native tree is recognized as a valuable timber producing plant in silviculture (Savill, 1991). Beech occupies about 75,000 ha or 4 % of the forest area of Britain (Locke, 1987).

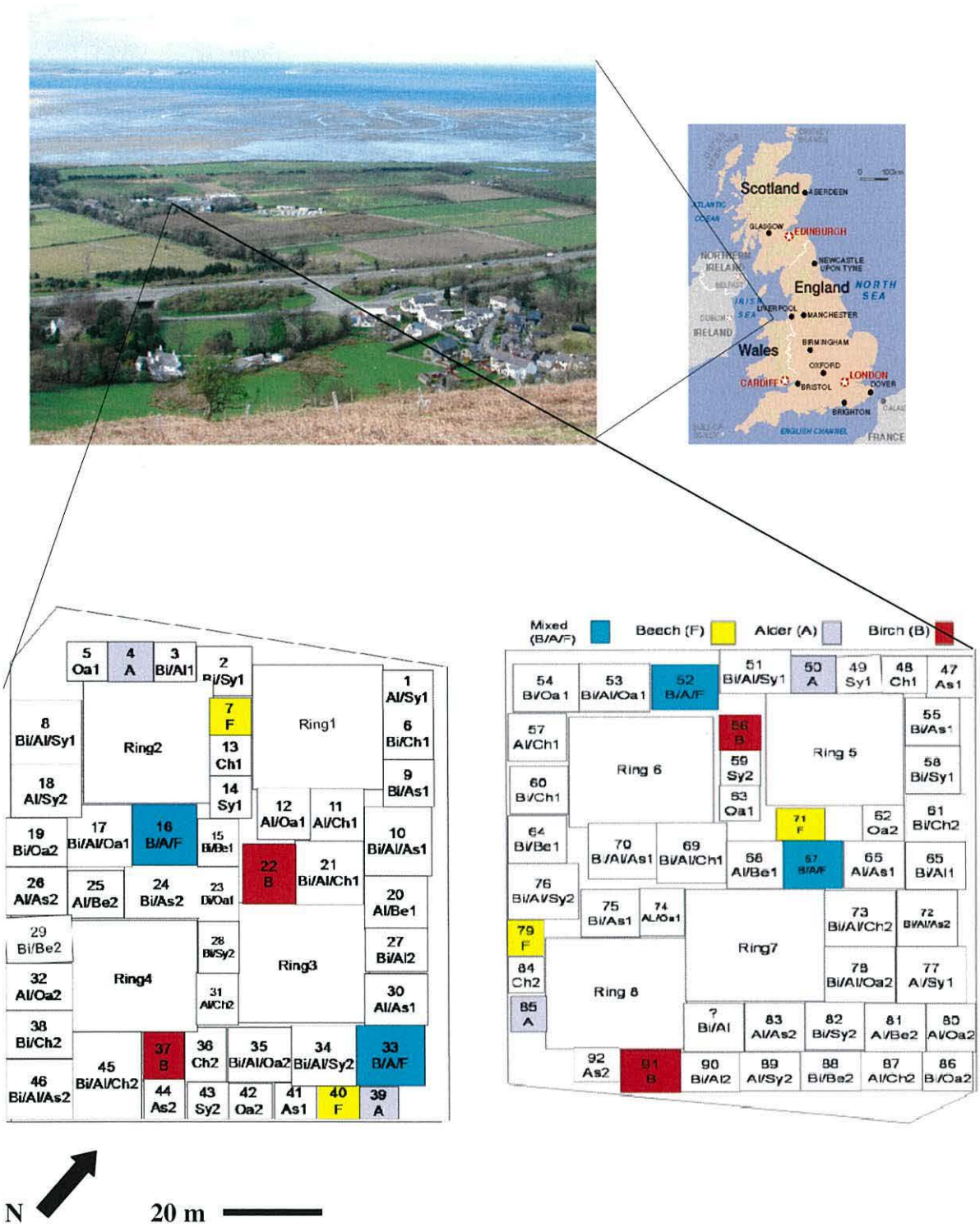


Figure 3.2 Location and study site, and plantation layout at Henfaes Research Centre. Plantation plots are split into two blocks within the 2.36 ha area. (Map and View sources: http://www.walesdirectory.co.uk, http://nvqlearning.com/UK_Flag_and_map.aspx).

4. Above and belowground biomass allocations in single and mixed stands of three native broadleaved trees

4.1 Introduction

Tree biomass is analogous to primary production as biomass accumulates atmospheric carbon through photosynthesis. Therefore the net primary production (NPP) is generally estimated by measuring plant biomass and thus considered as a basic parameter in ecosystem research (Landsberg and Gower, 1997). However, estimation of forests biomass has received much attention in recent years because of firstly, anthropogenic emissions of CO_2 are thought to be partially offset by increasing forest biomass (Nabuurs *et al.* 2007) and secondly, a change of biomass regionally is associated with important components of climate change (Lu *et al.* 2002). Biomass determines potential carbon emission that could be released to the atmosphere due to deforestation or conversion to non-forest land use. Another important role played by forest biomass is use as wood fuel, a substitute of fossil fuels, and providing wood products for more energy-intensive materials (IPCC, 2007).Therefore, accurate estimation of biomass is necessary for better understanding deforestation impacts on global warming and environmental degradation at one hand and ecosystem C sequestration and storage on the other.

Afforestation increases C accumulation through production of biomass and dead organic matter depending on the plant species and site conditions, and thus enhances carbon storage at the biomass level. Globally accumulation of biomass in afforestation varies between 1-35 t CO_2 ha^{-1} y^{-1} (Paul *et al.,* 2003; Richards and Stokes, 2004). The production and distribution of above and below ground biomass may change in response to variation of three broad ecological factors i.e. nutrient availability, physical properties of soils and climatic conditions (Scarascia-

Mugnozza *et al.* 2000). At a local scale, tree biomass accounts for most of the total plant biomass in a stand, varying with tree species, age of the trees, site conditions, and management practices (Finer *et al.* 2011). The wood density of a tree also varies in the radial and vertical directions according to a species-specific pattern (Hakkila 1979; Repola 2006). Species mixture can also influence the biomass production through the efficient use of resources to producing greater total stand biomass than the monoculture (Kelty, 2006).

Woody biomass is particularly important for long term C sequestration. Generally, as a rule of thumb, 1 m^3 wood stores ~ 0.92 t CO_2 and the woody biomass fixes C depending on its maturity and post harvesting use (Nabuurs *et al.* 2007). Although the use of wood product as biofuels results in the release of stored C immediately, it provides sustainable C benefits as a substitute of fossil fuel. Alternatively C may be fixed for hundreds of years if it is used for houses or furniture (Nabuurs *et al.* 2007). Unlike woody biomass, root biomass is rarely used as an energy source, but it provides many ecosystem services of which resource acquisition from the soil and contribution to C sequestration are the most crucial. Fine roots are recognized as the most dynamic part of the root systems and regulate belowground C flux and net primary production (Vogt 1991; Lukac and Godbold, 2011).

The above and belowground C is connected with soil C storage through the global C cycle (Deyn *et al.* 2008). Soil C mostly derives from decaying above and belowground plant tissues and root exudates, as a result the quality and quantity of C return from plant to soil can be related to plant growth rate (Chapin 2003; Lavorel *et al.* 2007). Therefore, for investigations of C dynamics in soil, it is a prerequisite to quantify the above and belowground biomass pools and their production pattern for proper understanding of the ecosystem processes. Different approaches and protocols have been formulated to assess forest biomass at local and regional levels such as forest inventory (Fang & Wang 2001, Fournier *et al.* 2003; Somogyi *et al.* 2007), radar (Rignot *et al.* 1994; Naeset 2002) and other remote sensing techniques (Drake *et al.* 2003;

Tackenberg 2007; Zheng *et al.* 2007). The most frequently used method is to harvest the model tree and to develop allometric equations, and then to use these to estimate biomass using regression models (De Angelis *et al.* 1981; Satoo 1982; Cannell 1982; Parresol 1999). However, the approach is destructive, laborious and time consuming. We used a tree harvesting method to develop allometric models for accurate estimation of aboveground biomass. The main objectives of the study are to develop up-to-date allometric models for the tree species under study and to estimate the above and below ground biomass of single and mixed species stands to evaluate the mixture effect on standing biomass and biomass production.

4.2 Materials and Methods

A. Estimation of woody biomass and development of allometric equations

4.2.1 Study sites and tree selection

In 2010 an experiment was carried out to develop the allometric relationships for the woody biomass of six year old stands following the tree harvesting approach.The experiment site, Henfaes Research Centre of Bangor University is located in the village of Abergwyngregyn, North Wales, UK. The forest plantation was established in 2004 on 2.36 ha area of the former arable land with eight native broadleaved tree species planted as single species and two or three species in mixtures.

Trees were selected from both blocks of plantation area eqially to obtain representative trees, covering the The total of 30 trees was selected as two trees from each single species plots of 12 and three trees from each mixed species plots of two. Selection was made on the basis of the diameter at breast height (DBH) to obtain a range of DBH. Before harvesting tree height, DBH and diameter at 22.5 cm were measured using a telescopic measuring pole and digital callipers. As most of the trees' circumference was not perfectly round shape, the geometric mean of the highest and lowest diameter was calculated to estimate DBH.

4.2.2 Harvesting tree and measuring dry weight

The trees were harvested in December 2010 and the branches, dry leaves (alder and birch were mostly leaf less), catkins were separated and the stems were cut into three sections, to determine moisture content along the length of the tree. The fresh weight of all separated parts was measured using an electrical balance (OHAUS, 5000 Series, Xtreme W, T51XW), bagged and dried in the oven at 80 ^{O}C until constant weight. Sub samples were collected from each lower, middle and upper part of the stems, branches and catkins and used for determination

of moisture content. The dry mass of different tree components was estimated from moisture percentage of sub samples.

$$DM = FM * \frac{DMsub}{FMsub}$$

Where,

DM = Dry mass of the plant material

FM = Fresh mass of the material

DMsub = Oven dry mass of the sub sample of the material

FMsub = Fresh mass of the sub sample of the materials

4.2.3 Estimation of woody biomass

Three models viz. power, exponential and logarithm were considered to select the equation that best predicts the relationship between woody biomass and tree variables (Table 4.2 and 4.3).The model that had low root mean square error (RMSE) and high coefficient of determination (R^2) and F value was considered to have an acceptable goodness of fit (Arevalo et al. 2007). To estimate aboveground woody biomass of the following power regression model was used for different tree variables (DBH, height, diameter at 22.5 cm and branch dry mass):

$y = a x^b$

Where y = the woody biomass of the tree (kg), x = the tree variables (DBH and diameter at 22.5 cm in mm, height in m), a and b are the parameters of the model (intercept and slope of the regression line respectively).

Data obtained from non-destructive biometric measurement (height, DBH and diameter at 22.5 cm) of the individual tree have been used in the allometric models to estimate biomass (Hood, 2006). On average, a total of 1,805 trees were measured during the March-May of each year of 2008, 2009 and 2010. These data were used to estimate the woody biomass of each year using allometric models obtained by destructive tree harvesting.

B. Fine root biomass and production

4.2.4 Measuring fine root biomass

The sampling of fine root biomass (≤ 2mm) was carried out in April-May 2009 and 2010 before starting the growing season using a soil corer (8 cm) from 16 mono and mixed species plots. Core samples were collected from three randomly selected location of each plot, equal distance from surrounding trees with depth intervals of 0-10 cm, 10-20 cm and 20-30 cm. In mixed species plot samples were collected from equal distance of birch, alder and beech stands. After harvesting, the samples were transported to the laboratory and stored in $4^{O}C$ fridge until washing. The whole core sample was collected on the set of mesh sieves (2-0.5 mm, Sierra *et al.* 2003) and thoroughly washed with tap water and sorted carefully by hand from floating water. The washed fine roots were dried at 65 ^{O}C to constant weight and the dry weight was determined.

4.2.5 Root biomass production and turnover

To estimate fine root (< 2 mm) production a root-mesh technique (Godbold *et al.* 2003; Lukac and Godbold, 2010) was used during the period of June – November 2010. In this method, a nylon mesh strip (7cm X 25 cm, 1mm mesh size) was pushed into ground vertically with a steel blade and hammer. Four strips were inserted in each of 16 single and mixed species plots. The mesh net was kept into soil for 6 months to allow penetration of surrounding new roots through the net. After the set time, the mesh was harvested using a narrow garden spade. As the mesh is two dimensional , we excavated a block of 4cm X 7cm (2 cm from both sides of 7 cm mesh), and the whole block was collected, carefully put into plastic bags and transported to laboratory. All soil adhering to root and mesh was removed with minimum disturbance, generally by the gentle flow of tap water. The fine roots that crossed through the net were cut

into 2 cm fragments (1 cm from both sides of the mesh) and dry weight was recorded after drying to a constant mass at 65 °C. Root biomass turnover rate was calculated as annual root production divided by the mean standing biomass.

4.2.6 Litter fall collection

Litterfall was collected using 40 litter traps (2 traps in each single and 4 in mixture plots) placed on June 2008. The traps consist of plastic pot (planter) of square opening (35 cm X 35 cm) with a plastic screen (4 mm mesh) attached at the bottom to allow the leaching of rain water from accumulated litter in the trap. Small holes were drilled into the bottom of each pot to drain water. Litter was collected at 15 days intervals in summer and at 1 month in other seasons. Collected litter was sorted into leaves, branch (< 2mm diameter), twigs and catkins. All components were placed in paper bags and oven dried to a constant weight at 70 ^{O}C and the oven dry weight was recorded.

4.2.7 Theoretical mixture

To determine the effect of growing species in mixture the average measured biomass from the three species mixture plots was compared to a theoretical mixture calculated from each of the species contributing to the mixture growing in monoculture. This was calculated from the parameters measured as the summed value of one-third of each of the single species plots expressed on an area basis and was termed as 'estimated'(predicted) biomass for mixsed stands. To evaluate the mixture effect on individual species, the biomass of each three species in mixture calculated separately and compared with same proportion of biomass in respective single stands.

4.2.8 Statistical analysis

Different non-linear regression models for tree allometry were assessed for the goodness of fit by comparing coefficient of determination (R^2), *F*- ratio and root mean squared errors (RMSE) of the models. ANOVA and normality test (K-S statistics) of data were performed using Sigma Plot -11(SPSS Inc. Chicago, IL). A paired sample t-test was performed to compare actual and predicted biomass in the mixed species plots. The standing fine root biomass and fine root production data were normally distributed among replicates and in all figures expressed as mean, SE (n=4). To examine the species and species mixture effects on fine root biomass and root production, one way ANOVA was conducted with SPSS 16.0 (SPSS Inc., Chicago, IL) and further post hoc test (Tukey) was done for multiple comparison of the mean and the level of significance $P < 0.05$ was accepted in all cases.

4.3 Results

4.3.1 Allometric models for estimation of woody biomass

The model trees were selected on the basis of DBH for the development of allometric equations. The DBH ranges of selected birch, alder and beech trees were 14.5-75.4 mm, 29.9-91.5 mm, 15.0-50.2 mm respectively (Figure 4.1). Other plant variables are given in Table 4.1. The tree variables considered in the present study were DBH, diameter at 22.5 cm, plant height and dry weight of branches. The curve fitting for these four variables against total woody biomass of birch, alder and beech is presented in Figures 4.2, 4.3, 4.4 and 4.5. The power, exponential and logarithmic models were considered to determine the relationship between plant woody biomass and tree variables. The properties of these models were given in Tables 4.2 and 4.3. Consedering high coefficient of determination (R^2), and F value; and comraratively small RMSE, two parameters power regression curve was the best fit for the plotted values (Arevalo *et al.* 2007). The power regression equations for four variables of three plant species are shown in Table 4.2. For birch and beech diameter at 22.5 cm was the best predictor of woody biomass (R^2 = 0.997, F= 2746.4 and R^2= 0.985, F= 517.5 for two species respectively). Although the coefficient of determination for DBH was close to that of diameter at 22.5 cm but the F value was much lower for DBH (1285.70 and 480.3 respectively). In contrast DBH of alder was the best predictor of woody biomass (R^2=0.995, F= 1491.3) compare to other three variables. Tree height and branch dry weight of all three species was showed the weakest relationships with woody biomass compare to the other two variables.

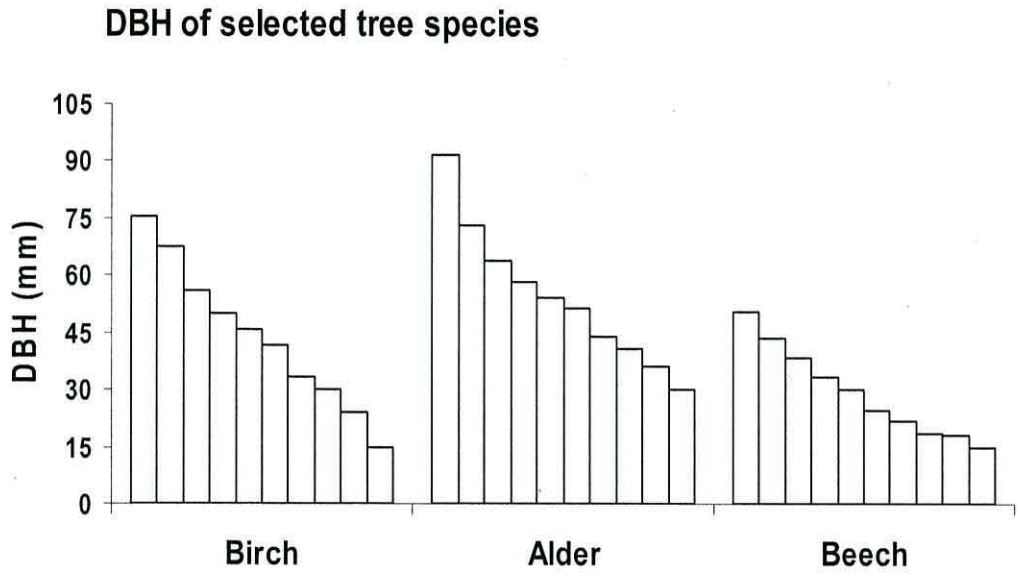


Figure 4.1 Selection of trees on the basis of diameter at breast height (DBH) for harvesting to develop allometric equations. Ten trees from each species were harvested on December 2010.

Table 4.1 Biometric parameters of the trees used for development of allometric equations to estimate woody biomass. Values in parentheses indicate SEM (n = 10).

Tree variables		Birch	Alder	Beech
Number of tree		10	10	10
DBH (mm)	**Highest**	75.45	91.46	50.25
	Lowest	14.58	29.95	15.02
Diameter at 22.5 cm (mm)	**Highest**	100.31	112.52	56.52
	Lowest	22.74	43.30	22.19
Height (m)	**Highest**	6.34	9.07	5.37
	Lowest	3.59	4.43	2.92
Total woody dry weight (kg)	**Highest**	12.85	17.51	5.20
	Lowest	0.45	1.21	0.54
Stem dry weight	**(% Woody dry wt.)**	74.52(2)	82.54 (1.4)	65.15 (2)
Branch dry weight	**(% Woody dry wt.)**	25.48	17.46	34.85

Table 4. 2 Allometric equations for birch, alder and beech to estimate woody biomass. General model $y = a x^{b}$, where y = woody biomass of plant, x = tree variables (here D, d, b and h denotes DBH, basal diameter (diameter at 22.5 cm), branch dry weight and tree height respectively), a and b are power regression coefficient.

Plant Species	Tree variables	Equations ($y = a x^{b}$)	R^2	F	*P*	RMSE*
Birch	DBH (mm)	$Y = 0.0008\ D^{2.2322}$	0.9938	1285.70	< 0.0001	0.3459
(*B. pendula*)	Basal diameter (mm)	$Y = 0.0002\ d^{2.3893}$	0.9970	2746.41	< 0.0001	0.2370
	Branches dry weight (kg)	$Y = 4.4302\ b^{0.7502}$	0.9345	114.18	<0.0001	1.1256
	Tree height (m)	$Y = 0.0001\ h^{5.8014}$	0.8773	57.227	< 0.0001	1.5406
Alder	DBH (mm)	$Y = 0.0006\ D^{2.2775}$	0.9946	1491.32	< 0.0001	0.3807
(*A.glutinosa*)	Basal diameter (mm)	$Y = 0.0001\ d^{2.6453}$	0.9884	682.81	< 0.0001	0.5610
	Branches dry weight (kg)	$Y = 6.3385\ b^{1.2229}$	0.9297	105.93	< 0.0001	1.3815
	Tree height (m)	$Y = 0.0048\ h^{3.5841}$	0.7898	30.07	< 0.0006	2.3900
Beech	DBH (mm)	$Y = 0.0071\ D^{1.6883}$	0.9836	480.28	< 0.0001	0.2151
(*F.sylvatica*)	Basal diameter (mm)	$Y = 0.0002\ d^{2.5770}$	0.9847	517.46	< 0.0001	0.2073
	Branches dry weight (kg)	$Y = 2.8883\ b^{0.8845}$	0.841	42.32	< 0.0002	0.6706
	Tree height (m)	$Y = 0.0396\ h^{2.8864}$	0.7189	20.47	< 0.0019	0.8912

*RMSE, root mean square error.

Table 4.3 Expoenetial and logarithm models to examine the relationship between woody biomass and DBH and basal diameter. General model $y = a\ e^{bx}$ (exponential) and $y = y_0 + \ln x$ (Logarithm), where y = woody biomass of plant (kg), x = tree variables {here D and d denotes DBH and basal diameter (diameter at 22.5 cm) in mm respectively}, a and b are regression coefficients.

Plant Species	Tree variables (mm)	Equation types	Equations	R^2	F	*P*	RMSE*
Birch	DBH	Exponential	$\mathbf{Y = 0.6242\ e^{0.0407D}}$	0.9757	321.47	< 0.0001	0.6854
(*B. pendula*)		Logarithm	**Y = -22.0148 +7.2838 ln D**	0.7744	27.47	< 0.0001	2.0891
	Basal diameter	Exponential	$\mathbf{Y = 0.5264\ e^{0.0323d}}$	0.9831	467.47	<0.0001	0.5706
		Logarithm	**Y =-27.6937 + 8.0970 ln d**	0.7964	31.30	0.0005	1.9845
Alder	DBH	Exponential	$\mathbf{Y = 0.8275\ e^{0.0337D}}$	0.9744	304.64	< 0.0001	0.8340
(*A.glutinosa*)		Logarithm	**Y = -48.0301+13.7332 ln D**	0.8775	57.34	< 0.0001	1.8243
	Basal diameter	Exponential	$\mathbf{Y = 0.5916\ e^{0.0304d}}$	0.9673	273.06	< 0.0001	0.9420
		Logarithm	**Y = -63.4545+ 16.4406 ln d**	0.8733	55.16	< 0.0001	1.8555
Beech	DBH	Exponential	$\mathbf{Y = 0.4777\ e^{0.0488D}}$	0.9485	147.59	< 0.0001	0.3811
(*F.sylvatica*)		Logarithm	**Y = -10.1198+ 3.7605 ln D**	0.9457	139.36	< 0.0001	0.3917
	Basal diameter	Exponential	$\mathbf{Y = 0.1842\ e^{0.0595d}}$	0.9807	407.38	< 0.0001	0.2332
		Logarithm	**Y = -14.9092+ 4.7496 ln d**	0.8823	59.98	< 0.0001	0.5764

* RMSE, root mean square error

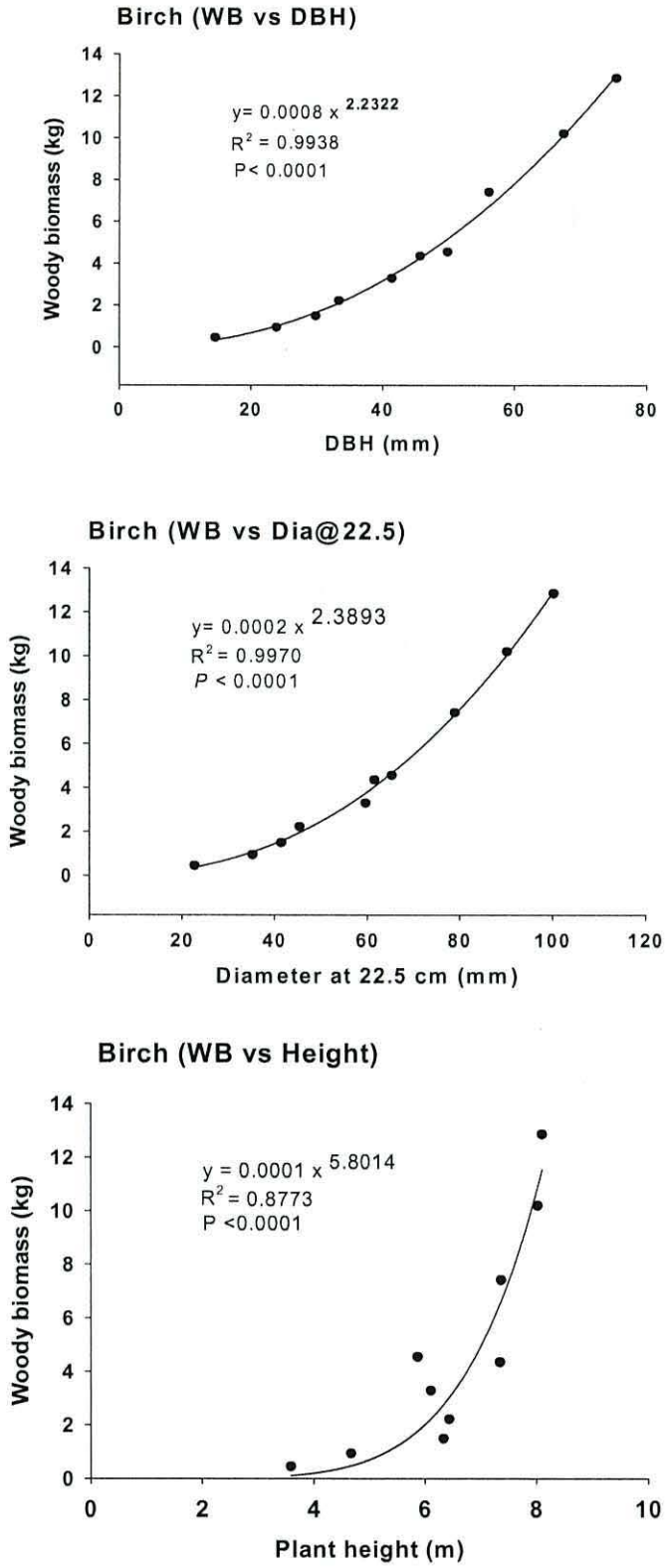


Figure 4.2 Allometric relationship between plant variables and woody biomass (WB) of birch plants, measured after the growing season of 2010. Data from 10 selected plants and fitted with a power function regression model. The regression equation, coefficient of determination and *P* values (using ANOVA) are inserted in each panel.

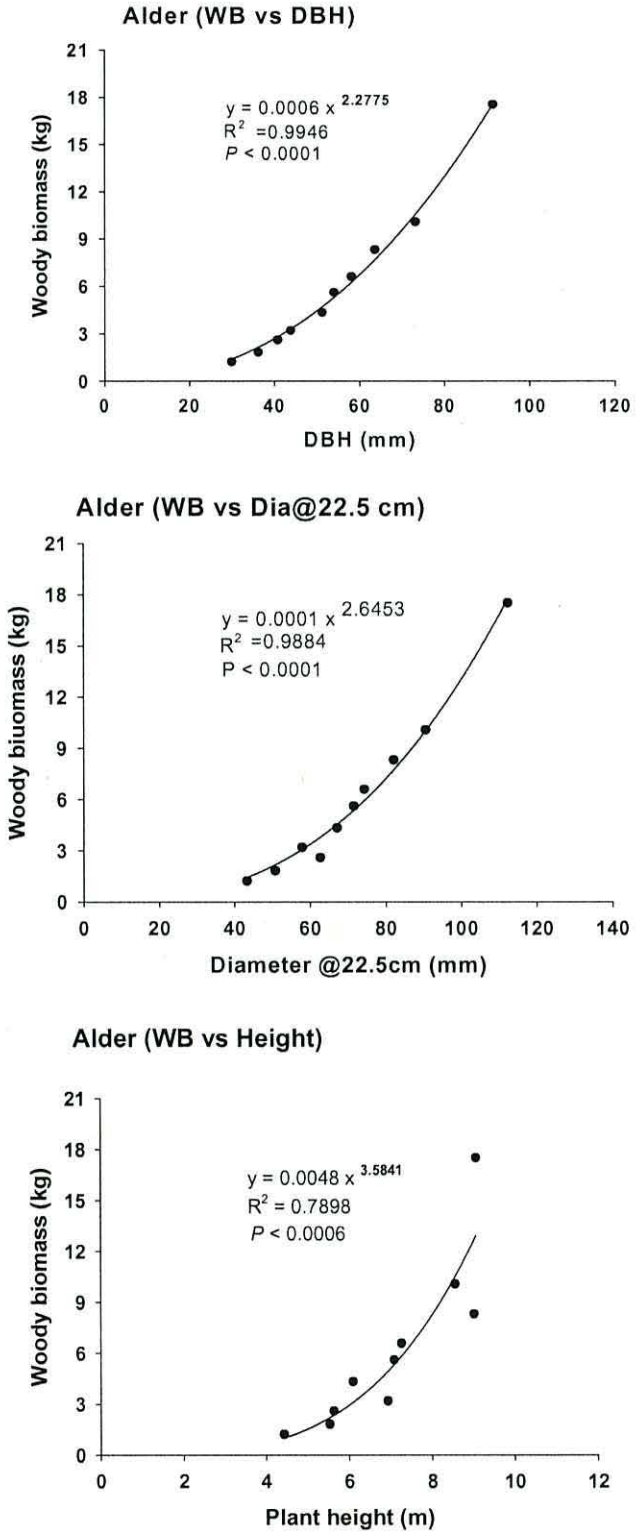


Figure 4.3 Allometric relationship between plant variables and woody biomass (WB) of alder plant, measured after the growing season of 2010. Data from 10 selected plants and fitted with a power function regression model. The regression equation, coefficient of determination and *P* values (using ANOVA) are inserted in each panel.

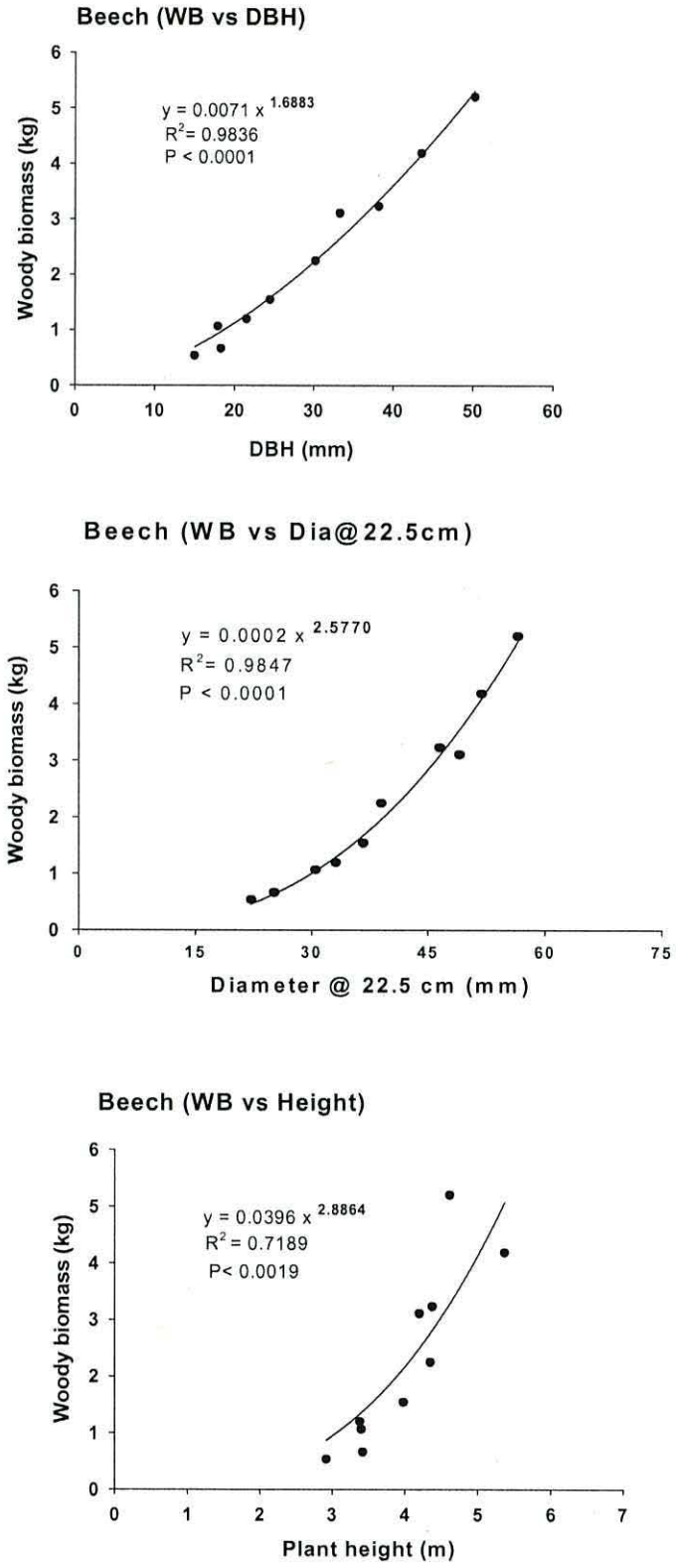


Figure 4.4 Allometric relationship between plant variables and woody biomass (WB) of beech plants, measured after the growing season of 2010. Data from 10 selected plants and fitted with power a function regression model. The regression equation, coefficient of determination and *P* values (using ANOVA) are inserted in each panel.

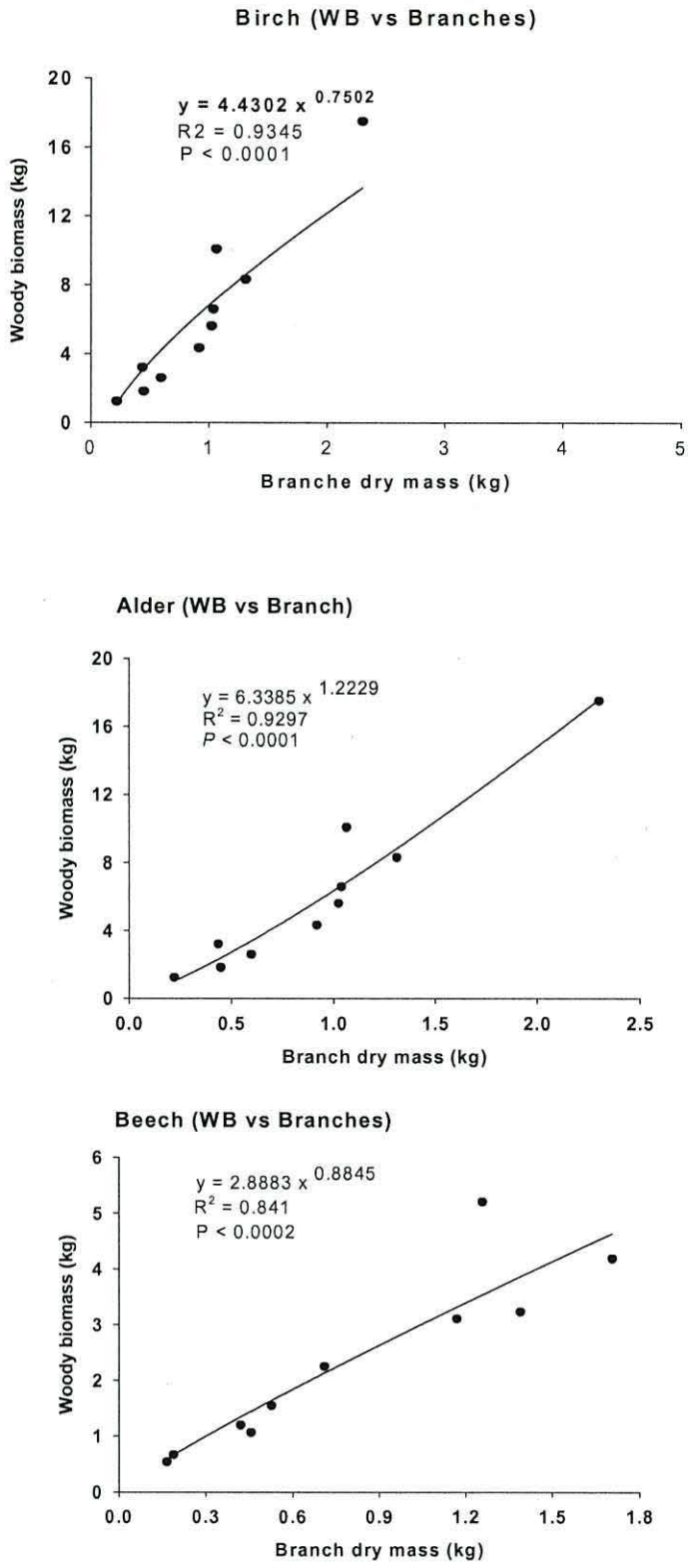


Figure 4.5 Allometric relationship between dry mass of branch and woody biomass (WB) of birch, alder and beech, measured after the growing season of 2010. Data from 10 selected plants of each species and fitted with a power function regression model. The regression equation, coefficient of determination and *P* values (using ANOVA) are inserted in each panel.

4.3.2 Woody biomass in different tree species and mixture

The aboveground woody biomass showed that the biomass in birch was the highest followed by alder, tree mixture and beech (Figure 4.6). In 2010, the standing woody biomass of birch was significantly higher (5.3 kg m^{-2}) than alder (P = 0.014), beech (P = 0.000) and the mixture (P = 0.006). Although during the year of 2009 and 2008, standing biomass in birch tended to be the highest among tree species, the variation with alder was not statistically significant. Data indicated that the yearly increment in biomass production during last two annual intervals (2008-2009 and 2009-2010) decreased considerably. During 2009 the woody biomass was estimated 4.8, 3.2, and 0.6 kg m^{-2} in birch, alder and beech plots respectively which was 30, 28 and 54 % higher than the previous year. In 2010 the increments were 10, 14 and 33 % higher for birch, alder and beech than 2009. In spite of the highest annual increment in woody biomass production during these 2 years, beech remained significantly lower in standing biomass than other two species at a 6 year stand age.

4.3.3 Mixture effects on woody biomass

The mean standing woody biomass in the mixed species plots were 3.4, 2.9 and 2.2 kg m^{-2} during the years 2010, 2009 and 2008 respectively (Figure 4.6) which were significantly higher than the beech plots, but lower than the birch. The annual increment was 30.8 and 18 % in mixed plot during last two years. The overall (stand level) biomass in mixed plot (actual) showed no significant variation compared with the estimated amount based on single stands of component species; however, the biomass in mixed species plot tended to increase at the end of three years studies (Figure 4.7).

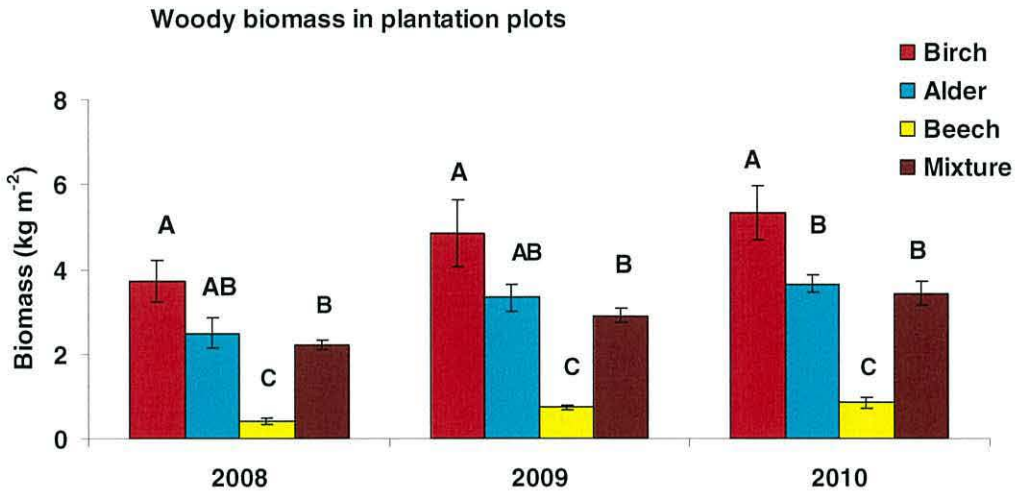


Figure 4.6 Aboveground woody biomass in single and mixed species stands of birch, alder and beech estimated using species-specific allometric equations during 3 growing season. Bars equal mean, SEM (n = 4). In the year group, bars without the same indices are significantly different ($P < 0.05$).

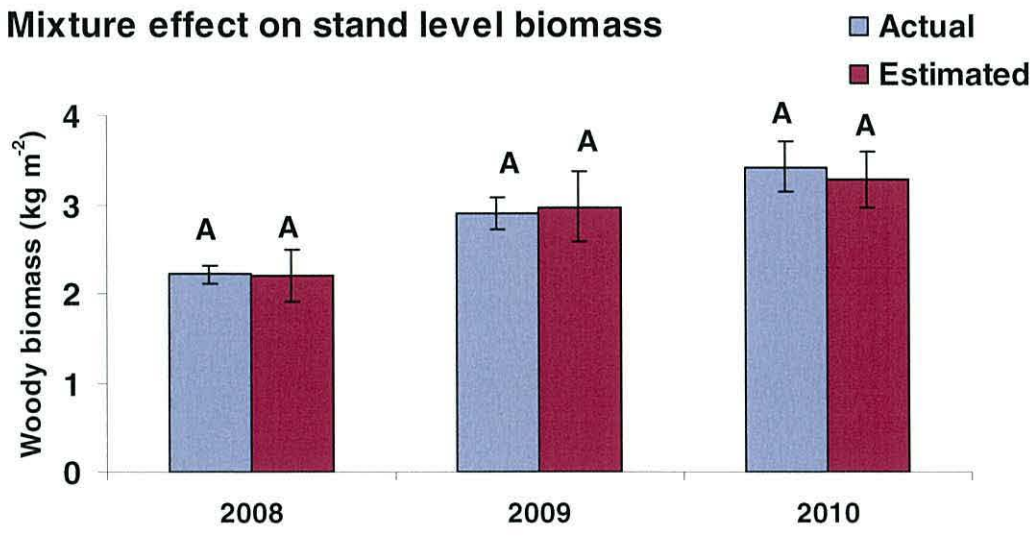


Figure 4.7 The actual (in mixed stand) and estimated (calculated from single stand) woody biomass during the three growing season to evaluate the overall mixture effect on biomass at stand level. Bars equal mean, SEM (n = 4). In the year group, bars without the same indices are significantly different ($P < 0.05$).

To evaluate mixture effect on particular species we calculated biomass of each species in mixture separately and compared it with the biomass of single stands. The analysis of growth performances of individual tree species (species level) in mixture revealed that birch and alder exhibited no significant differences between actual and estimated biomass in mixture that suggests additive mixture effects (Figure 4.8). However, alder tended to show a better performance in biomass production in the mixture. Although initially birch tended to response negatively in mixed planting, at the end of the experiment it accumulated woody biomass similar to that of the single stand (Figure 4.8). The woody biomass of beech was significantly reduced in mixed culture planting during the 3 years of the experimental period ($P = 0.013$, 0.009, and 0.02 for 2010, 2009 and 2008 respectively).

4.3.4 Estimation of fine root biomass

There was considerable variation in total (0-30 cm) accumulation of fine (< 2mm) root biomass among different plant species. The highest standing fine root biomass was recorded in alder (91 g m^{-2}) followed by birch (69.4 g m^{-2}) and beech (59.3 g m^{-2}) in 2010, which were 32, 10 and 67 % higher respectively than the previous year (Figure 4. 9). In the upper soil layer (0-10 cm) alder biomass was significantly higher than that of beech (P = 0.036). The vertical distribution of fine roots exhibited more or less same pattern of the greatest proportion of total fine roots (78-82 %) distributed in the upper 0-20 cm soil layer of three species stands; however, the higher beech root biomass tended to accumulate in the deeper soil layer (20-30 cm) compare with other two species. Root distribution in deep soil layer was significantly lower than in the surface layer in 2010. A similar trend was also observed in the previous year; however the differences were not always significant.

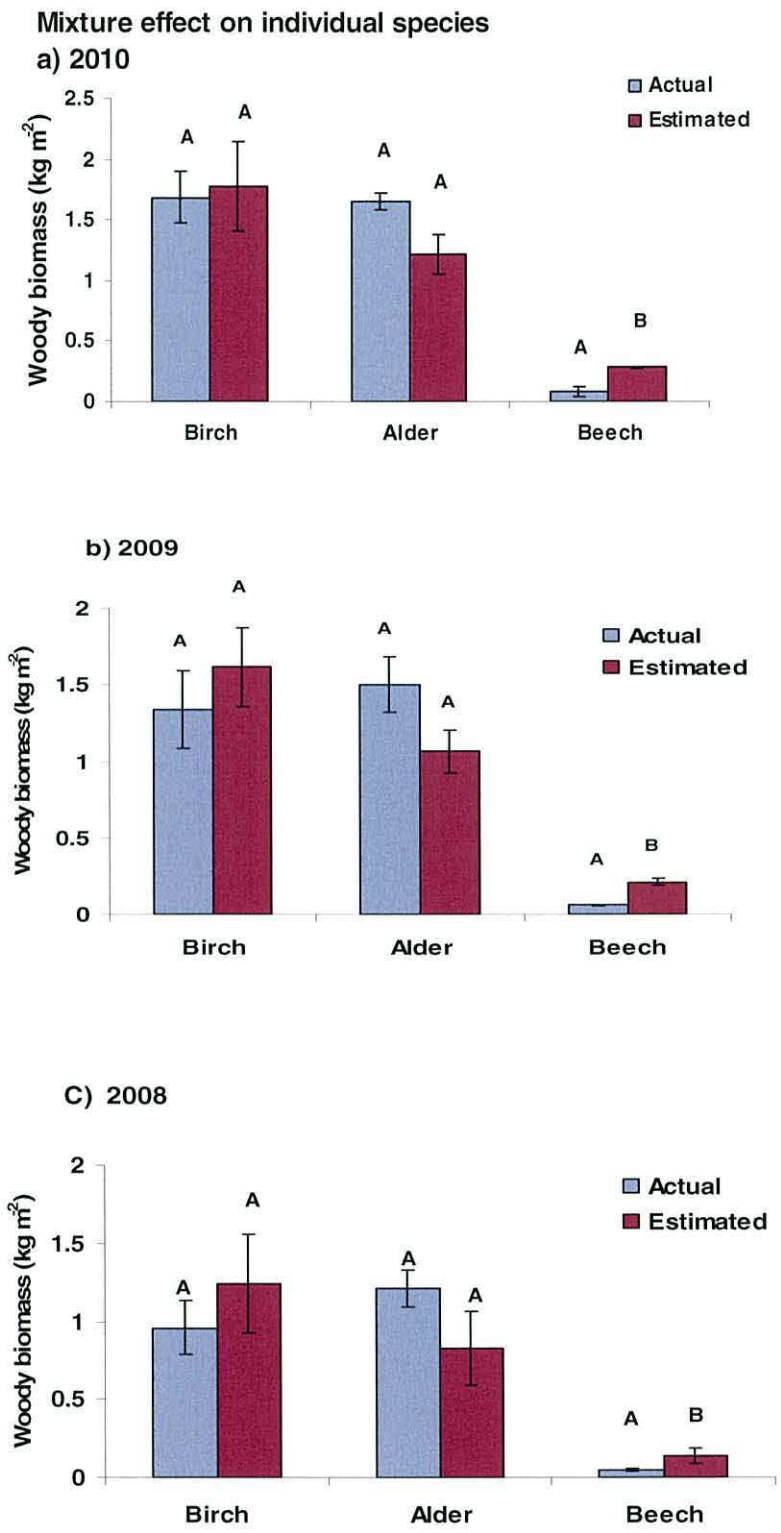


Figure 4.8 Mixture effect on the woody biomass of individual species.The actual (biomass of individual species in mixed stand) and the estimated (biomass calculated proportionally from single stand) woody biomass of the three growing seasons. Graphs showed the difference of standing biomass of a particular species between single and mixed plantings. Bars equal mean, SEM (n= 4). Bars of each species without the same indices are significantly different ($P < 0.05$).

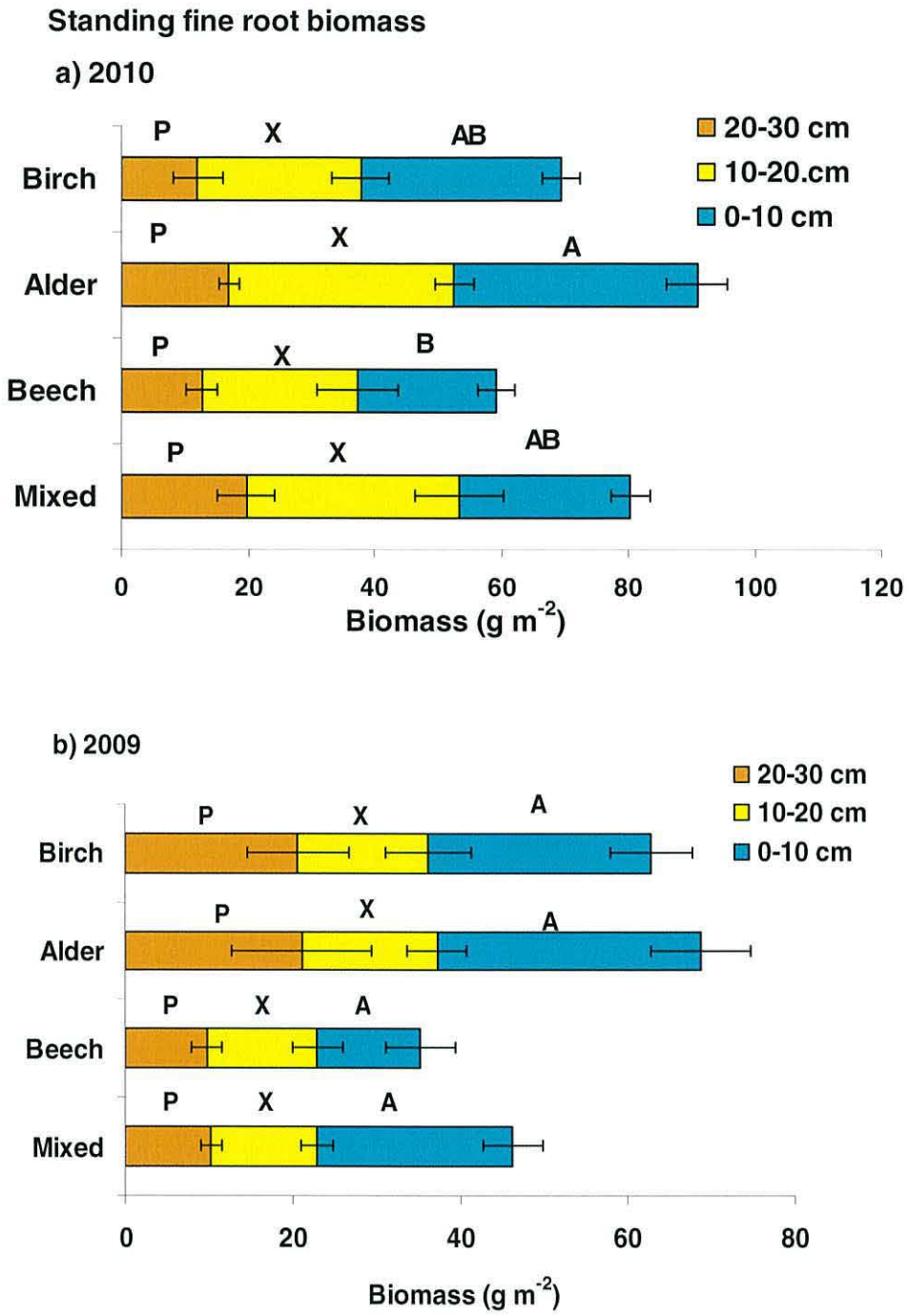


Figure 4.9 Standing fine root (< 2mm) biomass in single and mixed species stands of birch, alder and beech. Bars equal means, SEM (n = 4). Each bar has three segments representing mean biomass of three sampling depths. Multiple comparisons were made among mean root biomass of different species at particular depth. A, B and AB for comparison of different species at 0-10 cm, X for 10-20 cm and P for 20-30 cm depth. For a particular depth, bar segments without the same indices are significantly different ($P < 0.05$).

4.3.5 Fine root biomass in mixed stands

The total fine root biomass (0-30cm) in mixed plantation plots was 80.4 (14 SME) and 46.3 (6 SME) g m^{-2} in 2010 and 2009 (Figure 4. 9). Overall, the admixture of plant species showed no clear variation in fine root biomass in our experiment; however, the increment over one year was higher in the mixture (73 %) than the single species plantation. The actual root biomass in the mixture tended to be higher in 2010 than the amount estimated from single stands (Figure 4.10). Fine root mass was distributed slightly deeper in mixture than the single species with 24 % of the root system in 20-30 cm soil layer.

4.3.6 Fine root production and turnover rate

Fine root production (seasonal growth) during the growing season of June – November (2010) was 55.7 (19 SME), 72.1 (6 SME) and 26.6 (6 SME) g m^{-2} in birch, alder and beech stands in the 20 cm thick mineral soil layer (Figure 4.11). Although fine root production in alder is substantially higher in our experiment site, only the variation with beech was statistically significant (P = 0.047). In mixed species plot, the fine root production was slightly increased compared to monoculture. Alder in mixture produced 11.7 g m^{-2} higher fine root than that of the monoculture; however, in birch and beech the increments were smaller (3.1 and 3.6 g m^{-2} respectively).

The fine root turnover rate was estimated as the ratio fine root production and standing fine root biomass Root turnover rate was estimated for the year 2010 using standing biomass and root production data. The highest root turnover were, 1.1 y^{-1}(0.5 SME) for birch followed by alder, 1.0 y^{-1} (0.2 SME) and beech 0.6 y^{-1} (SME, 0.2). In mixture the overall turnover rate was same as in birch 1.1y^{-1} (0.2 SME). The species variations were not statistically significant.

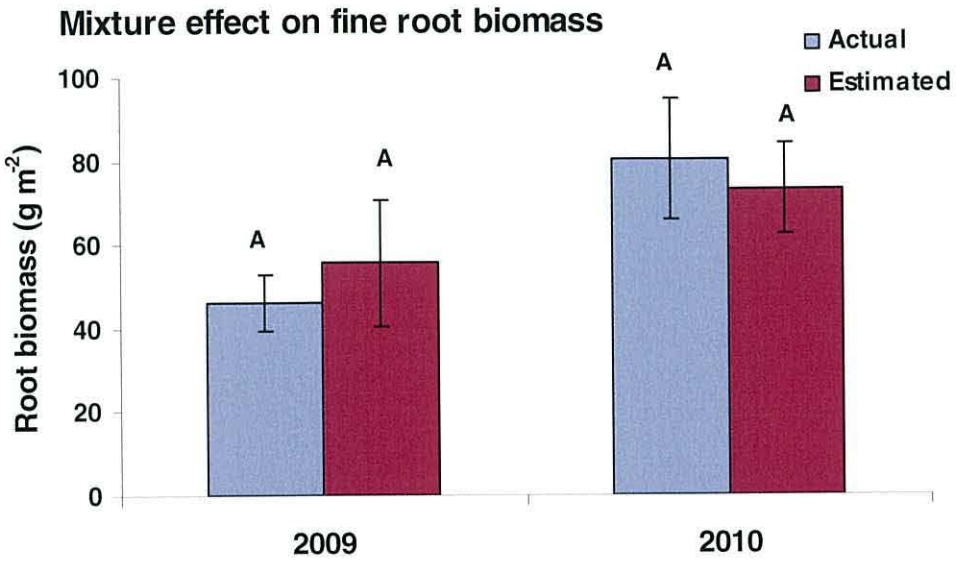


Figure 4.10 Stand level mixture effect on fine root biomass The actual (in mixed stand) and the estimated (calculated from single stand) fine root biomass during the two growing season. Bars equal mean, SEM (n = 4). Between the actual and predicted values, bars without same indices are significantly different ($P < 0.05$).

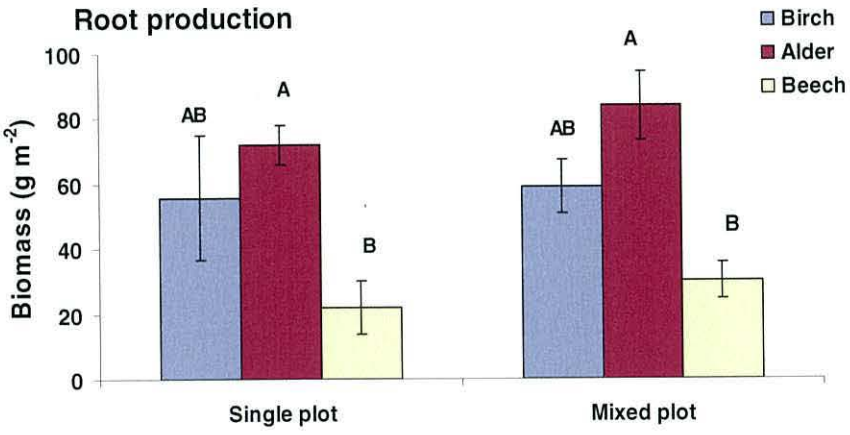


Figure 4.11 Fine root production during the growing season of 2010 (June–November) estimated by root mesh technique. In mixed plot, mesh was deployed to capture the root grown from specific plant (not overall). Bars equal mean, SEM (n = 4). In species type group, bars without same indices are significantly different ($P < 0.05$).

4.3.7 Litter fall

The total annual litterfall ranged between 178 and 472 g m^{-2} on dry weight basis the largest component was the foliar litter (> 94 % of total litterfall in in birch and beech stands was leaf litter and in alder stands it was 73 %) (Figure4.12). Considerable amounts of twigs and catkins (23%) were collected from alder stand. Although beech produced a significantly lower level of litter (P = 0.02) compared to birch and alder, the annual increment was the highest in beech (85 %). The increment in total litter production was lowest in birch (34 %); however, annual foliar litter production was 10 % higher in birch than alder. In the mixed stand a similar magnitude of litter flux was recorded for three plant species with a small quantity of litter from the beech.

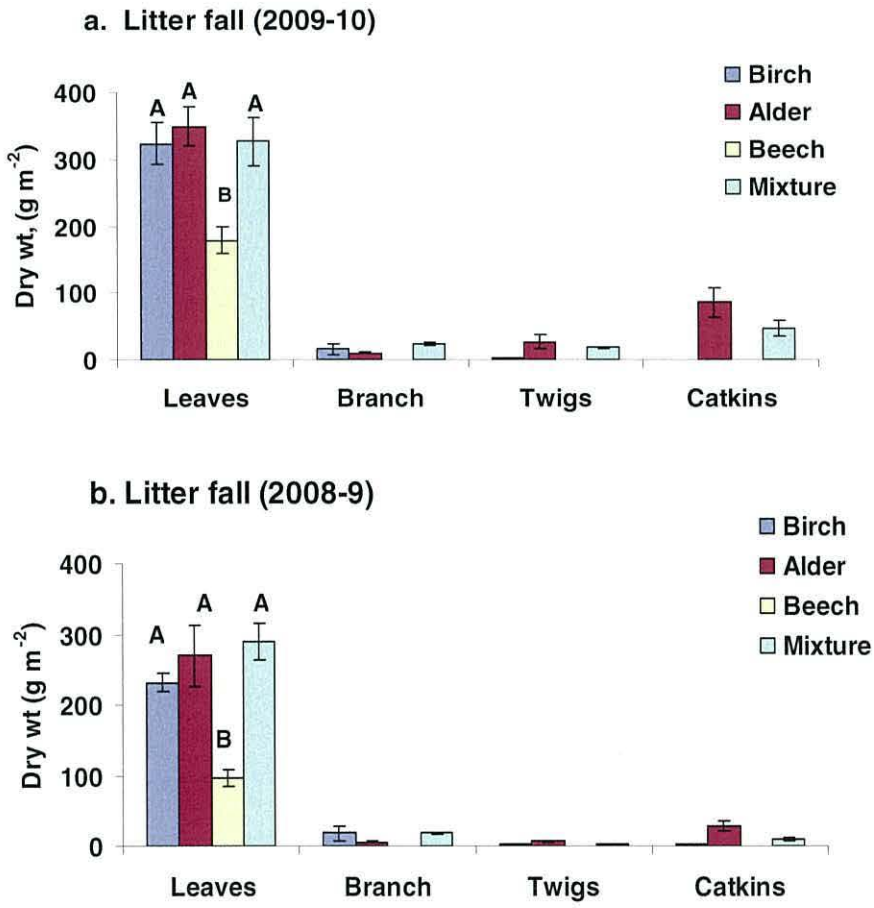


Figure 4. 12 Annual litter fall in different plant species stands and mixture for the year 2009 and 2010 (June-May). Bar equals mean, SEM (n = 4). Bars with similar indices are not statistically significant (P < 0.05).

4.4 Discussion

4.4.1 Allometric models for three tree species

The results of regression analysis confirmed that there is a strong relationship between the tree variables used in this experiment (DBH, diameter at 22.5 cm and plant height, branch dry weight) and the woody biomass. Similar models and approaches were applied to assess the total above-ground and woody biomass in the Netherlands (De Gier, 1989). The process consisted of tree sub- sampling to obtain individual estimates of dry weight, fresh weight and tree volume etc., and the development of regression models which were used for all trees with high accuracy (Mabowe 2006; De Gier 2003). Since allometric relationship for tree biomass are strongly influenced by local and regional pedo-climatic conditions (Karkkainen 2005), we compared our results to the previous study of harvesting the model trees of young birch , alder and beech grown in the same hyperoceanic temperate climatic conditions of North Wales (Hood, 2006). Our results suggest the best fit is a nonlinear power relationship between plant variables and the aboveground woody biomass, as shown by the goodness of fit of the models. The non linear models especially power functions are commonly used in biomass estimation because they strike a good balance between accurate predictions and low data requirements (Ter-Mikaelian and Korzukhin 1997). The previous studies at the same location and plant species indicated the use of power functions for biomass estimation with considerable goodness of fit. However many studies used other functions (polynomial, linear, logarithmic etc.) with complicated mathematical functions which sometimes do not improve the fitness of the model, but do further complicate the application of the biomass equation (Overman,1994).

In our experiment, the woody biomass of alder showed a good fit of the model with DBH which was in agreements with the previous findings of strong relationships between above ground biomass and DBH (Arevalo *et al.*, 2007; de Gier,2003; Ketterings *et al.*, 2001; Overman, 1994; Crow, 1978). Although the non linear relationships between DBH and biomass have been studied by many investigators for long time and found to be adequately predictable, our results suggest that for birch and beech the basal diameter (diameter at 22.5 cm) was the better predictor of woody biomass than other plant variables. In fact the coefficients of determination (R^2) for the two variables (basal diameter and DBH) were very close in the cases of birch and beech (R^2 for basal diameter and DBH were 0.9970 and 0.9938 for birch, and 0.9847 and 0.9836 for beech respectively). However considering other statistical indicators such as higher F value and minimum residual sum square (RSS) of the equations (Parresol, 2001), the DBH and basal diameter have been found appropriate to predict woody biomass of alder and birch and beech respectively. The basal diameter as a predictor of woody biomass might be due to the lower growth of young birch and beech than alder, as Williams and Mc Clenahen (1984); Ter-Mikaelian and Parker, (1999); Schmidt *et al.* (2009) suggested that, the basal diameter was the most important parameter especially for the young trees and explained more than 95 % of the variability of different biomass compartments. In the previous study, the woody biomass of all the 3 plant species was correlated with basal diameter when the trees were two year old (Hood, 2006). We assumed that as the growth increase with the plant age, DBH becomes more efficient than basal diameter in predicting the woody biomass in alder.

Two major sources of uncertainty associated with the development of allometric equations, are sampling and regression errors. Sampling error includes considering the trees whose DBH is well beyond the range of model tree size used to develop the equation (Aboal *et al.* 2005), and the number of harvested trees. We selected the model trees on the

basis of increasing level of DBH to minimize sampling errors. Some investigators used a combination of basal diameter and total plant height as independent variables to increase the potential applicability of the equations to cover different locations (Cole and Ewel 2006; Schmid *et al.* 2009). However, for the trees used here the addition of height did not improve the allometric models (data not shown, and Smith 2011). Thus for the estimation of woody biomass in the small scale plantation site we used only DBH in our allometric models.

4.4.2 Plant woody biomass in monoculture and mixture

The aboveground woody biomass of birch, alder and beech showed a consistent pattern of birch > alder > beech over the last 3 years. Our estimated standing woody biomass in birch (4.8 kg m^{-2}) and alder (3.1 kg m^{-2}) agrees with the published data. Johansson (1999) estimated 3.3 kg m^{-2} of woody biomass in seven year old stand of *Betula pendula* (Roth) grown on abandoned farmland in Sweden. In Finland, Hytonen *et al.* (1995) assessed 3.4 and 2.4 kg m^{-2} of woody biomass (leafless) in six year old stands of downy birch (*Betula pubescens*) and grey alder (*Alnus incana*) respectively. Wittwer and Stringer (1985) reported 4.2 kg m^{-2} of woody biomass in black alder (*Alnus glutinosa*) for five year old trees in a temperate forest on the Ohio River floodplain. The mean annual production of woody biomass in a 6 year old birch stand was 0.81 kg m^{-2} in our experiment. Uri *et al.* (2007) found 1.2 kg m^{-2} of annual aboveground biomass production in an 8 year old stand of *Betula pendula* in naturally regenerated farmland in Estonia. Although standing biomass and biomass production vary widely due to spatial variations, the consistent data indicate the good predictability of the allometric equations used in the experiment. The considerable decline in the annual production of biomass in the 3 plant species particularly in birch and alder is due to the effects of tree density. In similar but slightly denser plots (80 cm spacing) at the same site, leaf area index the increase in leaf

area index with stand development reach an asymptote in 2008. Competition for light is among other factors known to limit production (Elowson 1996). As a dominant late successional species, European beech (*Fagus sylvatica*) accumulated the lower biomass but exhibited the higher annual woody biomass production rate than the other two species. Overall, our analysis indicates no clear mixture effects on the accumulation and production of woody biomass in mixed species stands; however, individual species at tree level, particularly alder, tended to show positive effects in mixture. This finding contrast with the general pattern of higher biomass accumulation in the mixed species stands compare to single (Kelty 1992). The main benefit of mixed culture planting over monoculture is presumably the efficient use of site resources due to combination of species with substantial variation in characteristics such as shade tolerance, height growth rate , crown structure, root depth etc. (Kelty 1992). In addition, incorporation of N fixing species in mixture may increase N availability in the soil due to addition of nutrient rich litter from the fixer species which can be used by other species (Kelty 2006). However, due to competitive effects of faster growing N fixing species problems may occur for other species in mixture when planted at the same time (Binkley, 2003). In our study, faster growing N fixing alder was the higher canopy species and birch was sub-dominant in mixture which might have reduced the beneficiary effects of mixture (Kelty, 2006). In our mixed plot no clear stratified canopy layer was formed (mean height of birch and alder was 5.83 and 6.18 m respectively in mixed plot) which might be diminished the mixture effect. Parrotta (1999) reported no significant difference in total biomass production between mixtures and monocultures of *Eucalyptus robusta* and N fixing species *Casuarina equisetifolia* when grown in mixed plantation at 1:1 proportion due to strong competition between the trees.

Another cause of the lack of mixture effect might be tendency of birch not to respond to soil inorganic N (NH_4^+). It has been reported that higher amounts of NH_4^+ in the soil does not cause a large NPP or biomass production for *Betula pendula* in acidic soils, where NH_4^+ is usually dominant form of inorganic N (Esmeijer-Liu *et al.* 2009; Troelstra, 1990). This might be a further reason why birch does not increase biomass production when planted with alder. At the present experimental site, the level of both NH_4^+ and NO_3^- increased in the soil solution (Hoosbeek *et al.* 2011); however, N fixed by alder did not greatly contribute to the total N pool in the soil at these stands (Millet *et al.* in review).

The most pronounced mixture effect in our experiment was a significant decrease in beech biomass. It was clear from the result that due to a decline in beech biomass the overall mixture effect tended to be negative or have no effect. The species trait of persisting in the understory for a long time without appreciable growth (Johnson *et al.* 1997) might be responsible for low biomass accumulation in beech stands of mixed plot. The lower competitiveness of beech due to limited ability to acclimate to different light environment (Givnish, 1988; Kuppers, 1994). Beech is recognized as shade tolerant and can survive well in the understory.

4.4.3 Fine root biomass in monoculture and mixture

The main objective of this study was to estimate the standing fine root biomass pool in the birch, alder and beech plantings, and to investigate whether the species mixture affected the fine root biomass pool. Our data of two growing seasons indicated that the increasing standing fine root biomass is in the order: alder < birch < beech. However, there was no clear spatial variation in the fine root system of the 3 plant species with in 0-30 cm of soil profile after 6 years of growth. In addition to species identity, many soil, stand and climatic factors influence the variation in fine root biomass among different tree species (Finer *et al.* 2011). We estimated 69 g m^{-2} (11 SME) of fine roots in the six year old birch

stand. Kalliokoski *et al.* (2010) reported 75 g m^{-2} fine root biomass in 0-30 cm soil layer in *Betula pendula* saplings in Finland. This variation might be due to stand age (Mekkonen and Helmisaari, 2001). The fine root biomass of alder and beech was estimated to be 91 and 59 g m^{-2} in our study site. Uri *et al.* (2007) estimated 87 g m^{-2} fine root biomass in 10 years old grey alder stands on former arable land in Estonia.

Our results indicated that 78-82% of fine roots were concentrated between 0-20 cm soil depth which was consistent with previous findings. Makkonen and Helmisaari (1999) found 87 % of fine root biomass of Scots pine (*Pinus sylvestris*) in the top 20 cm of mineral soil layers. Uri *et al.* (2007) reported that 76.2% of fine roots of silver birch was located in the upper 20 cm soil layer. This is because generally the distribution of fine roots in the soil profile is influenced by nutrient rich soil patches, and the highest plant nutrients concentration is found in the top soil (Morris 1996, Schmid and Kadza 2005, Uri *et al.* 2007). Cermak *et al.* (1993) and Nadezhadina *et al.* (2006) suggested that the plant roots system can rapidly change their spatial pattern of water uptake as the distribution of fine roots is connected with the water availability and sensitivity to drought. Location specific soil properties such as soil type, stoniness and impermeable layers etc. can also influence the vertical distribution of fine roots in the soil (Schenk and Jackson, 2002).

In mixed species plots, the overall fine root biomass was 80.4 g m^{-2} which was slightly higher than the calculated predicted values. At this stage we can only assume that the fine root biomass pool in species mixtures tended to be higher than single species plots and no clear synergistic effect was observed. In fact the below ground competition and interactions among different species in mixture is much more complicated than in monoculture, and needs a more detailed study. The dynamics of fine root distribution in mixture may be greatly influenced by factors such as interactions between species (i.e. competition for resource acquisition and thus change in the distribution pattern as

mentioned earlier), mycorrhizal associations which influence the availability of resources, or species specific associations between soil fungi, microbes and plant roots (Zobel *et al.* 1997). For example, it has been reported that the belowground competitiveness of beech in a mixture can push the root systems of other species toward the surface, and thus the beech fine roots occupy a large portion of rooting zones (Leuschner *et al.* 2001; Schume *et al.* 2004).

The estimation of structural root biomass often rely on allometric equations which are generally derived from limited data set (Law *et al.* 2001) and many observations are excluded due to the difficult sampling methods (Hart *et al.* 2003). Because of high spatial variability of course root, excavation was reconized as the most accurate methods to estimate course root biomass (Retzlaff *et al.* 2001). However, due to the time and labour intensive sampling methods, estimation of structural roots was not included in the present study.

4.4.4 Root production in single and mixed stands

The growth of fine roots is a continuous, round the year process depending on the simultaneous occurrence of internal resource (carbohydrates) allocation and the availability of soil nutrients at the immediate vicinity of fine roots (Lukac and Godbold, 2011). Thus the production of fine root is closely related to plant growth. Our result showed the highest fine roots production in alder (72 g m^{-2}) followed by birch and beech. Lee and Jose (2003) estimated 144 g m^{-2} (23 SEM) annual production of fine roots in seven year old loblolly pine (*Pinus taeda* L) stand by the in-growth core method in a temperate plantation in Florida. On the other hand, results from a mixed stand showed that there was a slightly higher fine root production of three species in mixture compared to single species plots. A greater fine root production in mixed stands compare to pure was also reported by Fredericksen and Zedaker (1995) in an experiment with pure and mixed stand of three year

old loblolly pine, red maple and black locust in South-eastern United States. Positive effects of admixture on fine root production might be because of the capacity of plant species to improve their resource acquisition by niche- partitioning in the mixture in comparison with monocultures (Kelty, 2006; Forrester *et al.* 2006). Reduction of competitive overlap due to differences in root distributions in mixed stands was also postulated by some investigators (Fredericksen and Zedaker 1995). Contrasting results of lower fine root production in 15 year old mix stands of Sitka spruce and Scots pine (97 g m^{-2}) compare to pure stand of Sitka spruce (181 g m^{-2}) in upland heath was reported by McKay and Malcolm (1988) which might be due to interspecific competition between component species in mixture to capture nutritional resources (da Silva *et al.* 2009).

Methodological variations can be a source of variation during the assessment of fine root production (Majdi and Andersson, 2005; Lukac and Godbold, 2010). Although estimation of fine root production through the root mesh technique is superior to other methods, there is a possibility of underestimating the production if new roots do not penetrate through the mesh (Hirano *et al.* 2009).

4.4.5 Root turnover rates in single and mixed stands

. The mean turnover rate was highest in birch (1.1 y^{-1}) and the lowest in beech (0.6 y^{-1}) in our experiment. In mixed stands the turnover rate was same as in birch (1.1y^{-1}). Lee and Jose (2003) estimated fine root turnover rate of 0.54 y^{-1} in a 7 years old loblolly pine (*Pinus taeda* L) stand in a temperate plantation. Godbold *et al.* (2003) estimated fine root biomass turn over between 0.28y^{-1} and 1.0 y^{-1} in Norway spruce stand in a temperate forest in Germany. Montagnoli *et al.* (2009) reported that fine root turnover rate of 1.04 and 0.83 y^{-1} at the sampling depth of 0-10 and 0-30 cm respectively in a beech (*Fagus sylvatica*) stand at Alps Valley, Italy . Two major causes that might contribute to the

inconsistent findings are firstly, species specific differences and secondly methodological variations (Majdi *et al.* 2005). At a tree level, fine root production and turnover is generally regulated by starch and sugar deposition in the roots, root maintenance respiration rates and root temperature (Marshall and Waring 1985). Different fine root diameter that is used in minirhizotron and ingrowth core methods may influence the estimation of fine root turn over (Finer *et al.* 2011).

The main purpose of quantifying the root turnover is to assess the C input to the soil. In forest ecosystems, the extrametrical mycelium of mycorrhizal fungi associated with plant fine roots is also recognized as a large C pool (Wallander, 2006). Lukac and Godbold (2010) reported that most of the tree species in boreal ecosystems contain ectomycorrhizae in the root systems and therefore, the hyphal production should also be quantified for more accurate assessment of C flux through fine root turnover.

4.5 Conclusion

Although labour is expensive and time consuming, the destructive tree harvesting approach is still a widely use method of biomass estimation. Using DBH and basal diameter have been found as best predictors of plant aboveground woody biomass. Using the species specific allometric equations, higher woody biomass was found in birch compare with alder and beech, suggesting the potential of birch trees for long term C sequestration in aboveground parts. Although at the stand level, fine root biomass and fine root production tended to be higher in alder, the annual turnover rate was slightly higher in birch. The response of species mixtures on belowground biomass and turnover rate was slightly positive compare to single species stands which might be crucial in long term C storage in our experiment site.

5. Studies on decomposition of single and mixed leaf litter using laboratory incubation and litterbag approaches.

5.1 Introduction

Leaf litter decomposition plays the key role in soil nutrient cycles by regulating carbon and other nutrients fluxes from plants to soil. It is the integral part of global C budget (Aerts, 1997). Decomposition is a transformation process for C, mainly from the biosphere to the lithosphere or the atmosphere. All components of the forest ecosystems, directly or indirectly, are involved in or influenced by this process. It provides many ecosystem services such as recycling nutrients, renews soil fertility, and is a driver of carbon sequestration (Wall and Virginia, 2000). The consequences of litter decomposition are widespread. Most of the essential plant nutrients are released during different stages of decomposition and subsequently used by plants and microbes, whilst carbon is released to the atmosphere during a wide time frame through complex biogeochemical processes (Didham, 1998). Long-term storage of organic C in soils occurs by humification and fixation on soil mineral surfaces (Kramer *et al.* 2003). Rates of humification are dependent upon the quality of litter inputs, but also on nitrogen availability (Neff *et al.* 2002). Substrate quality, soil microbial decomposer and environmental conditions are three major factors that influence the rate of decomposition (Berg and McCaugherty 2003; Vitousek *et al.* 1994; Swift *et al.* 1979).

Litter decomposition and nitrogen mineralization have long been studied as an important link in the nutrient cycles of ecosystems (Van Vuuren, *et al.* 1993; Vitousek *et al.* 1994). Recently the role of litter decay on global climate change and carbon sequestration has received tremendous attention, due to the fact that impacts of elevated atmospheric CO_2 concentration and globally rising temperature may have an effect on decomposition process and consequently on C cycles in terrestrial ecosystems (Fierer *et al.* 2005; IPCC, 2007; Sokolov *et al.* 2008). During litter decomposition a considerable amount of greenhouse gases, especially CO_2, is released to the atmosphere through the respiration process, which is a driving factor in global climate change. Thus, factors that enhance the degradation process may also be responsible for greenhouse gas emission (Berg and McClaugherty, 2005). Soil can sequestrate C through humification and long term storage capacity. Stability of humus is also important in predicting the global atmospheric C budget (Schlesinger and Andrews, 2000). Therefore appropriate knowledge about organic matter decomposition, especially leaf litter decomposition, is indispensable for understanding and managing C storage in forest ecosystems.

The importance of dissolved organic matter (DOM) in soil biochemical processes is now well recognized (Park *et al.* 2002). Leaching of soluble organic materials has been considered as one of the main processes in litter decomposition. The soil solution contains various quantities of dissolved organic matter (DOM) which includes dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and soluble phenolic compounds etc, parts of which are derived from plant litter during decomposition. Recent studies have indicated that the turnover of dissolved organic nutrients in soils is a major pathway of nutrient cycling (Jones *et al.* 2005; Kalbitz *et al.* 2000). Therefore, characterization of soil solutions derived from decomposed litter during the course of decay is crucial for understanding the decomposition dynamics, and is a valuable tool in controlled

environmental studies. We studied litter decomposition using a litterbag method, but as litter bag studies provide no information about the dynamics of dissolved organic substances, we also a used laboratory incubation experiment to characterize DOM release during decay processes.

Most of the decomposition research has addressed the decay process of litter of individual species (Gartner and Cardon, 2004). However, the potential interactions among leaf litter of different species during decomposition may change the decay rate and nutrient release, and consequently influence the soil carbon stock (King *et al.* 2002; Berg and McClaugherty, 2005). Mixing leaves from species with differing resource quality and leaf structure may change the chemical environment and physically alter the total litter surface where decomposition is occurring (Mc Arthur *et al.* 1994; Salamanca *et al.* 1998; Hector *et al.* 2000). These interactions can also affect decomposer abundance and activity (Hanse, 1999; Wardle, 2002).

In the present experiments, we examined the decomposition of birch (*Betula pendula*), alder (*Alnus glutinosa*) and beech (*Fagus sylvatica*) leaves separately and in mixture, both in laboratory controlled conditions using an incubation method and in the field by using litter bags. The main objectives of the study are to examine the decay rate and release of nutrients and dissolved compounds during decomposition under single and mixed species woodlands.

5A. Laboratory incubation experiment

5A.1 Materials and Methods

5.1.1 Soil and litter collection

Soils: The soil was a Dystric Cambisol (Brown Earth), collected from a depth of 0-10 cm from the mixed plantation block of birch alder and beech in Henfaes Research Centre of Bangor University. Stones, plant materials and tree roots were sorted manually and removed and the soil thoroughly mixed. Soil was then sieved by a 2 mm sieve and stored at 4°C until used. Soil moisture and the maximum water holding capacity were determined by the gravimetric method. Some physico-chemical properties of the soil were presented in Table 5.1.

Collection of leaf litter: Leaf litter was collected in mid September 2008 from litter trap (as described in Chapter 4, Section 4.2.6), placed on the single plantation blocks of birch (*Betula pendula*), alder (*Alnus glutinosa*) and beech (*Fagus sylvatica*). Fresh litter was dried for 48 hours at room temperature (20 ^{0}C), petioles were discarded, cut into small pieces (approx.1cm X 1cm), weighted and then stored in paper bags at room temperature.The moisture content in leaf litter was determined gravimetrically by drying at 80 ^{0}C for 48h.

5.1.2 Treatments and soil incubation

A laboratory microcosms consisting of resealable plastic bags (25cm X 15cm) and soil with added leaf litter. An equivalent of 100 g air dry soil was used to study the decomposition of leaf litter. Soil was moistened to 70% of water holding capacity which was maintained throughout the incubation period by adding distilled water as necessary. Leaf litter (2g) of birch, alder, beech and mixed birch, alder, beech were placed into the

plastic bags and leaf pieces were covered with soil. The mixing ratio was 40:50:10 for birch, alder and beech respectively, approximately proportional to the litter fall of the mixed species plots. One bag with only soil was treated as the control. Six sets of microcosms were incubated at 10 ^{0}C for periods of 0, 3, 6, 9, 12 and 15 weeks incubation. Three replicates were used for each type of leaf litter and control for each sampling time intervals. During each sampling event, the whole contents (soil plus litter residues) in individual bags were transferred to PTFE centrifugal extraction cups, and centrifuged for 30 min at 4000 rpm, and the supernatants were collected and stored at -18 ^{0}C until further analysis.

5.1.3 Chemical analysis of soil solution

EC and pH: The pH and electrical conductivity (EC_{sol}) of the soil solutions were determined by a Jenway pH meter and Jenny conductivity meter.

Ammonium (NH_4^+) in soil solution was determined colorimetrically by the salicylate-nitroprusside method using a PowerWave XS microplate Spectrometer (BioTeck Instuments Inc.) for absorbance readings at 667nm (Mulvaney 1996).

Nitrate (NO_3^-) was determined colorimetrically using a PowerWave XS microplate Spectrometer (BioTeck Instuments Inc.). In this method NO_3^- was first reduced to NO_2^- followed by the reaction with N-1-napthylethylenediamine to produce chromophore. Absorbance was read at 540nm.

Total Dissolved Nitrogen (TDN) and ***Dissolved Organic Carbon (DOC)*** in soil solutions were determined with Shimadzu TOC-V (Total organic carbon analyser) and TNM-1(Total Nitrogen Measuring unit, Shimadzu Corp. Japan). In this method 50µl of soil solution was injected into a combustion furnace at 720 ^{0}C with subsequent detection of N_2O using a chemi-luminescence detector.

Dissolved Organic Nitrogen (DON) was calculated as the difference between TDN and DIN (Dissolved Inorganic Nitrogen) where DIN=$NO_3^- + NH_4^+$

Total phenolics were determined colorimetrically using Folin and Ciocalteau's reagent.

Total organic C and N: Total C and N in oven dry powder samples of fresh leaf, litter and soils were determined by TruSpec® CN analyser as mentioned earlier.

5.1.4 Mixture effect (Actual and predicted values)

To study the effects of mixing leaf litter on release of various water soluble compounds during decomposition, the amount released from mixed litter treatment, termed as actual value, was compared with the sum of proportional amounts released from the single litter treatments of component species. In the present study, the proportion of birch, alder and beech leaf litter in mixture was 40:50: 10. Therefore, the predicted (estimated) amount of any compound in mixture was calculated as follow (Salamanca *et al.* 1998):

Predicted (estimated) = (Amount in single treatment of birch x 40 %) + (Amount in single treatment of alder x 50 %) + (Amount in single treatment of beech x 10 %).

If the actual amount of any component is higher than predicted amount, the mixture effect was considered to be the positive and if predicted value is higher than actual, the mixture effect was evaluated as negative (Table 5.2).

5.1.5 Statistical analysis

Release of water soluble organic compounds from five treatments (control, birch, alder, beech and mixed litter) after different time intervals were compared among all different combinations following *Post hoc* multiple comparison test using Tukey HSD. To assess the relationship between NO_3^- and electrical conductivity in soil solution, Pearson

Correlation Coefficient (r) was detemined. Paired sample t-test was used to compare the actual and predicted values to assess the mixture effects. The variations among different treatments were considered to be significant at $P<0.05$. All statistical analyses were performed using SPSS (Version 16.0).

Table 5.1 Some physico-chemical properties of soil and leaf litter used in litter bag experiments. Mean, (SEM).

	Soil	**Fresh leaf litter**		
		Birch	**Alder**	**Beech**
Texture (%)				
Sand (0.05-2mm) :	48.16			
Silt (0.002-0.05mm :	33.59			
Clay (< 2μm) :	18.25			
Textural Class :	Loam			
pH (H_2O)	5.42 (0.04)			
EC (μS cm^{-1})	118.30 (37)			
Moisture content %	4.24 (0.72)	68.11 (0.26)	67.60 (1.14)	62.01 (0.14)
Organic Carbon (%)	2.88 (0.16)	53.15 (2.78)	52.52 (2.81)	51.34 (1.40)
Total Nitrogen (%)	0.30 (0.01)	2.90 (0.15)	3.50 (0.35)	3.05 (0.37)
C/N ratio	9.6	18.49 (1.87)	15.53 (2.13)	17.25 (1.90)
Lignin (%)	ND	27.2 (1.12)	13.4 (1.27)	33.8 (1.46)
Total elemental analysis (mg g^{-1})				
Phosphorus	0.33 (0.06)	1.60 (0.25)	1.95 (0.11)	1.27 (0.03)
Potassium	10.10 (0.17)	9.00 (1.10)	5.62 (0.21)	3.72 (0.10)
Calcium	1.83 (0.36)	5.65 (0.59)	10.74 (0.36)	4.14 (0.19)
Magnesium	ND	1.43 (0.03)	3.28 (0.33)	2.88 (0.45)
Manganese	1.62 (0.07)	0.50 (0.06)	0.87 (0.01)	0.52 (0.02)

ND: Not determined.

5A.2 Results

5.2.1 Release of dissolved inorganic nitrogen (DIN)

Dissolved inorganic nitrogen (DIN) consists of nitrate (NO_3^-) and ammonium (NH_4^+) in the soil solution. The initial NO_3^- concentrations in the control and different leaf amended soil solutions ranged between 33.3 to 23.6 mg L^{-1}. During the first 3 weeks of incubation, a rapid decrease in NO_3^- concentrations was observed in all leaf litter treatment; however, in the control treatment it was higher than others. Nitrate in solution from alder-leaf treated soil increased sharply after 3 weeks and reached 734 mg L^{-1} after 15 weeks which was significantly higher than other three litter types ($P < 0.001$) (Figure 5.1). After an initial decline, other leaf litter gradually released higher levels of nitrate, where as the release from the litter mixture was the highest followed by beech and birch; however, these variations were not statistically significant. Soil solution from control treatment showed a progressive increased in NO_3^- over the course of incubation period which was significantly higher than all litter types except alder (Figure 5. 1).

Similar pattern of an initial decline and then an increase with time was found in case of ammonium but the quantity was lower than nitrate. Alder showed the highest, but a fluctuating NH_4^+ concentration over the incubation (ranging from 11.1 to 0.9 mg L^{-1}) (Figure 5.2). Although initially alder and the leaf mixture released higher amounts of NH_4^+ (5.7 and 4.0 mg L^{-1} respectively) than other treatments, the variations were not significant until 6 weeks of incubation. After 9, 12 and 15 weeks alder released significantly higher amounts of NH_4^+($P = 0.000$, 0.016 and 0.001 respectively).

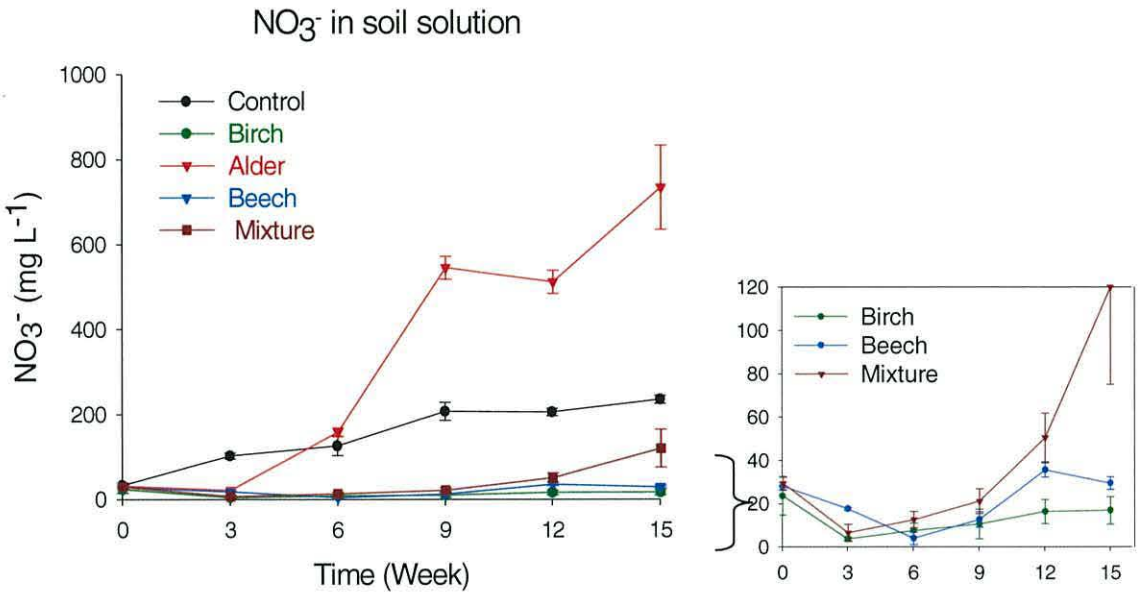


Figure 5.1 Concentration of dissolved NO_3^- in the soil solution of different leaf litter treatments incubated in the laboratory for 15 weeks at 10 °C. All values equal means, SEM (n=3). Concentrations close to horizontal axis are shown separately in the inset.

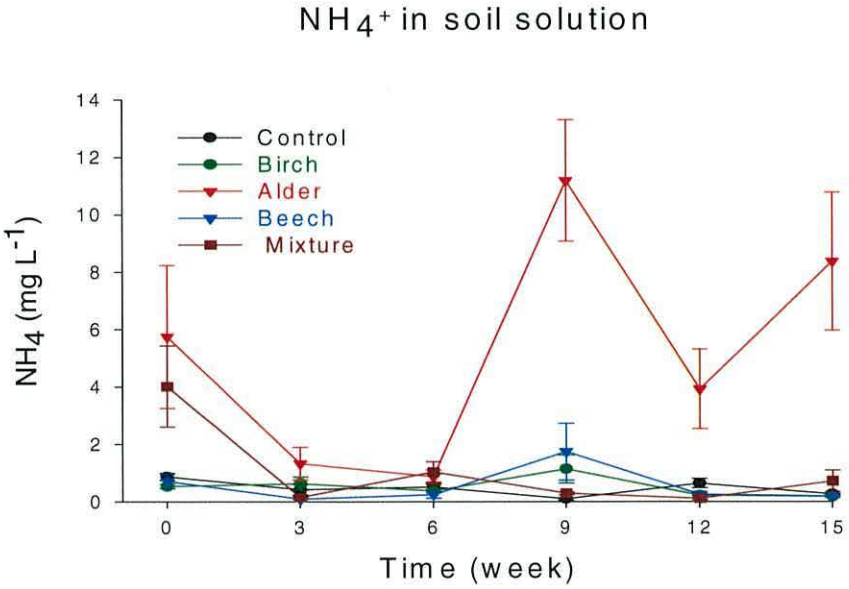
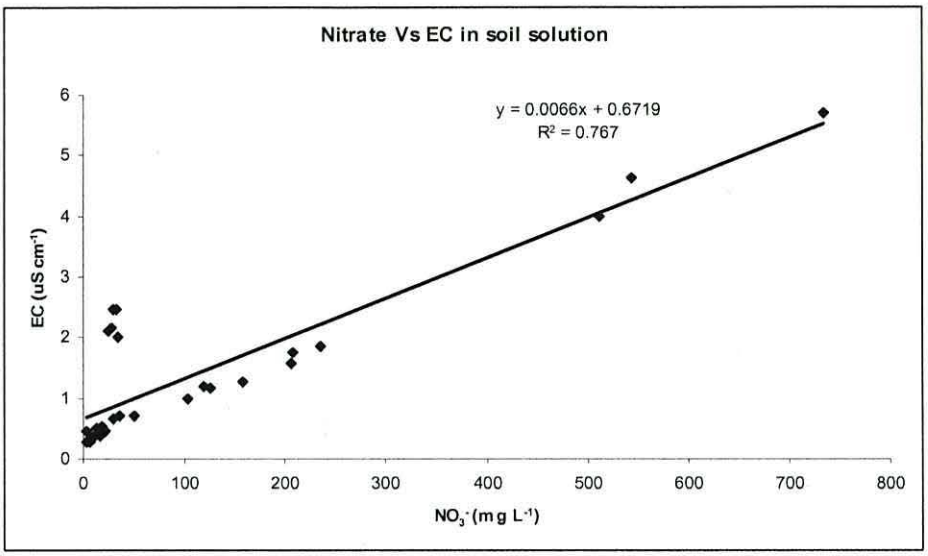


Figure 5.2 Concentration of dissolved NH_4^+ in the soil solution of different leaf litter treatments incubated in the laboratory for 15 weeks at 10 °C. All values equal means, SEM (n=3).



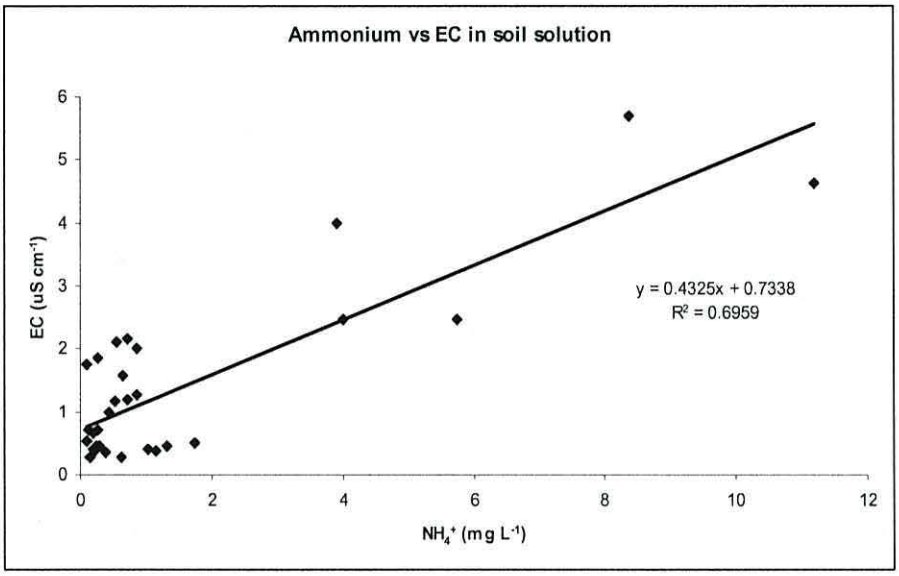


Figure 5.3 Regressions between electrical conductivity (EC) and inorganic nitrogen ions (NO_3^- and NH_4^+) in the soil solution of different leaf litter, extracted during the laboratory incubated experiment.

A positive relationship between the electrical conductivity (EC) of the soil solution and NO_3^- and NH_4^+ ions was observed. Pearson Correlation Coefficient indicated clear relationship ($r = 0.88$, $P<0.01$) between NO_3^- and EC in soil solution and regression analysis showed linear relationship with coefficirnt of determination, $r^2 = 0.78$ (Figure 5.3). However, NH_4^+ showed a weaker relationship with EC than NO_3^- ($r^2=0.69$).

5.2.2 Dissolved organic nitrogen (DON)

The dissolved organic nitrogen (DON) concentration in the soil solution is shown in Figure 5.4. After an initial decline, all treatments showed significantly higher concentrations of DON at the end of the experiment than at the beginning, except birch leaf which released slightly lower DON than at the start of the incubation. The variation in DON content due to litter types was not significant initially, but after 3 and 15 weeks differences were statistically significant (P = 0.048 and 0.013 respectively) and after 15 weeks the cumulative concentrations were 65.0, 41.9, 27.7 and 11.0 mg L^{-1} in soil solution from alder, leaf mixture, beech and birch respectively.

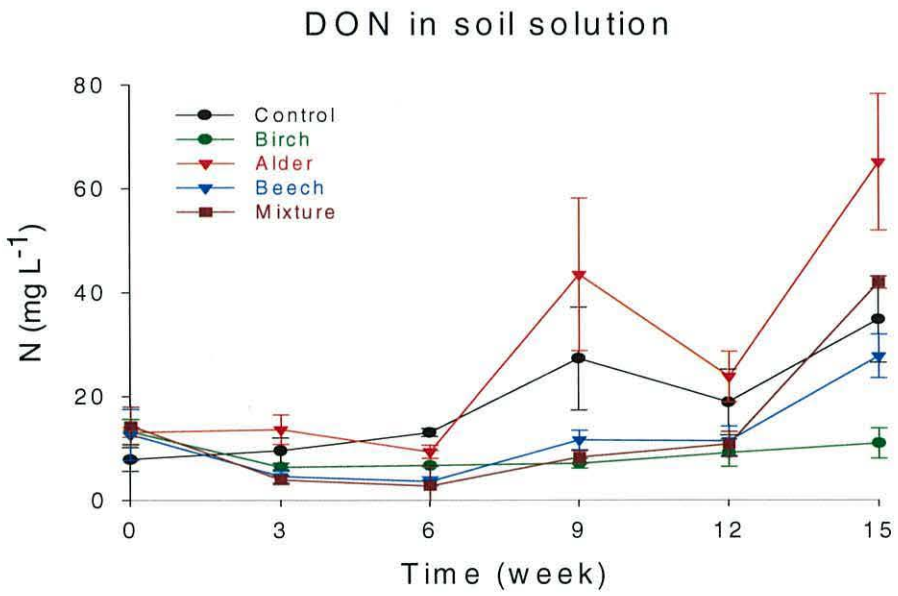


Figure 5.4 Concentration of dissolved organic nitrogen (DON) in the soil solution of different leaf litter incubated in the laboratory for 15 weeks at 10 ^{0}C. Values shown are means, SEM (n=3).

5.2.3 Release of water soluble C and polyphenolics

The different leaf litter treatments followed the same pattern of rapid increase in dissolved organic carbon (DOC) initially and then varied inconsistently over the incubation period. After 15 weeks of incubation, the cumulative amounts of DOC were 93.6, 79.3 and

44.2 mg L^{-1} for birch, alder, beech litter respectively (Figure 5. 5) which were more than 13, 3 and 8 times higher than the respective initial contents. Birch and alder exhibited increasing trends throughout the experiment with significantly higher values at some sampling intervals. In contrast DOC in beech remained relatively constant over the 15 weeks.

The cumulative amount of water soluble phenolics in the soil solution of the different leaf litter treatments is shown in Figure 5. 6 .The phenolics varied considerably between litter types with significantly higher values in birch than alder at initial two sampling times. The soluble phenolics content in birch and alder increased with the length of the incubation. On the other hand soluble phenolics in beech were higher than alder for up to 6 weeks of incubation, and after 9 weeks it declined considerably. The release of soluble phenolics in different foliage treatments after 15 weeks exhibited the following order: birch > alder > litter mixture >beech; however, only the difference between birch and beech was statistically significant.

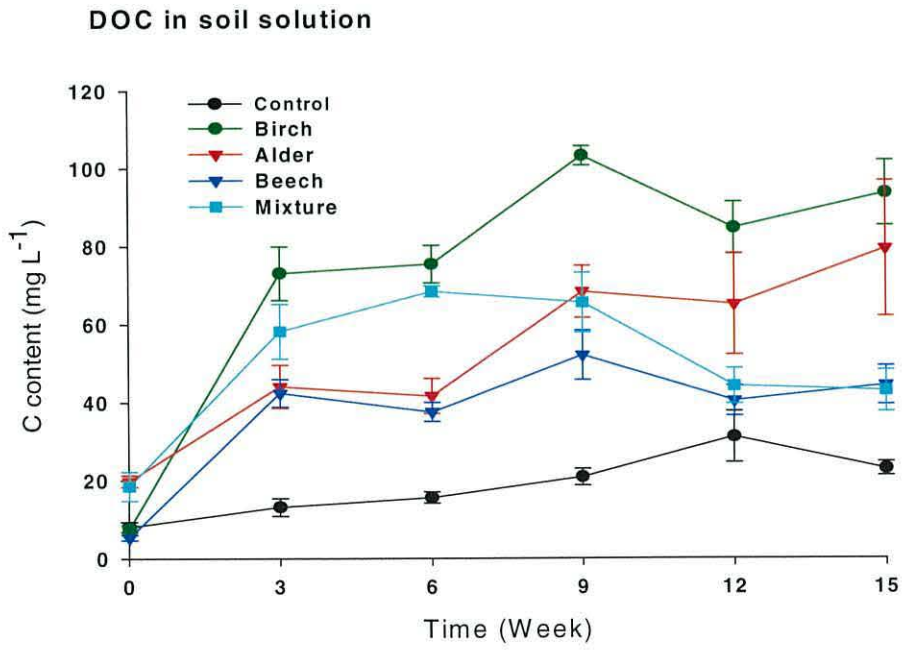


Figure 5.5 Concentration of dissolved organic carbon (DOC) in the soil solution of different leaf litter treatments incubated in the laboratory for 15 weeks at 10 °C. All values equal means, SEM (n=3).

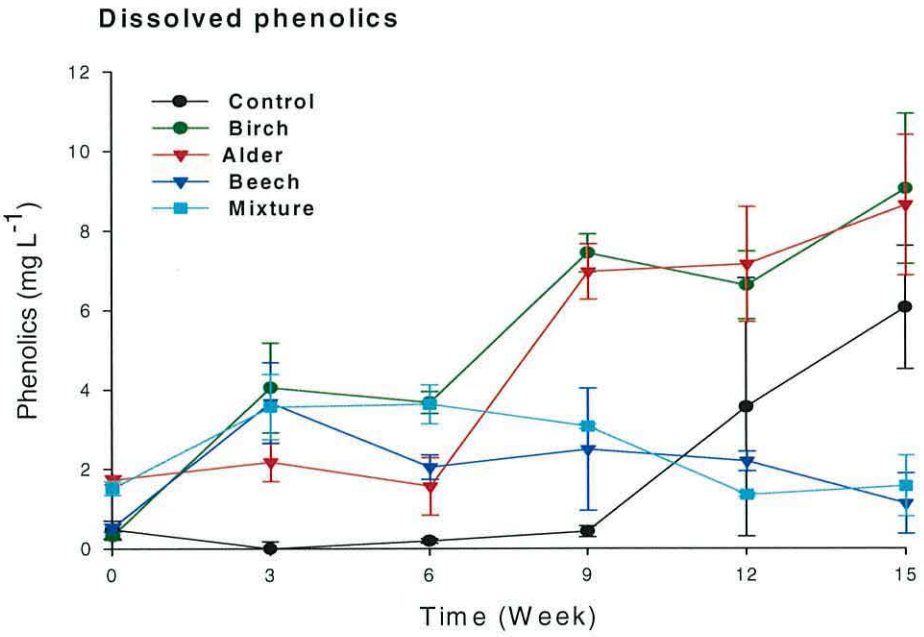


Figure 5.6 Concentration of dissolved phenolics in the soil solution of different leaf litter treatments incubated in the laboratory for 15 weeks at 10 °C. All values equal means, SEM (n=3).

5.2.4 Effects of litter mixture on water soluble compounds

Data from single species litterbags were used to calculate the predicted value for mixture according to proportions of each component in mixture. The estimated and actual quantities of DIN, DON, DOC and water soluble phenolics are presented in Table 5.2. The predicted amounts of DIN and DON were higher than the actual values in contrast, DOC and soluble phenolics showed higher actual values during the first phase (0-6 weeks) and higher predicted values during the second phase (9-15 weeks) of decomposition. The differences between actual and predicted amounts in mixture were not statistically significant in most of the cases, except NO_3^- after 6 week and soluble phenolics after 9 weeks of incubation (Table 5.2). Paired sample t-test revealed that NO_3^- released from the litter mixture was significantly reduced after 6 weeks of incubation (P=0.012, 0.005, 0.011, and 0.018 after 6, 9, 12 and 15 weeks respectively). Similarly the release of water soluble phenolics decreased significantly in the mixed treatment after 9 weeks (P=0.013, 0.002, 0.001 after 9, 12, and 15 weeks respectively).

Table 5.2 Mixture effects on release of NO_3^-, NH_4^+, dissolved organic nitrogen (DON), dissolved organic carbon (DOC) and water soluble phenolics from leaf litter during 15 weeks incubation. Values indicate differences between actual and predicted** amounts (+ Higher in actual, - Higher in predicted).

Incubation time (week)	Organic compounds released from leaf litter (mg L^{-1})				
	NO_3^-	NH_4^+	DON	DOC	Soluble Phenolics
0	+1.34 NS	+ 0.85 NS	+1.21 NS	+5.02 NS	+0.47 NS
3	-7.24 NS	- 0.77 NS	-5.9 NS	+2.73 NS	+0.5 NS
6	-69.74 *	+0.42 NS	+4.91 NS	+13.75 NS	+1.18 NS
9	-256.86 *	-5.93 NS	-17.45 NS	-15.06 NS	-3.63 *
12	-215.56 *	-1.97 NS	-5.9 NS	-26.31 NS	-4.62 *
15	-257.04 *	-4.56 NS	+2.23 NS	-38.64 NS	-6.48 *

* Statistically significant ($P < 0.05$), NS= Non significant

** Predicted amount of organic compound for mixture = Sum of 3 portions of single treatments; (birch x 40%), (alder x 50%) and (beech x 10%) as the proportion of three leaf litter in mixture was 4:5:1. For example, at the starting (0 week), dissolved NO_3^- in soil solution of birch, alder, beech and mixture were 23.60, 31.58, 28.04 and 29.38 mg L^{-1} respectively (Figure 5.1). Therefore, predicted value was {(23.6 x 40%=9.44) + (31.6 x 50% =15.8) + (28.0 x 10% = 2.8)}= 28.04.

The actual value i.e in mixture was 29.4 mg L^{-1}. In this case actual value is higher than predicted value and the difference was 29.38- 28.04 = + 1.34.

5A.3 Discussion

5.3.1 DIN release during decomposition

Initial decreases in both NH_4^+ and NO_3^- up to 3 weeks might be due to conversion of NH_4^+ to NO_3^- and immobilization of NO_3 by soil microbial community. As a typical pattern of decomposition, formation of unstable NH_4^+ by breakdown of protein (R-NH) and nitrification and then again immobilized into fungal and microbial protein have been observed for many species of leaf litter, as evaluated by Triska and Sedell (1976). Substantial amounts of NH_4^+ in alder and mixed litter before starting decomposition indicate the existence of a higher quantity of nitrogenous soluble compounds leaching from alder leaf litter. Taylor and Barlocher (1996) reported higher leaching of soluble compounds from air-dried leaves of red alder (*Alnus rubra*) (33% of dry mass after 72 h) compare to European beech (*Fagus sylvatica*) (17%) and white birch (*Betula papyrifera*) (12%).

Decomposing substrates with high nitrogen content particularly nitrogen fixing plant materials are considered to be of high quality, resulting in rapid release of nitrogen by microbial decomposers (Weeraratna, 1979; Palm and Sanchez, 1990). As much as 3 times more NO_3^- was released by alder leaf compared to the control, and indicating faster nitrogen mineralization in alder leaves. This result confirmed the assumptions that alder is a relatively faster decomposing species with high extractable nitrogen particularly nitrate (Binkley, *et al.* 1992; Petersen and Cummins, 1974). In contrast to alder, nitrogen mineralization in birch, beech and mixed litter was partially blocked probably because of the higher concentration of inhibitory substances like lignin and phenolics (Palm and Sanchez, 1991).

The role of microbial competition for NH_4^+ may also be responsible for the level of nitrate accumulation in the soil as suggested by Jones *et al.* (2004). In the case of available C, the heterotrophic NH_4^+ immobilization process dominates over autotrophic nitrification resulting low NO_3^- accumulation in soils; however, in conditions of depleted availability of C, no more NH_4^+ immobilizes but nitrifiers can still oxidise NH_4^+ to produce NO_3^-, resulting in high accumulation of NO_3^- in the system. We assumed that the first situation may have occurred in case of birch, beech and mixed litter due to slower breakdown process compared to alder. On the other hand, rapid decay in alder litter might cause a scarcity of C at the later stage of decomposition resulting in high accumulation of NO_3^-.

DON in soil solution varied depending upon species, but overall concentration was much lower than DIN (Magill *et al.* 2000). A similar pattern in release of DON was observed but the quantity was very low compare to DIN indicating either low production of DON or dominancy of low molecular weight labile DON enhancing the rate of ammonification and nitrification in the soil (Jones *et al.* 2004). Our results showed 6-7 mg L^{-1} DON in the leaf litter at the start of the incubation period suggesting some DON was present in the soil before incubation. As reported by Jones *et al.* (2004) we hypothesized that conversion of low molecular weight DON to NH_4^+ and consequently NH_4^+ to NO_3^- did not limit the rate of nitrogen mineralization in alder. In contrast, high molecular weight DON especially polyphenol bound nitrogen might inhibit microbial activities and further reduced the ability of nitrogen mineralization in birch and beech leaf litter (Li *et al.*, 2009).

5.3.2 Dissolved Organic C (DOC) and water soluble phenolics

Our results showed that cumulative DOC concentration in soil solution from all leaf treatments increased with incubation time and was significantly higher in birch at some sampling intervals. Findings are consistent with these suggested by others (Solander and Kitunen. 2002) that the highest DOC was released from birch leaf litter in a laboratory incubation experiment with silver birch, Norway spruce and Scots pine in Finland. The possible reason might be chemical composition of leaf litter (Johansson, 1995; Harris and Safford, 1996). The DOC concentration in beech soil solution remained relatively constant over the 15 weeks of incubation experiment. Similar result of relatively constant DOC concentration in leachate from American beech (*Fagus grandifolia*) was observed in a laboratory based decomposition experiment by Magill *et al.* (2000). Our results suggested that lower DOC released by alder than birch might be attributed to high activities of microbial decomposer, which may decrease DOC due to respiratory losses of DOC as CO_2 (Moore and Dalva, 2001).

The relatively higher water soluble phenolics in birch than other leaf litter in our study indicates that an effective protection exists in birch from decomposition and nitrification (Horner *et al.* 1988). This is consistent with previous studies that have shown relatively higher concentration of water soluble phenolics in *Betula pendula* (11 mg tannic acid equivalent (TAE g^{-1}) than *Fagus sylvatica* (8.5 mg TAE g^{-1}) was reported by Kuiters and Sarink (1986) in a leaching experiment by periodic collection of leaf litter (Oct-Dec-Jan) in Netherlands. Phenolic compounds can inhibit microbes by interacting with extracellular enzymes, protein substances and possibly other N containing compounds, as acting as toxins in microbes (Bradley, 2000; Karus *et al.* 2003; Kanerva *et al.* 2006).

Phenolic in the solution of beech soil was gradually decreased over the length of incubation period. Disappearance of soluble phenolics might be partially due to microbial

decay of easily degradable phenolic fractions particularly in beech leaf litter (Bernhard-Reversat *et al.* 2003). Generally in fresh plant tissue almost all phenolics compounds occur in combined form with sugars as either glycosides or esters (Harborne, 1964), however Whitehead *et al.* (1983) repoted that substantial proportion of water soluble phenolics occurred in the beech leaf litter as free form which might cause rapid biodegradation and subsequent decreased in cumulative content.

5.3.3 Dissolved compounds in mixed litter

Cumulative concentration of DIN and DON in mixed litter was always in 2nd position in order of concentration (i.e alder > mixed litter > beech > birch). In case of DOC and total phenolics the order of concentration was birch > alder > beech/mixed litter. As the proportion of three plant litter in mixture was 4:5:1 (for birch: alder: beech) it is apparent from our results that the mixture effects were mostly additive. The actual (values of mixed litter experiment) and predicted (proportionally calculated from pure litter) values also suggested that most the effects were additive. However, after 6 and 9 weeks, the mixture effect was negative in case of NO_3^- and soluble phenolics respectively (Table 5. 2) which indicates possibilities of biphasic decomposition pattern during the decay period.
The evaluation of mixed litter N dynamics using the predicted values from single species litter has been criticized as inappropriate by some investigators because of the dynamic nature of N (leaching, breakdown and release phases) may vary temporally and spatially which can complicate the accurate assessment N during decomposition (Blair *et al.*, 1990, Colpaert, and Tichelen, 1996).

5B *In situ* Litter bag experiment

5B.1 Materials and Methods

5.1.1 Experiment sites

Study area and soils: The experiments were carried out at Bangor University research farm (Henfaes), at the village of Abergwyngrageyn , Bangor, UK. Local climate was recognized as Hyperoceanic with annual mean temperature and rainfall of 11.5 ^{O}C and 1034 mm respectively. The experimental area includes former arable lands, afforested with native broadleaved species of birch (*Betula pendula*), alder (*Alnus glutinosa*), beech (*Fagus sylvatica*) and mix of three since 2004. The soil has been classified as Dystric Cambisols (Teklehaimanot *et al.* 2002) with postglacial alluvial parent materials. Some physico-chemical properties of the soils and chemical composition of leaf litter are shown in Table 5.1.

Weather data: The mean monthly air and soil temperature and rainfall data was obtained from weather monitoring units of the experiment site (Campbell Scientific Ltd., Shepshed, UK).

5.1.2 Preparation of litterbag and litter collection

Preparing litterbag, litter collection and placement of bags: Litterbags were constructed using 1mm nylon mesh, sewn along three sides of the rectangular shape bags with dimensions of 20 cm X 15 cm with a plastic tag at corner of the bag. This mesh size is suitable for free access of most of the soil macro-invertebrates and also small enough to prevent excessive loss of litter fragmentation (Bradford *et al.* 2002). A total of 180 bags were used for 12 plantation plots, nine replicate bags for each 4 species type (birch, alder, beech and mixed) for five time intervals.

Fresh mature leaves were picked from the trees in mid-June 2010, petioles were discarded and laminae were cut into equal size pieces (approximately 3cm X 3cm) for each species. A portion of ~5.0 g fresh litter was put into the bag immediately and the openings of bags were carefully stapled to close. For mixed litter, leaves were mixed at 4:5:1 ratio for birch, alder and beech respectively on the basis of litter fall estimation from respective mixed species blocks (for 5.0g fresh mixed sample, the amount of birch, alder and beech leaves were 2.0, 2.50 and 0.50 g respectively). Representative sub samples litter from each species and mixture were brought to laboratory to determine moisture content and other parameters. The litter bags were placed on the plantation floor of respective species types, fixed with a metal peg to prevent the movement and covered with thin soil layer to make sure that all litter in the bag come in contact with the soil. The bags with pure leaf litter were placed on the randomly selected spots, equal distance from surrounding trees and the mixed litter bags were on the centre of triangular area consists of birch, alder and beech plants in the mixed species plots.

5.1.3 Collection and processing of decomposed litter

The bags were deployed on June 2010 and were harvested at 3, 6, 10, 15 and 21 weeks after placement of the bags. The collected litterbags were put in the sealed plastic bags and carried to the laboratory immediately and dried at room temperature before removing the litter from bags. The whole content in the bags was transferred to clean trays and soil, roots and insects were carefully separated from the remaining leaf litter. Soil adhering to the litterbag was removed as much as possible using a soft brush. The cleaned litter was dried at 60 ^{0}C in the oven for 72 h and moisture content of the litter as estimated by weighing before and after oven drying. A subsample of 0.5-1.0 g was burned at 450 ^{0}C for over night in the muffle furnace to estimate ash content in the litter. As there were

variable quantities of soil particles amassed within the remaining leaf litter, the estimation of mass loss and other chemical analysis of remaining litter were done by ash free basis.

5.1.4 Chemical analysis

Lignin: The leaf samples were dried at 70 ^{0}C for 24 hours and then ground to fine powder (sieved with 0.85 mm sieve).The lignin content of the leaves were determined by modified method of Effland (1977). Three hundred mg of dried leaf sample was hydrolyzed with 4 ml of 72% H_2SO_4 in a water bath at 30 ^{0}C for two hours. The content was washed into a 250 mL conical flask using 28 mL of water for each mL of 72 % H_2SO_4 to make 4% w/w solution. The flasks were autoclaved at 120 ^{0}C for one hour and the extracts were drawn through a pre weighed sinter crucible by rinsing the flask with hot water. The sinter crucibles were dried at 105 ^{0}C for four hours, cooled in a desiccators and weighed. The acid insoluble lignin was determined gravimetrically.

Elemental analysis of leaf litter: Major nutrient elements (P, K, Ca, Mg, and Mn) in plant leaf samples were analyzed by Total Reflection X-Ray Fluorescence Spectroscopy (TXRF) methods using S2 PICOFOX XRF Spectrometer (Bruker AXS Microanalysis GmbH, Berlin, Germany). In this method a uniform suspension of fine leaf material (ground using ball mill and passed through 76 μm sieve) was prepared using a 20 mg leaf sample and 1% Triton, $\{C_{14}H_{22}O(C_2H_4O)_n\}$ solution. A portion of 10 μg of As (1000 μg mL^{-1}) was added to the suspension as standard. After thoroughly mixing, 5 μl of suspension was placed onto a quartz disc. The sample disc was dried (~80 ^{0}C) and placed in the instrument to run the sample.

5.1.5 Data processing and Statistical analysis

Due to considerable level of soil contamination we analyzed the remaining litter as ash free weight basis. The ash free weight of litter remaining in the litter bag was calculated according to the following equation suggested by Schuman and Belden (1991):

$$W1 = Wt \left[\frac{As - At}{As - A1} \right]$$

Where, W1 is dry weight of the litter remaining in the litterbag, Wt total dry weight of the litterbag content (litter + soil), At is the ash percentage of the litterbag content, As and A1 are the ash percentage of soils and fresh litter respectively.

We used the following single negative exponential decay model (Olson, 1963) to compare overall decay pattern of different species leaf litter: $Y= Y_0 e^{-kt}$ where, Y = Per cent mass remaining after certain time, Y_0 initial litter mass, t is the time, and k is the decay rate constant. The statistical assessment of decay curve fit was done by using Sigma Plot 10 for Windows (SPSS, Inc.).

The absolute decay rate was calculated using the following formula (Janssen 1984)

$dy/dt = -k\ y = (-k)\ (Y_0\ e^{-kt})$, where k is decay constant and y is per cent mass remaining after a certain time.

The effects of individual species and mixture on mass loss and decay rate were tested by one way ANOVA. The Tukey HSD test was used to locate the specific individuals having significant differences. To assess the mixture effects, the actual and estimated values were compared using paired sample t test. The results of statistical tests were considered as being significant at $P < 0.05$. All statistical analyses were carried out using SPSS 16 software package (SPSS, Inc.).

5A.2 Results

5.2.1 Weather

The litterbag experiment was conducted between June and November 2010 and the mean monthly maximum and minimum temperature during this period at the experimental sites were 11.1 °C to 17.8 °C. However the soil temperature (0-10 cm) was slightly lower ranged from 15.4 °C to 16.8 °C. Over 6 months of experimental period the total rainfall was 507 mm.

5.2.2 Substrate quality and soil chemistry

The alder leaf litter had the highest total initial N concentration and birch leaves contained the lowest while beech was intermediate (Table 5.1). The C content in birch, alder and beech litter was 53.18, 52.52, and 51.34 % respectively, but the differences were not statistically significant. Slightly higher P and Ca were found in alder leaf while K was higher in birch leaves. Significantly higher Mg and Mn were found in alder leaf materials than birch leaf materials. The acid insoluble lignin contents in were 27.2, 13.4 and 33.8 % in birch, alder and beech, leaves litter respectively. Lignin content in alder was significantly lower than that of birch and beech.

5.2.3 Mass loss and decay rates

The litterbags were buried on the top soils of respective plantation floors which caused significant contamination of soil mineral particles with decomposed litter especially after 10, 15 and 21 weeks of decomposition. Therefore, all mass losses from litter have been estimated on a ash free dry weight basis.

The loss of leaf litter mass from litterbags during the 6 months of the decomposition period is presented in Figure 5.7. After 3 weeks, the highest loss of litter mass was recorded from alder leaf (47.4 % initial litter weight) followed by birch (44.1%) and beech (24.3%) and the lowest mass loss was observed from the litter mixture (10.9%). ANOVA showed that the overall differences of loss among these 4 types of litter observed during first four sampling times were statistically significant (P=0.00), but after 21 weeks the differences were not significant (P=0.82). However further post hoc test (Tukey HSD) revealed that variation between mass loss of birch and alder litter were not significant after 3 weeks (Table 5.3). More or less similar trends have been observed after 6 and 10 weeks of decomposition periods with significantly higher mass loss in alder litter which continued up to 15 weeks. At this stage, the leaf mixture lost the lowest mass (84%), which was significantly lower than alder and beech (P=0.00 and 0.014, respectively). At the end of 21 weeks, the litter mass loss were 92, 98.4, 92.2 and 87.9 % from the bags of birch, alder, beech and mixed litter respectively which were not significantly different.

Two phases have been recognized during the entire decomposition period in relation to mass loss. The initial phase characterized by faster loss of leaf necromass than the slower second phase and for alder litter it extended until 10 weeks, and for birch, beech and the leaf mixture it was 15 weeks (Fig 5.7). Decomposition process throughout the experiment has been fitted in the first order exponential equations with $R^2 > 0.94$ and $P < 0.006$ for all single and mixed litters (Figure 5.8) (Table 5.4). Considering the good coefficient of determination (R^2), high intercept values (near to 1) and small sum and mean square values of residuals suggested that decomposition dynamics fitted to the exponential model. The highest decay constant (relative decay rate) was found in alder leaf litter, followed by mixed litter, beech and birch with significant level $P < 0.01$.

The absolute decay rate after different time intervals is presented in Table 5.3. After 3 weeks of decomposition, the decay rate in alder and birch litter were significantly lower than beech and the mixed litter, with the highest rate in alder and the lowest in the mixed litter. A similar magnitude of decay has been observed after 6 weeks, however the decay rate of alder litter was significantly slower than other species and the mixture after 10 and 15 weeks of decomposition, and after 21 weeks although the rate was slowest in alder, it was not significantly different from beech.

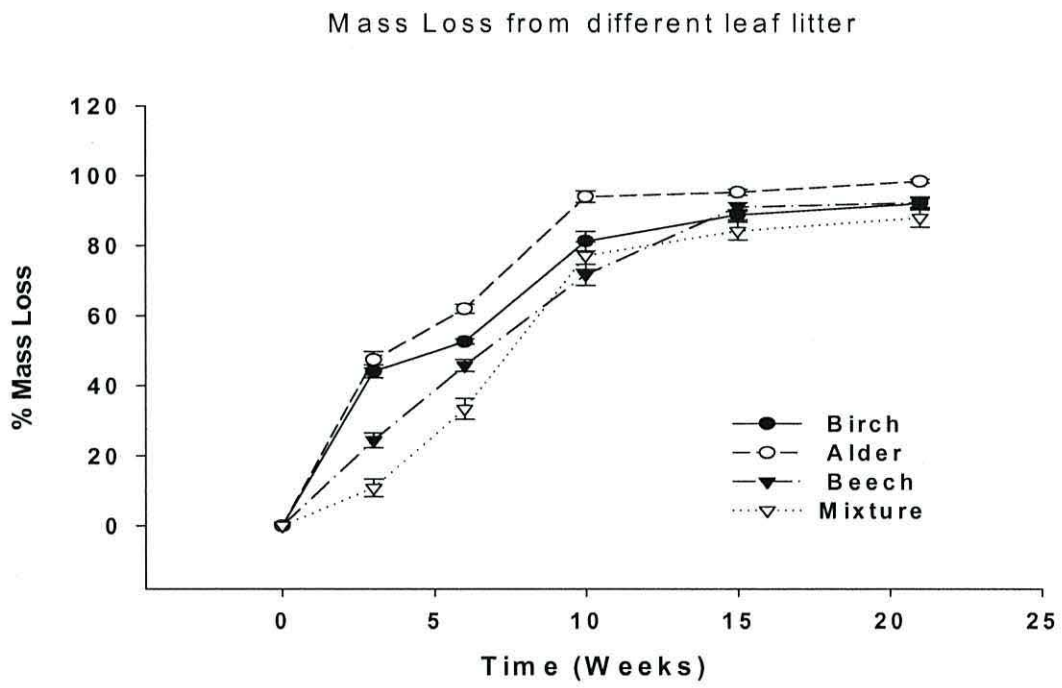


Figure 5.7 Mass loss from the litter bags with leaf litter of different broadleaved species decomposed for 21 weeks under the respective species stands. All values equal means, SEM (n=9).

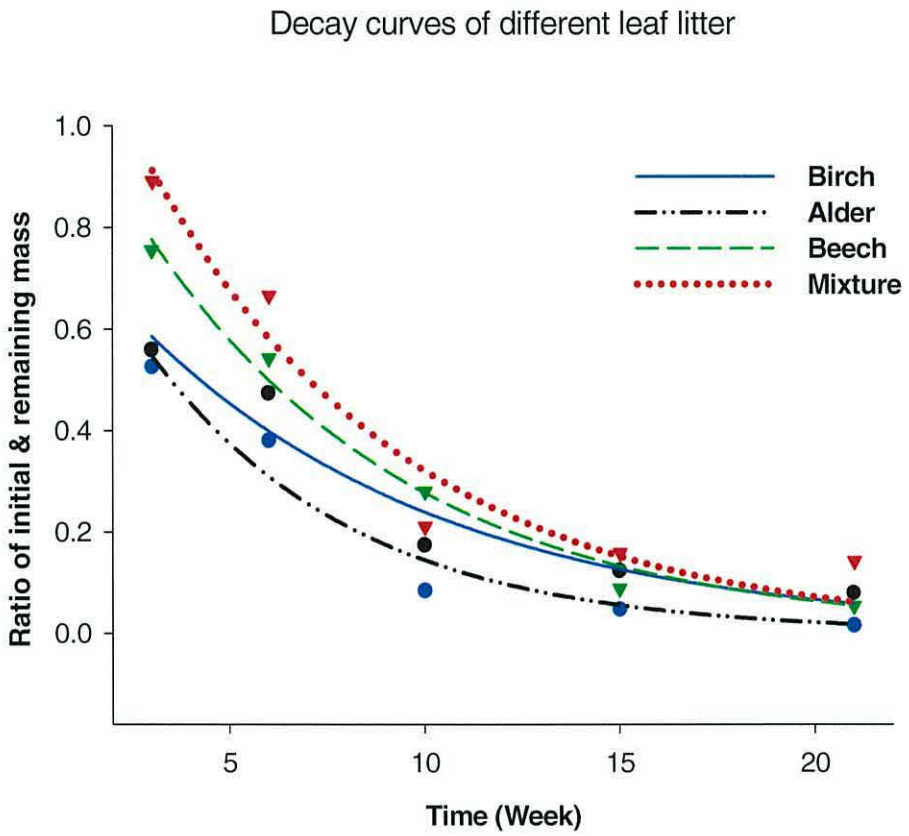


Figure 5.8 Decay pattern (non linear regression, $y = ae^{-bx}$) of different leaf litter types and their mixture. The detailed properties of each curve are given in the Table 5.4.

Table 5.3 Mass loss and absolute decay rates at different sampling intervals. Values equals mean, SEM in parentheses (n = 9). Values with similar indices are not statistically significant (P<0.05).

	Weeks									
	3		6		10		15		21	
Litter type	% Mass loss	Decay Rate (week^{-1})	% Mass loss	Decay Rate (week^{-1})	% Mass loss	Decay Rate (week^{-1})	% Mass loss	Decay Rate (week^{-1})	% Mass loss	Decay Rate (week^{-1})
Birch	44.06a (1.84)	-0.10745 a	52.56 a (0.72)	-0.05889 ab	81.24a (2.79)	-0.02943 a	88.78ac (1.49)	-0.01699 ac	92.02a (1.85)	-0.00876 ac
Alder	47.36a (2.37)	-0.11119 a	61.90 b (1.25)	-0.06099 a	93.98b (1.65)	-0.01984 b	95.24bd (0.85)	-0.00926 b	98.36a (0.55)	-0.0041 ab
Beech	24.43b (2.10)	-0.06979 b	45.74 a (1.66)	-0.05496 b	71.61a (3.05)	-0.03434 a	90.97cd (1.22)	-0.01399 a	92.20a (1.33)	-0.0071 cb
Mixture	10.85 c (2.52)	-0.03318 c	33.43 c (2.95)	-0.04426 c	77.05a (3.92)	-0.03215 a	84.11a (2.54)	-0.01847 c	87.88a (2.63)	-0.01256 a

Table 5. 4 Exponential regression models for decay pattern of 3 leaf litter and the mixture. In statistical analysis residual sum square (SS) and residual mean square (MS) are very small and F is high indicating good fit of the model to predict variables. In the equation, Y= percentage of weight remaining after certain time t.

	Leaf litter			
Equations	***Betula pendula***	***Alnus glutinosa***	***Fagus sylvatica***	**Mixed litter**
	$Y= 0.8595\ e^{-0.1278\ t}$	$Y= 0.9751\ e^{-0.191\ t}$	$Y= 1.209\ e^{-0.1474\ t}$	$Y= 1.4292\ e^{-0.1496\ t}$
R^2	0.94	0.96	0.99	0.94
Residual SS	0.0110	0.0092	0.0043	0.0260
Residual MS	0.0037	0.0031	0.0014	0.0087
F	49.105	64.874	250.634	51.4542
P	0.006	0.004	0.0005	0.005
Decay Rate Constant (-k) ($week^{-1}$)	0.1278 (0.025 SEM) , P=0.01	0.1910 (0.040 SEM), P =0.01	0.1474 (0.014 SEM) , P =0.002	0.1496 (0.04 SEM) , P =0.005

5.2.4 Decay in mixed leaf litter

Decay rates in mixed litter were significantly slower in the initial stages than litter of the other species, but gradually increased with time and were faster than pure alder and beech after 15 weeks. The estimated mass remaining and decay rates of litter mixture have been calculated on the basis of percentage of litter remaining in the single species litter bags, and the proportions of each species types originally added to obtain the litter mixture. The mass remaining at different time intervals over the decomposition period were significantly higher in the mixed litter bags (actual) than the calculated values (estimated) (Figure 5.9). After 3 and 6 weeks of decomposition, the actual mass remains were about 33% (P=0.00) and 23% (P=0.00) higher than the estimated, however after 10 weeks the actual mass remains closer to the expected values (7 , 7.6 and 9.7 % higher actual values than estimated after 10, 15 and 21 weeks respectively) (Figure 5.9). Although the actual decay rate constant (k) for the mixed species litter was higher than that of predicted values the variation was not statistically significant (P=0.092). The absolute decay rates in the actual mixed litter were lower than the estimated rates after 3 (more than 3 times lower in actual) and 6 weeks however, after 10 weeks higher decay rates have been recorded in actual mixed litter than estimated rates throughout the experiment (Figure 5.10), and paired samples t-test showed that the differences between actual and predicted absolute decay rates were statistically significant. This indicates both negative and positive non-additive effects of litter mixture during the decomposition.

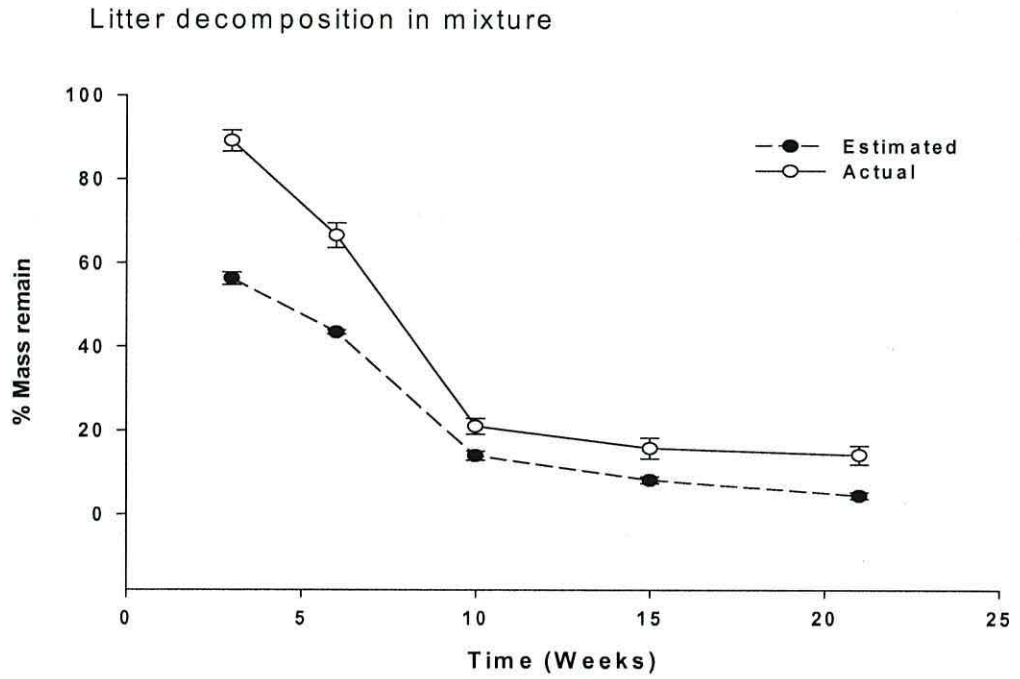


Figure 5.9 Actual (mass remain in the mixed litter bags) and estimated (calculated from mass remain in single leaf litter bags) values of mass remain in mixed species litterbags. All values show mean, SEM (n = 9).

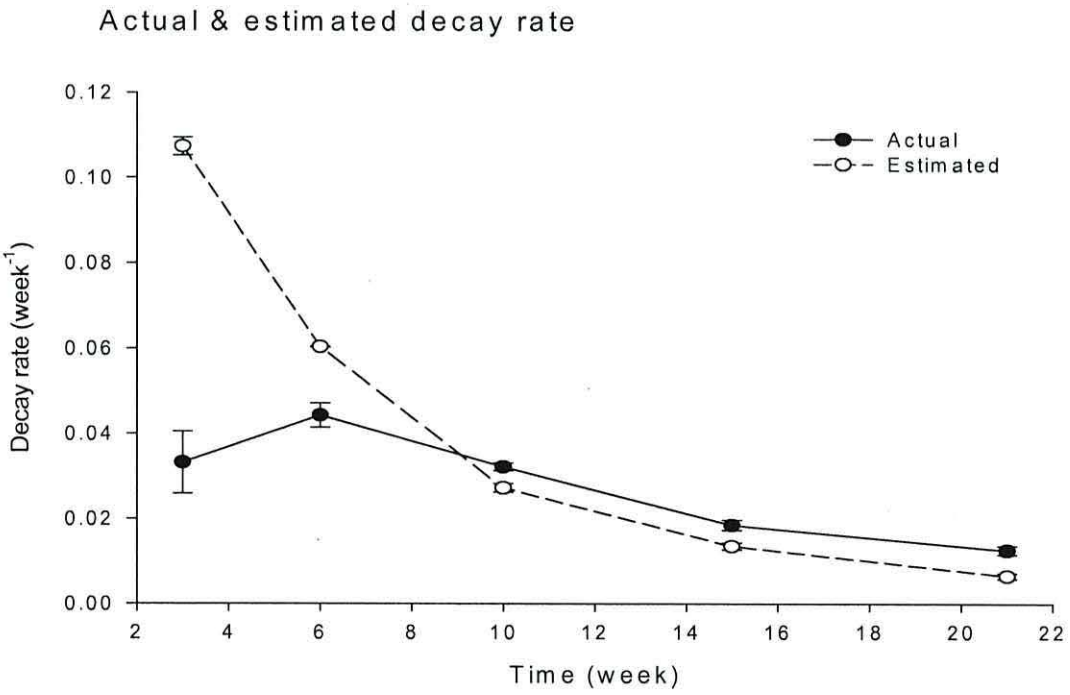


Figure 5.10 Actual (decay rates in mixed litter bags) and estimated (calculated from decay rates in single litter bags) decay rate in mixed species litterbags. All values show mean, SEM (n = 9).

5.2.5 Carbon, nitrogen dynamics and litter C/N ratio

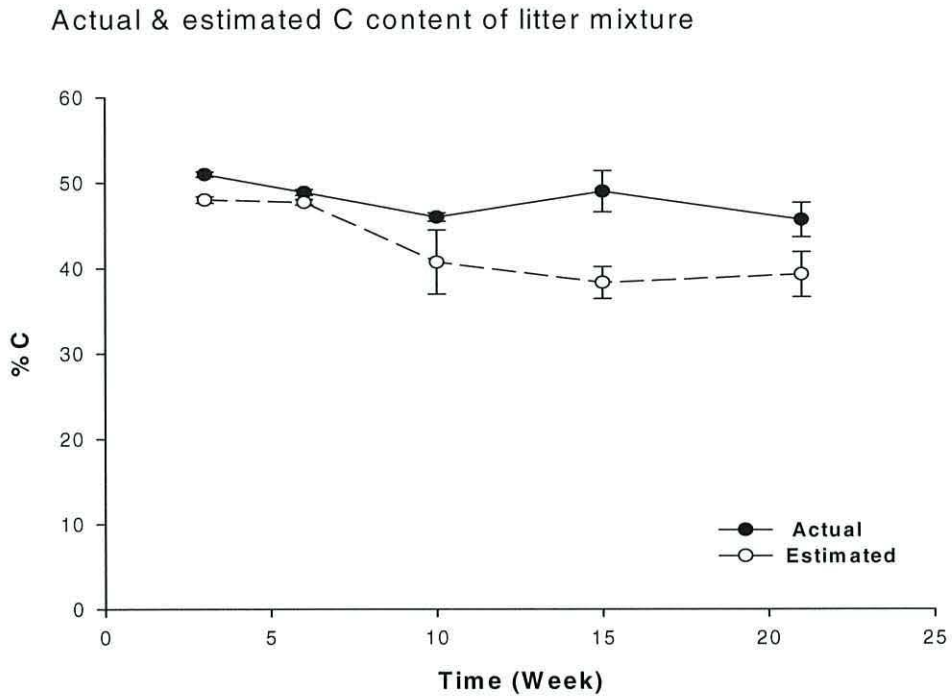
Among the single species birch had the highest amount of carbon in fresh leaf litter (53.1%) followed by alder (52.5 %) and beech (51.3%) which gradually decreased in birch and beech litters until 15 weeks (Figure 5.12). However in alder and beech although C contents declined at the end, they fluctuated over the course of the decomposition. After 21 weeks of decomposition, the C contents in the litter decreased by 11.9, 10.0, and 3.4% for birch, alder and beech litter respectively which, however, were not statistically significant. In the mixture of leaf litter, initial carbon content was 52.8% (1.60 SEM) and after 21 weeks it was 45.7% (2.02 SEM). The estimated C contents, calculated on the basis of data of pure species litters, and the actual C in the mixed leaf litter bags are presented in Figure 5.11. The actual C contents were higher than the estimated values over the 21 week experimental period. The variations between actual and estimated C content were very low, however, after 6 weeks, higher differences have been observed as decomposition proceeded and after 12 weeks it was significantly higher (P=0.048), which indicates interactive effects of the litter mixture on carbon dynamics.

In the fresh leaf, the highest per cent nitrogen was recorded in alder (3.5 %) followed by beech (3.0 %) and birch (2.9%) and after 21 weeks the amounts were increased to 3.8, 4.0 and 3.5 % in alder, birch and beech litter respectively. Nitrogen concentrations in the leaf litter of different species and species mixture fluctuated over the course of experiment (Figure 5.12). Initially, a steady increase in total N concentration of all litter occurred over 6 weeks of decomposition. After 6 weeks, decreasing trends in total nitrogen content have been observed in birch, alder and the leaf mixture for rest of the experiment period, but in beech this trend continued up to 15 weeks and slightly increased at the end of experiment. Although there is an increase in nitrogen content in leaf litter

irrespective of species types, the variations were not statistically significant at the end of experiment in comparison to initial total N contents, except in mixed litter where it significantly increased after 21 weeks ($P = 0.028$).

The actual and estimated nitrogen concentration of mixed litter slightly increased as the 21 weeks of decomposition proceeded, although after 6 weeks it initially declined (Figure 5.11). After 6 weeks the actual amounts of nitrogen in litter mixture were significantly higher than the estimated values (P=0.012), and this trend continued for the rest of the decomposition time, which indicates slower release of nitrogen from the mixed litter in comparison to pure leaf litter.

Figure 5.12 shows the changes in C/N ratio in the leaf litters of three different species and their mixture at five sampling dates of the decomposition experiment. In fresh leaves and the mixture, the C/N ratios were 18.5, 17.3, 16.4 and 15.4 for birch, beech, mixture litter and alder respectively. The C/N ratio of all four litter types exhibited similar trends of rapid decrease initially during 6 weeks and then steady increase until 21 weeks. The changes in C/N ratio clearly showed two phases of dynamics: a sharp decrease until 6 weeks and a second stage of steady increase until end of the experiment. Among the four litter types, beech leaf litter showed the highest C/N ratio after 3 weeks and at 15 and 21 weeks it was significantly higher than others. After 21 weeks, the highest C/N ratio detected in beech (13.6) followed by mixed litter (12.0), birch (12.0) and alder (11.4).



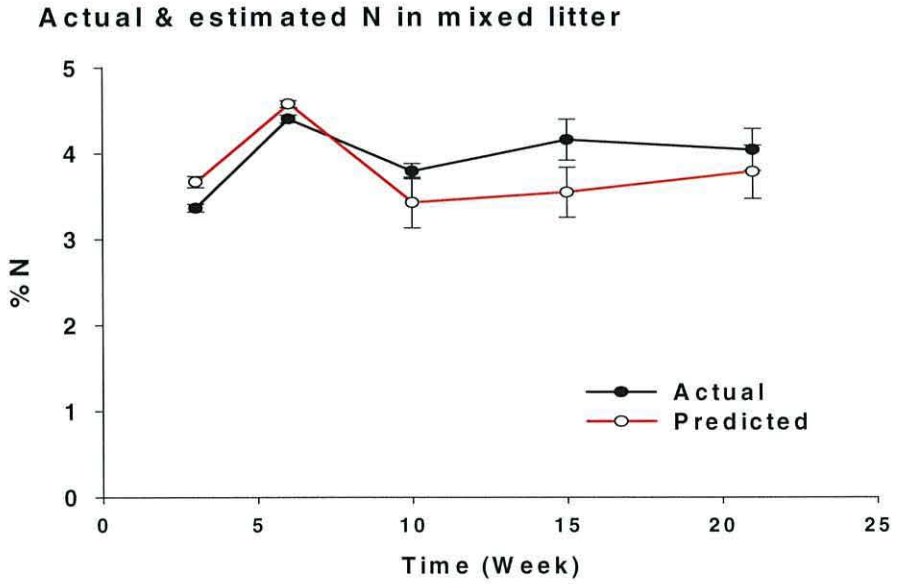


Figure 5.11 Actual and Estimated carbon and nitrogen in the mixed species litterbags. The estimated values were obtained by calculating from the data of single species litterbag proportional to the each leaf litter in mixed bag. All values show mean, SEM (n = 9).

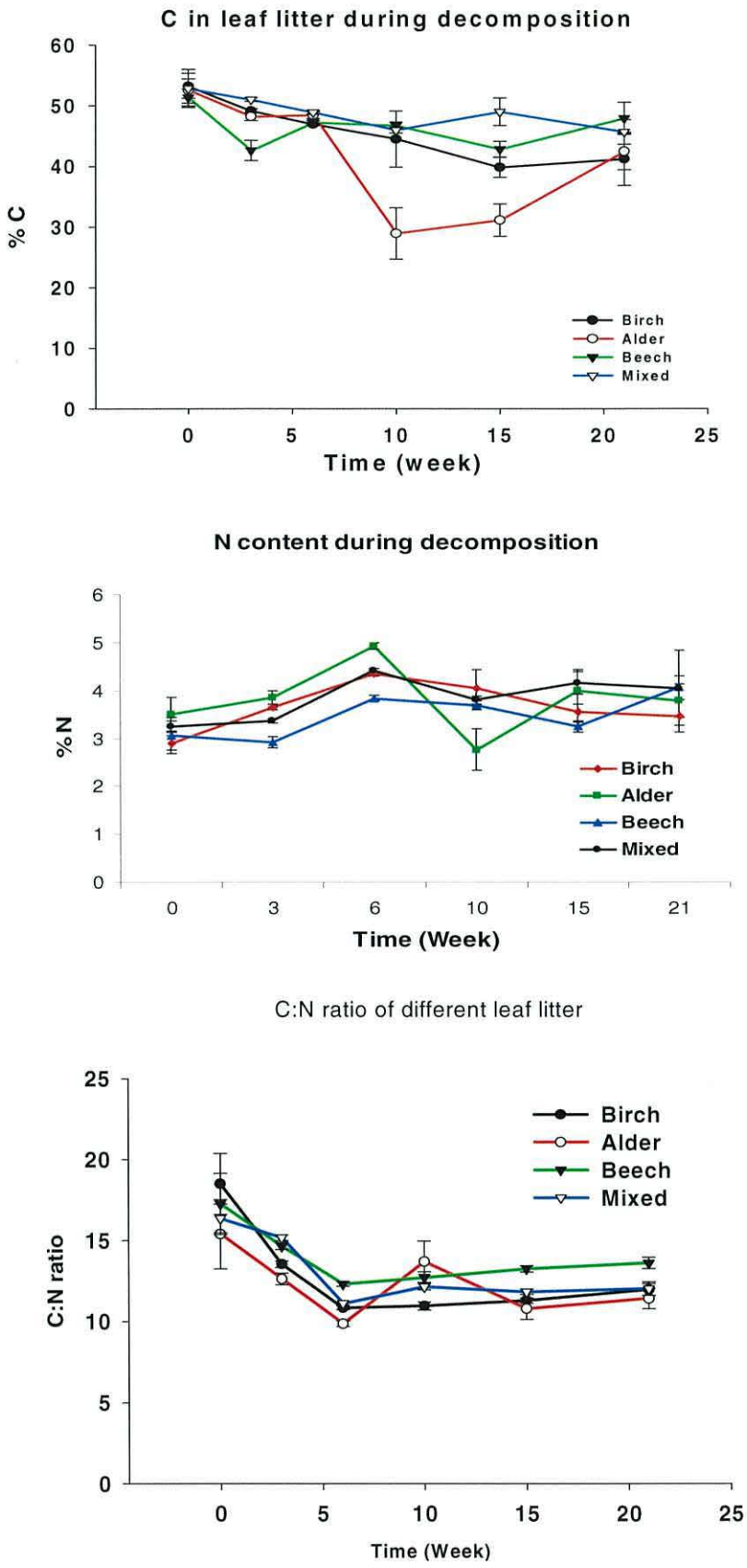


Figure 5.12 Carbon, N and C:N ratio in the decomposed leaf litter collected at different time intervals. All values equal mean, SEM (n= 9).

5A.3 Discussion

5.3.1 Weight loss and decay rate in single leaf litter

The mass loss pattern in our experiment clearly indicates two stages of decomposition during the study period. The rapid mass loss in all species during 0-10 weeks can be considered as first phase, and after 10 weeks relatively slow mass loss of second phase support the typical weight loss pattern of decomposition (Berg, 1986; Alhamd *et al.* 2004). This is because during the initial phase the soluble substances and non-lignified carbohydrates such as cellulose, hemicellulose are decomposed by saprophytic fungi and in the second phase lignin and lignified materials are the dominant decomposition substrates (Alhamd et al, 2004). Our results confirmed that the highest mass loss from alder litter (47%) after 3 weeks of decomposition followed by birch and beech. At the earlier stage weight loss was mainly due to release soluble compounds and major nutrients. This is in agreement with the findings of Berg and McClaugherty (2008) who reported higher amount of (30% of litter mass) of water soluble substances in alder leaf litter compared to birch, and consequently 40-50% initial mass loss and abrupt shift from early to late stage occurred during the decomposition of grey alder leaf litter. At the end of experiment (after 21 weeks) mass loss from alder leaf was as high as 98%. Similar magnitude of 94% weight loss in alder leaf after 20 winter weeks of decomposition was observed by Cornelissen (1996). Higher N level and low level of inhibitory compounds might be a reason for the higher weight loss (Kjoller et al, 1985).

Initial litter chemistry is thought to be influential in regulating the decomposition rate at different stages in various ecosystems and species (Hoorens *et al.* 2003; Tateno *et al.* 2007). The litter chemistry of birch, alder and beech partially support the above hypothesis (Table 5.1). The overall litter decomposition rate was slightly slower in birch

leaf litter that might be due to the higher phenolic content of birch (Giertych *et al.* 2006) which in combination with lignin protect amino acids and proteins from microbial enzymatic attack (Isaac and Nair, 2005; Kanerva *et al.* 2008). Influence of polyphenols on inhibition of fungal growth and activity was reported by some investigators (Hoorens *et al.* 2003). Colpaert and Tichelen (1996) reported a low decomposition rate in beech leaf litter because of the recalcitrant nature of lignocellulose matrix of beech litter and very limited access of saprophytic fungi to the leaf nitrogen pool of fresh beech litter, during a 6 month decomposition experiment. However, Hoorens *et al.* (2010) suggested that decay rate might be controlled by a unique combination of species-specific identity traits which act as a determinant for litter decomposition rather than one or few chemical parameters.

Our analysis indicated that the initial lignin content (beech > birch > alder) might have some influence on substrate decay rate particularly at the second stage of decomposition. Data from leaf chemistry revealed that lignin content is substantially lower in alder leaf and similar in birch and beech (non significant). Cromack (1973) concluded in his decomposition studies with mixed hardwoods that "lignin content of a given tree species leaf litter at the time of litter-fall is an important biological property influencing that species' rate of decomposition". The inherent cause may be the behaviour of lignin as an interfering factor in the enzymatic degradation of cellulose and other carbohydrates (Alexander, 1977). Similar results of slow decomposition rates due to high initial lignin levels was found by Melillo and Aber (1982) in their decomposition experiment with six species of hardwood leaves including beech and yellow and paper birch.

The overall decomposition rate seems to be faster in our experiment although in case of alder it is not uncommon as Kjoller *et al.* (1985) reported that the decomposition of alder (*Alnus glutinosa*) litter was complete within six months. Another cause of rapid disappearance of litter in the present study was the early stage of leaf development at the

sampling time of birch and alder leaves. Oleksyn *et al.* (2000) reported the higher plant nutrients such as N, P, and K during the formation stage of birch leaf than maturity or senescence stage, which might be enhanced the rapid biodegradation of leaf litter. We assumed that the local climatic conditions especially, rainfall might influence the leaching loss of partially decomposed substances from litterbag to some extent, as the total rainfall during the experimental period (June –November) was relatively higher (about 507 mm). We used 1 mm size mesh for litterbag to minimize the fragment loss and to prevent earthworm access. We succeed in the latter as no earthworms were found inside the litter bag; however, some other authors used < 1mm size mesh to retain the maximum amount of decomposed litter in the bag (Schadler and Brandl, 2005). Nevertheless, we assumed that the effect associated with the mesh size of litter bag is negligible between 1 mm and < 1 mm mesh size.

5.3.2 Nutrient dynamics and C: N ratio

The percentage nitrogen concentration in leaf litter slightly increased at the end of decomposition period, with statistically significant increase in beech litter. This might be due to accumulation and or immobilization of N by translocation and microbial growth respectively (Swift *et al.* 1979). Generally, the increased N originating from nutrients coming from other sources outside the litter concentrates in bacteria and fungal biomass that is attached to the leaf litter (Heal et al, 1997). In addition, N concentration may increase due to the formation of stable and less microbial accessible N compounds during lignin degradation (Nommik and Vahtras 1982). Higher concentration of N in the beech litter after 21 weeks than initial stage might be attributed to slow decomposition rate and limited access of fungal community to beech leaf N especially at the later stages of

decomposition (Rutigliano *et al.* 1996). The decline in C concentration during the initial phase clearly related to degradation of carbohydrate and phenolic compounds (Schlesinger, 1985). Litter with a low initial C: N ratio may have a rapid decomposition rate than higher C:N ratio material, which was supported by our results. C: N ratio is generally used as an index of predicting N dynamics and hence a predictor for degradability of organic matter (Tripathi *et al.* 2006). A relatively low C:N ratio indicated more stable organic substances in the later phase of decomposition course in our study.

5.3.3 Decomposition in mixed litter

Hoorens *et al.* (2010) found that when a relatively faster decomposing species was added to a slower species, this generally slowed down the decomposition of a mixture. In general, a negative non additive mixture effect i.e. decreases in litter decomposition due to mixture was observed in our experiment. Recently, similar results of antagonistic mixture effect was reported by Coq *et al.* (2011) in a greenhouse study with litter mixture of 4 tropical tree species from French Guiana. Although the overall mixture effect was antagonistic, when we examined the decay rate at each sampling interval, a low decomposition rate (antagonistic) in the first phase and relatively higher rate (synergistic) in the later phase of mixed litter, could be clearly demonstrated (Figure 5.10). There are several hypotheses explaining the antagonistic effects of litter mixture on decomposition. Fenn and Kirk (1981) and Melillo *et al.* (1989) suggested that high concentration of inorganic N may depress the rate of lignin decomposition in mixtures causing a decrease in net decay rate. Blair *et al.* (1990) reported that abundance of fungi and bacteria in mixed species litter bags was either similar or lower than the single species litter bags, and suggested that heterogeneous litter substrates may change the composition and abundance of the soil microbial community leading to an increase or decrease in litter decomposition

and N mineralization. Competition for substrate among different decomposer organisms in the litter mixture may affect the decomposition process. Antagonistic interactions between fungi and bacteria in the beech leaf litter and consequently a more than 50% reduction in mineralization rate has been reported by Muller *et al.* (1999).

Plant secondary compounds such as poly phenols and monoterpenes present in litter of any species in mixture could reduce the rate of decomposition and N mineralization in mixture by inhibiting the activity of the decomposer community (White, 1986; Nilsson *et al.* 1999; Hattenschwiler *et al.* 2005). We assumed that agonistic effects in mixture might be attributed to high polyphenolic content in the birch leaf litter as Giertych, *et al.* (2006) reported 60% higher total phenolics in the leaves of European birch (*Betula pendula)* than black alder (*Alnus glutinosa*) collected from seven year old trees in Poland.

The results indicate that a temporal change of mixture effect on decomposition rate is possible resulting simultaneous occurrence of both synergistic and antagonistic effects during the course of decomposition. Hattenschwiler (2005) examined the mixed leaf litter of six temperate tree species and found that mass loss at early stage of decay was either no change in three species, increased in one or decreased in two compared to the same pure species leaf litter and concluded that synergistic, antagonistic and additive are not necessarily exclusive interactions, but rather can occur together. In the present experiment, individual species were not measured separately in mixture bags but as a whole, therefore changes due to interactions can not be reflected in predicted values from pure leaf litter. However, as the objectives of the experiment was to evaluate the litter decomposition performances in single and mixed species plots in our field condition, the results of entire mixed litter decomposition was interpreted as a combined net effect of litter mixing. In addition the amounts of litter of component species, used in the mix bag was proportional

to litter inputs in the field, rather than equal amounts of each component, to emphasize the field conditions of the experiment sites. To examine whether the interactions among litter are occurring in mixture it is necessary to analyze component litters in mixture separately (Gartner and Cardon 2004).

5.2 Conclusion

Litter decomposition is an inherently complex phenomenon as a wide range of biotic and abiotic factors affect the processes continuously, causing changes in the rate and environment of decomposition during the decay course. Release of dissolved organic materials (DOM) in soil solutions was measured during laboratory incubation study. The magnitude of DOM released, was consistent with the mass loss and decay rates, calculated from data of litter bag experiment in the field. The litterbag study showed an antagonistic mixture effect on litter decomposition in mixed stands. The factors influencing the negative effects on mixed litter was not clear from our study, which needs a more detailed study, especially on decomposition residues of component litters for better understanding of mechanisms involved in the antagonistic effect of mixing litter.

6. Soil organic carbon storage in single and mixed stands of three broadleaved tree species

6.1 Introduction

Anthropogenic perturbation is causing a gradual increase in atmospheric CO_2 (IPCC, 2007). Two major compounds that are involved in the immediate release of CO_2 to atmosphere and that are boosted by human activities, are simple carbohydrates and hydrocarbons (fossil fuel). Carbohydrates in plant biomass are synthesized photosynthetically by fixing atmospheric CO_2, therefore, the management of forest biomass has a potential for reduction of atmospheric CO_2 to some extent. As a result, the higher production of biomass through afforestation / reforestation has been recognized as an effective, low cost option for mitigation of climate change impacts (IPCC, 2007). Expanding tree biomass may also increase the carbon stock in soil as 70% of soil organic C derives from plant biomass (Batjes, 1996; Jobbágy and Jackson, 2000). Thus the potential of forests for C sequestration partially depends on the C storing performances of soils.

Soil is the second largest reservoir of C in the natural ecosystem after the oceans. In the soil ecosystems, C generally exists in two major forms depending on the soil type: a relatively dynamic organic pool as soil organic matter (SOM) and inorganic forms mainly as carbonates, both are linked to atmospheric CO_2 through the processes of C cycle (Cheng and Kimble, 2001). Recently the interest of scientists and policy makers has increased dramatically about the pool size and budget of soil organic carbon (SOC). This is due to the establishment of the Kyoto Protocol aiming to reduce emission and/ or enhance

sequestration of CO_2 (Cheng and Kimble, 2001). The balance between the input of organic matter mainly from vegetation in the forest ecosystem, and losses mainly due to decomposition-respiration and leaching, determines the net C storage potential in the soil (Ostle *et al.* 2009). Therefore soil C storage not only depends on biomass inputs but also on subsequent biogeochemical processes, which can be improved by appropriate management techniques. Thus soil can be a sink for atmospheric CO_2.

Among different land use based ecosystems, forest soil contains about one third of soil organic carbon (Jazen *et al.*, 2004). At an ecosystem level, the storage and sequestration of SOC in forest ecosystems are primarily controlled by two broad aspects, C input from primary production and residence time (Thompson *et al.* 1996). The accumulation of organic C in forest soils depends on a number of factors such as plant biomass (above and belowground), litter quality, and soil microbial activities, management practices and climatic factors such as temperature, precipitation and fire (Lal, 2005). Tree species affects soil C stocks primarily through litter fall and root functions such as turnover, exudates etc, and it is hypothesized that under relatively homogenous biophysical circumstances, different tree species accumulate and fix different amount of carbon in the forest floor and mineral soils (Schulp *et al.* 2008). Relative to monocultures, the interactive effects of mixed species stands on SOC could be linear, antagonistic or synergistic, or dependent upon the intrinsic properties of the trees (De Deyn *et al.* 2008). For example, incorporation of N-fixing species in mixtures may influence the C sequestration in soils (Harpe, 1977; Kaye, 2000).

Many studies on global and regional SOC budgets particularly C storage in forest soils have been carried out worldwide (Eswaran *et al.* 1993; Kern 1994; Batjes 1996). Most of these studies focused on natural forest (Chen *et al.* 2000; Fang *et al.* 2004). Recently, research studies on SOC stocks in abandoned agricultural lands have receiving momentum but the findings are widely inconsistent. The response of new plantings especially mono and mixed culture on SOC stock is still unclear. Another important aspect that remains poorly understood is the vertical distribution of the SOC pool in relation to tree species (Jobbagy and Jackson, 2000), which can have major consequences for SOM turnover rates (Rumpel and Koegel-Knabbe, 2011). Therefore, the main objectives of this study were to estimate SOC pools in new plantings of three UK native broadleaved tree species and to evaluate the responses of species composition across the vertical dimensions.

6.2 Materials and Methods

6.2.1 Study sites

The location of the Henfaes Research Centre, Bangor comprises high mountains with steep slope, broad valley and flatter land adjacent to the coast.. The topography of the area includes a shallow slope on a deltaic fan of approximately 1-2° towards northwest, at an altitude of 4-14 m above sea level with the Hyperoceanic climate (Teklehaimanot and Sinclair, 1993).The seasonal temperature vary between -3 to 10 ^{O}C in Winter and 12 to 25 ^{O}C in Summer and the annual rainfall of about 1000 mm. The area was covered by typical mixed oak woodland and was transferred to agricultural system from Roman times onward (Avery, 1990).

6.2.2 Soils and woodlands

The soils developed under more or less well drained condition, non calcareous and from unconsolidated parent material traditionally grouped in Britain as Brown earth (Clarke, 1940) which are classified as Dystric Cambisols according to FAO system and recognized as Rheidol series (Teklehaimanot *et al.* 2002). The parent material of soils was originated from two sources: postglacial alluvial deposits.

The forest plantation was established on 2.3 hectare of ex arable lands in March, 2004 with a range of broadleaved tree species included the single and mixture plots of birch (*Betula pendula*), alder *(Alnus glutinosa*) and beech (*Fagus sylvatica*). In the present study, monoculture and mixed culture stands of the three species were used with 4 replicate plots for each species types.

6.2.3 Field sampling

Soil sampling was conducted during July-September 2008. Samples were collected from the single species planting blocks of birch (*Betula pendula*), alder (*Alnus glutinosa*) and beech (*Fagus sylvatica*) and mixed blocks of these three species as well from ungrazed grassland areas adjacent to planting blocks. Each single species plot, species mixed plot and the grassland had four replicated blocks, two in each field, giving a total of 20 plots. Samples, taken at a central point were equidistant from the three surrounding trees, and in case of mixed plots they were equidistant from three different tree species. From each sampling point composite bulk soil samples were collected from seven soil depths : 0-10, 10-20, 20-30, 30-40, 40-50, 70-80 and 100 cm. Samples from the upper 5 layers (0-50 cm) of each blocks were collected by excavating a 50 x 50 x 50 cm pit which were further used to estimating the rock volume of each plot. Soil samples from deeper layers (50-100 cm) were collected using an auger. Soils were placed in reseable plastic bags, labelled and immediately transported to the laboratory stored in 4 ^{O}C cold room prior to further processing.

6.2.4 Estimation of rocks volume

All bulk soils of each 50 x 50 x 50 cm pits were collected separately, and all rocks and stones (> approx. 2 cm) carefully hand sorted and collected separately. To account for stones sizes of < 2 cm, three replicated bulk density samples for upper three layers were used (the collection methods have been described in Bulk density section). All rocks and stones were washed and volume was measured by water displacement methods. The weight of dried rocks was recorded using electric balance. Rocks volume for 1 m soil depth was estimated by extrapolating values for 50 cm soil depth.

6.2.5 Analysis of soil physical properties

Sample preparation: Refrigerated soil samples were air dried (at 22 ^{O}C for 48 h) and ground with a mortar and pestle to pass through a 2 mm sieve. A portion of soil was further ground to pass a 0.5 mm sieve. All processed soil samples were stored in air-tight plastic bags at 4 ^{O}C.

Soil texture: Soil samples for particle size analysis were collected from 7 different depth intervals (0-10, 10-20, 20-30 , 30-40, 40-50 , 70-80 and 100 cm) of 4 randomly selected spots from birch, alder, beech and mixed plots.

The particle size distribution of soils was determined by a simplified method combining wet sieving and sedimentation steps proposed by Kettler et al, (2001). In this method soils were pre-treated with 30% H_2O_2 (2:1 ratio of Soil: H_2O_2) to remove organic matter. The soils were then mixed with 3% calgon solution (Sodium hexameta phosphate, [HMP, $(NaPO_3)n$]) at a ratio of 1:3 and shaken on a horizontal shaker for 2 h . The soil slurry was then passed through a 0.053 mm sieve to collect the sand fraction. The suspension containing silt and clay fractions was collected in a 800 ml beaker, stirred thoroughly to suspend all particles and then the silt particles allowed to settle in undisturbed conditions at room temperature (at 20 ^{O}C) for a sedimentation period of >1.5 to <6 h. After the sedimentation period the suspended clay fractions were siphoned off to separate them from the settled silt particles. The settled silt fraction was then dried in the beaker at 105 ^{O}C to constant weight.

The per cent sand, silt and clay were calculated as follows:

$$\text{Sand \%} = \frac{\text{Oven dry sand mass}}{\text{Original sample mass}} \times 100\ \%$$

$$\text{Silt \%} = \frac{\text{Oven dry silt mass}}{\text{Original sample mass}} \times 100\ \%$$

(Original sample mass was obtained after correction for the moisture content).

Clay % = 100-(Sand % + Silt %)

Textural class of soils was determined using soil textural triangle.

Bulk densities: The bulk density of the soil was determined by a core method using stainless steel core (100 cm^3). Samples were collected from an undisturbed levelled ground using a core sampler. Removing the extra soil and protruding roots, the whole contents was transferred to a pre weighed aluminium tray and dried at 105 ^{O}C to a constant weight and the oven dry mass of the soils recorded. The volume of the core was measured and recorded accordingly.

As the soils contained small stones (>2 mm), the soil was washed on a 2 mm sieve with flow of water and all stones collected. The volume of the stones was estimated by water replacement using a measuring cylinder and the mass of the stones was recorded after drying.

Bulk density of stony soils was calculated as follows (Cools and De Vos, 2010):

$$BD\ (g\ cm^{-3}) = \frac{Ms}{Vc} = \frac{M\ s{+}r - Mr}{V\ s{+}r - Vr}$$

BD = Bulk density of the soil (g /m3)
Ms = Oven dry Mass of only soil taken with core sampler (g)
Vc = Volume of the only soil in the core (cm3)
M s+r = Dry Mass of the soil sample with rocks taken with core sampler (g)
V s+r = Volume the core (soil with rocks) (g)
Mr = Mass of the rocks (g)
Vr = Volume of the rocks

6.2.6 Analysis of soil chemical properties

Soil pH and Electrical Conductivity (EC): Soil pH and EC were determined in a 1: 2.5 ratio of soil: water suspension using an EC and pH meter (Jenway 4010 EC meter and Orion 410A pH meter).

Soil moisture: Soil was dried in the oven at 105 ^{O}C to constant weight and moisture content was determined gravimetrically.

Organic matter: Soil organic matter was determined following the Loss on Ignition method (LOI). Oven dry (OD) samples were heated in muffle furnace at 450 ^{O}C overnight and per cent organic matter was calculated as follows:

$$\% \text{ Soil Organic Matter (SOM)} = \frac{\text{Weight of OD sample-Weight of ignited sample}}{\text{Weight of OD sample}} \times 100$$

Organic carbon and total nitrogen: Total C and N in soil were measured by dry combustion technique using a CN analyser (TruSpec® CN, LECO Inc.). Interference due to inorganic carbonates may cause error in the estimation in calcareous soil. However, soil pH is a good indicator for calcium carbonates and the pH range from 7.8 to 8.2 indicates the presence of inorganic forms of C (McLean, 1982). As a safety measure, a pH of 7.4 is considered as limit above which the sample should be treated to remove carbonates (Schumacher, 2002). The pH of our soil samples ranged 5.4-6.3, hence no carbonates were present.

Calculation of C storage: Soil C storage was calculated using following equation:

$$\text{Soil C storage (g m}^{-2}) = \frac{C*(100-R)*D*100}{BD}$$

Where,

C = % Carbon in soils
R = Volume of rocks in soils (% of soil volume)
D = Soil depth (cm)
BD = Bulk density of soils (g cc^{-1})

C storage in 1m soil profile (g m^{-2}) = $\sum (D_1 ..... D_{10})$

Mixture effects: The predicted value in mixed plots was calculated from single stands according to the proportion of trees in mixture. The proportion of birch (B), alder (A) and beech (F) in mixture was 1:1:1. Therefore the predicted value for mixture is (B/3 + A/3+ F/3), where B, A and F indicate values from single species stands.

6.2.7 Statistical analysis

In the plantation experiment, 16 single and mixture plots were arranged as a completely randomized design and split into two blocks. The normality and homogeneity of variables were checked using Kolmorgorov-Smirnov (K-S) test and Levene's test respectively. Differences in variables were tested with 4 replicate values using one way ANOVA following SPSS v 14.0 (SPSS Inc.) to examine overall effects of different treatments (species, depth etc.). Further Post hoc procedures were followed to compare all different combinations of the treatment groups (pairwise). In this case, Tukey'HSD were used (honestly significant difference) to explore exactly which combination differs significantly. To evaluate mixture effect actual and predicted values were compared using paired sample t test. To explore relationship between clay content and soil C, non-linear regression analysis (exponential) was performed using SPSS.

To establish a relationship between cumulative C stock and soil depth, the following three regression models were considered:

a. Exponential model, $y = a\,e^{bx}$

b. Logarithmic model $y = y_0 + a \ln(x)$

c. Power model $y = a\,x^{b}$

(Where y = Soil depth, x = Cumulative C stocks, a, b and y_0 = Constant parameters).

Comparing the coefficient of determination (R^2) of three models, the single exponential growth model ($\mathbf{y = a*e^{bx}}$) was found the best fit to establish relationship between soil depth and C content (Table 6.3). Moreover, among the different models, the exponential C depth model is the most widely accepted (Zinn et al. 2005; Minasny et al. 2006). The test of significance was done for all relationships using ANOVA and the difference between the treatments was accepted as being significant at 5% level ($P < 0.05$).

6.3 Results

6.3.1 Soil organic matter and bulk density

Soil organic matter (SOM) content decreased gradually with increasing soil depth and at the deepest soil layer (1 m depth) was only < 50 % of that in the surface soils. This pattern was observed in all tree species blocks and grassland soils (Figure 6.1). ANOVAs revealed that the variations in SOM along the depth profile were highly significant for all species types, the mixture and grassland soils (P = 0.00 for all vegetation types). Litter inputs caused variations in the SOM contents of forest floor especially in the upper 4 soil layers (0-40 cm). The soil under birch contained the highest SOM followed by alder, tree mixture, grass and beech, and the differences were statistically significant (P <0.05 for all 4 depths). However, differences at the deeper soil depths (40-100 cm) were not statistically significant. Pair wise comparison of SOM across different tree species showed that the soil under beech had significantly low organic matter content than that under birch and alder at 0-10 cm (P = 0.002 and 0.024 respectively). 10-20 cm (P=0.007 and 0.018 respectively), and at 20-30 cm only when compared to birch (P =0.01).

Although the soil under birch had the highest percentage of organic matter (7.5 %) among all plantation blocks and grassland soils, the variations were only statistically significant with beech and grassland at 0-10 and 10-20 cm; beech, grassland and tree mixture soils at 20-30 cm; and only beech at 30-40 cm depths (in all cases P <0.05). SOM content in grassland soils was significantly lower than birch soil at all 3 upper soil depths (0-30 cm) and alder at only 10-20 cm depth (Figure 6.1).

Soil bulk densities differed significantly between grassland and all tree plantation soils at 0-10 cm ($F_{4, 14} = 8.09$, $P < 0.05$) and only beech soil at 10-20 cm ($F_{4,14} = 3.59$, $P < 0.05$) (Table 6.1). No significant effect of vegetation was found in bulk densities at 20-30cm depth. Vertically, bulk densities increased with increasing soil depth in all tree plantation and grassland soils. However, in most soils statistically significant difference was found between soils of top two layers.

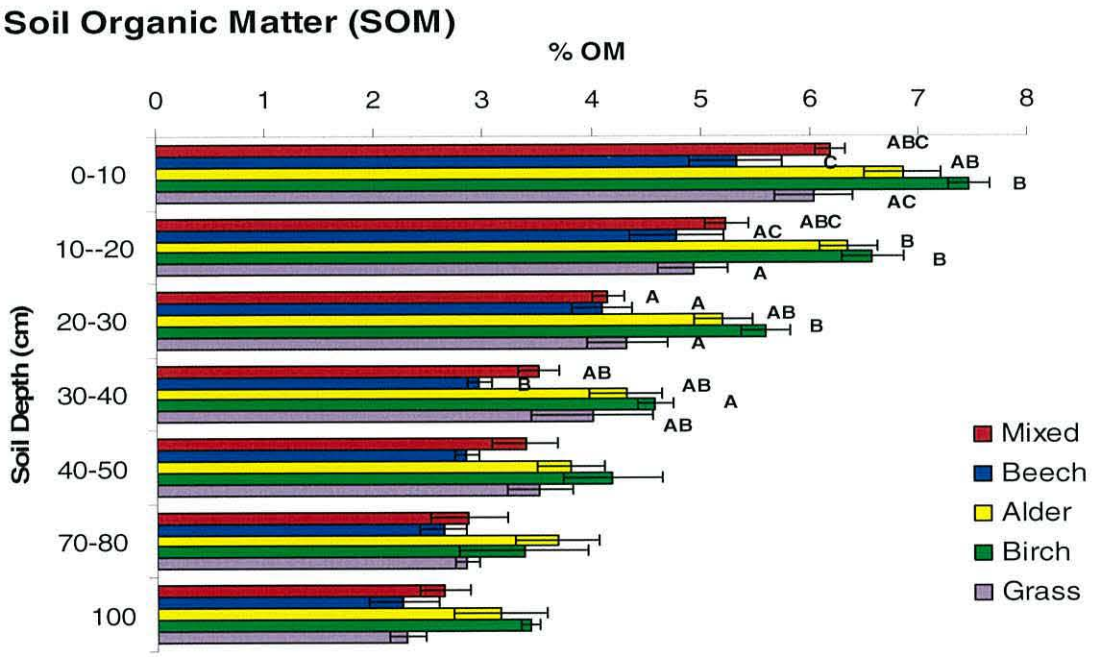


Figure 6.1 Soil Organic matter under different tree species and grassland soils, estimated by loss on ignition. Bars equal means, SEM. Bars without the same indices are significantly different between species at a particular depth ($P<0.05$).

Table 6.1 Area, stand size and soil volume characteristics of 16 plantation blocks. (Mean, Values in parentheses indicate standard error of mean (SE M) in bulk density column).

Species	Data from 4 plots of each stands types			Soil bulk density ($g\ cm^{-3}$)		
	Area m^2	Tree per stands	Stone volum. (%)	0-10 cm	10-20 cm	20-30 cm
	64	81	20	1.19	1.26	1.36
Birch	64	89	9	(0.02)	(0.03)	(0.04)
(*Betula pendula*)	64	70	14			
	120	119	15			
Alder	120	133	16	1.21	1.29	1.23
(*Alnus glutinosa*)	64	79	8	(0.02)	(0.02)	(0.08)
	80	85	16			
	56	69	10			
Beech	81	86	7	1.18	1.32	1.40
(*Fagus sylvatica*)	56	79	12	(0.03)	(0.03)	(0.04)
	72	83	10			
	64	79	10			
Mixed (Birch, Alder	169	217	7	1.20	1.28	1.35
& Beech)	169	212	5	(0.01)	(0.03)	(0.02)
	169	195	27			
	156	161	8			
Grassland			12	1.04	1.16	1.27
				(0.02)	(0.03)	(0.04)

6.3.2 Organic carbon concentration in soil

The mean content of soil organic carbon and nitrogen were decreased significantly downward in the soil profiles from 0-100 cm irrespective of species types (Figure 6. 2). The highest organic C content (3.3%) was found at the top layer of soil under birch followed by alder and the tree mixture (2.8%), grassland (2.7%) and beech (2.1%). More or less same order of magnitude was observed in the soil up to 30 cm depths. ANOVAs revealed that tree species had significant effects on C contents of upper two soil layers 0-10 cm ($F_{4,15}$=3.29, < 0.05) and 10-20cm ($F_{4,15}$ = 4.54, <0.05). Further Post Hoc tests (using Tukey HSD) revealed that birch soils significantly differed in organic C with beech soils at both layers (P =0.021 and 0.020 respectively). In contrast, in the deeper soil segments (30-100 cm), the differences in SOC due to vegetation were not statistically significant.

Table 6.2 Physico-chemical properties of soils in experimental site. (Mean, values in parentheses indicates standard errors of mean, n = 4).

Soil Depth	% soil fractions			Textural Class		Electrical conductivity (EC)	pH (H_2O)
(cm)	Sand	Silt	Clay			$\mu S\ cm^{-1}$	
0-10	48.1 (1.4)	33.6 (0.9)	18.3 (2.2)	Loam		130.5 (11.4)	5.41 (0.07)
10-20	49.3 (2.3)	33.1 (0.6)	17.6 (2.1)	Loam		67.2 (6.1)	5.6 (0.1)
20-30	49.5 (2.47)	33.4 (0.56)	17.2 (2.05)	Loam		48.9 (3.93)	5.8 (0.1)
30-40	49.4 (3.1)	34.8 (0.9)	15.8 (2.3)	Loam		38.9 (3.5)	5.9 (0.04)
40-50	51.7 (4.8)	32.3 (2.5)	15.9 (2.6)	Loam		36.0 (3.5)	6.0 (0.1)
70-80	62.3 (5.2)	25.7 (3.9)	12.6 (2.4)	Sandy Loam		30.6 (2.8)	6.2 (0.04)
100	62.9 (2.6)	24.9 (2.3)	12.1 (1.9)	Sandy Loam		29.9 (2.8)	6.3 (0.1)

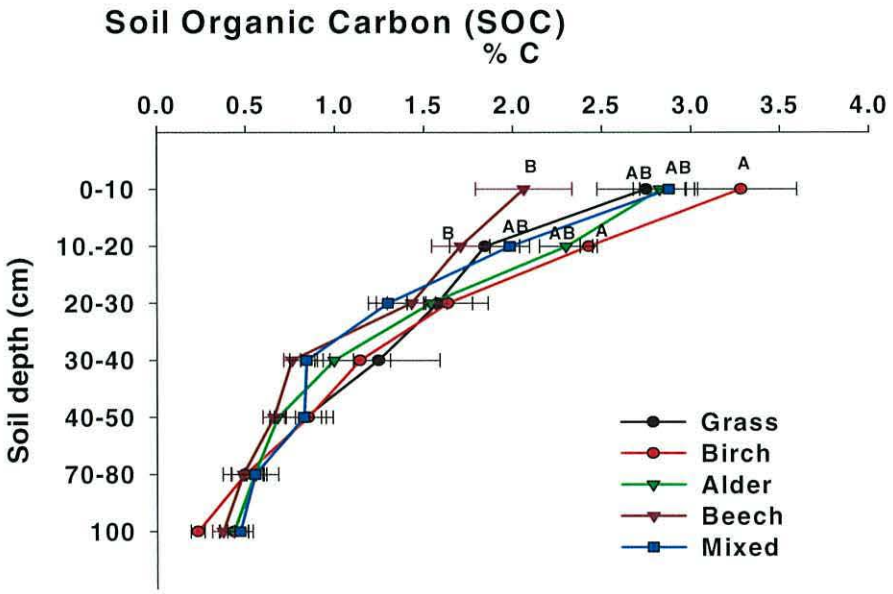


Figure 6. 2 Vertical distribution of SOC concentration under different tree species and grassland, determined using a CHN analyser. Bullet symbols equal means, SE. Symbols without the same indices is significantly different (*P*=0.05).

Planting of the tree mixture did not show any significant effects on soil C content. The actual (mixed plots) and predicted (estimating from single species plots) values of C suggested that SOC in mixed culture planting slightly increased at the top soil layer but decreased or same at the lower layers (Figure 6. 3). However, none of these variations were statistically significant.

The vertical distribution of SOC under the different tree species and grassland significantly decline over depth (Figure 6.2). C concentrations decreased sharply with increasing soil depth up to 1m by 83-92%. Mineral soils including the forest floor (0-10 cm, in this case) contain the highest amount of organic C (27-32 % of total profile C) compare to other layers.

The relationship between SOC concentration and per cent clay content was examined using textural clay content data of different depth intervals. A significant exponential relationship was observed in soils of birch, alder and mixed soils ($P < 0.05$), however the relationship in beech soil was not statistically significant (Figure 6.4).

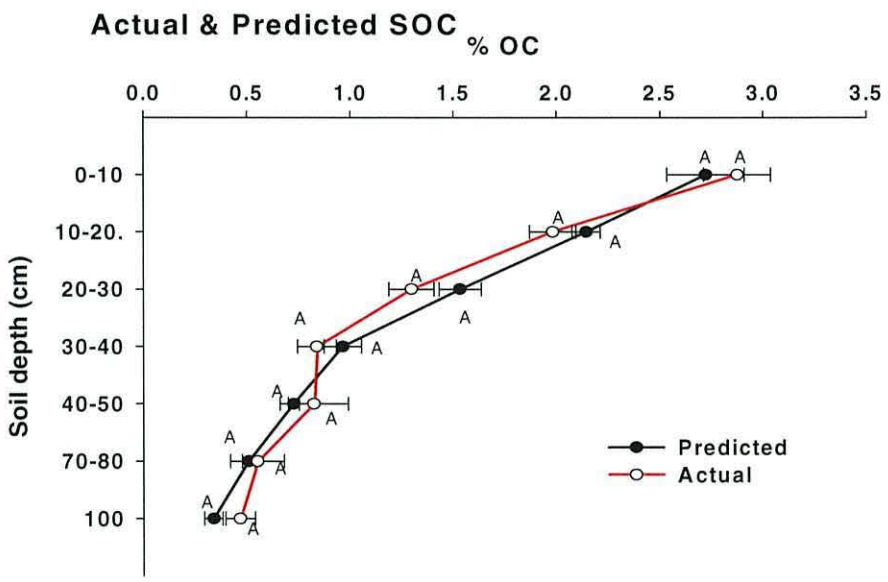


Figure 6.3 Comparison between the actual C (in the soil of the species mixture) and predicted C (obtained by calculating from single species planting soils). Symbols equal means, SE. Symbols without the same indices is significantly different (P=0.05).

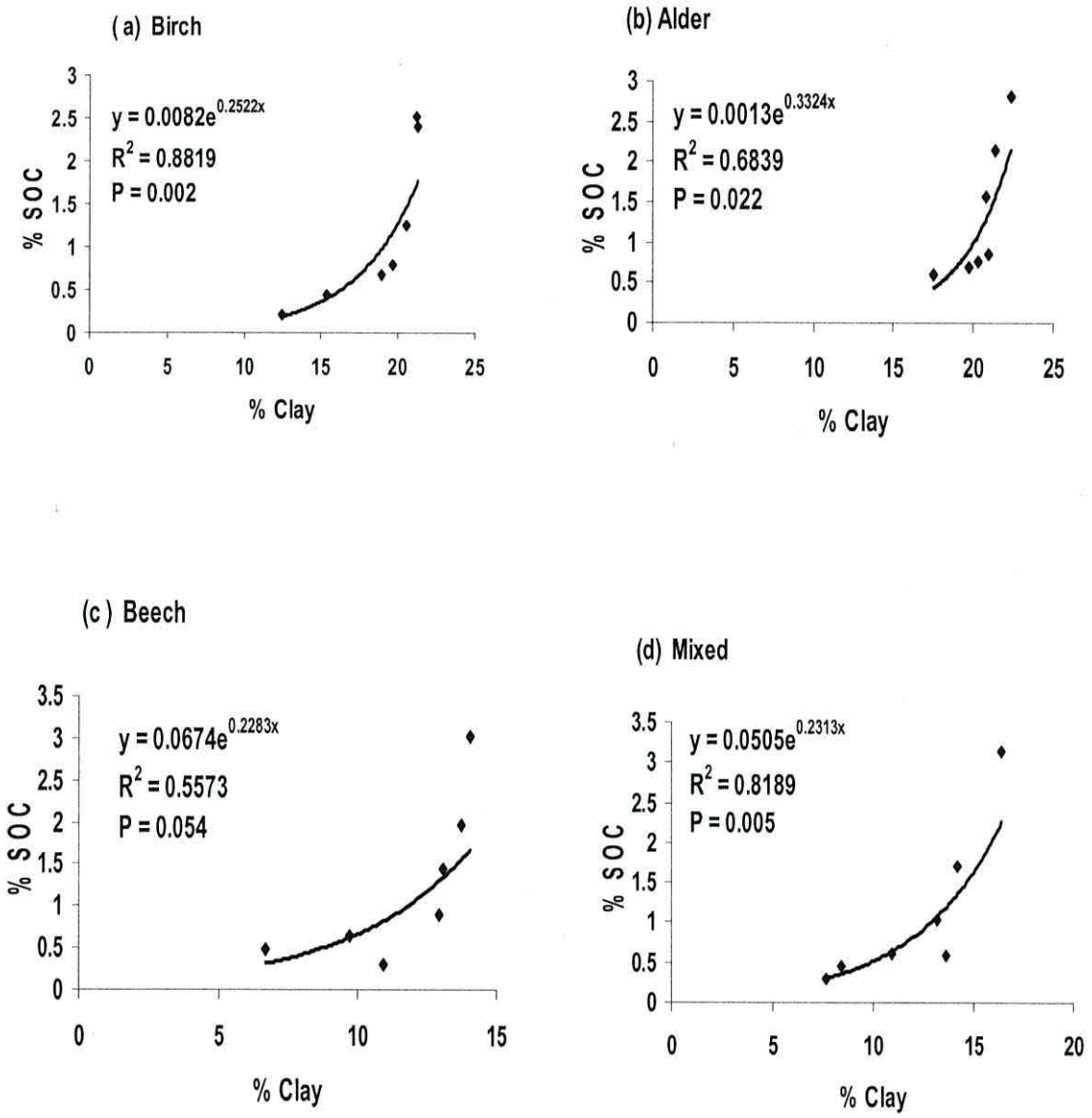


Figure 6.4 Exponential relationship between clay and organic C content in the soils from different plant species stands. The coefficient of determination (R^2) indicates goodness of curve fitting and hence strength of relationship.

6.3.3 Nitrogen and C: N ratio

Total nitrogen in the surface soils were in the range 0.35 - 0.23 % across the different tree species and grassland, with this being highest in the grassland soils, followed by birch, the mixture, alder and beech, however, only the variation between birch and beech was statistically significant (P =0.02). Although the nitrogen concentration significantly declined with depth in both grassland and tree planting plots, the decline in the grassland was relatively small at the deeper soil layers (Figure 6.5). A significantly higher concentration of total nitrogen was found in grassland than beech soil at 30-40 cm (P =0.034) and 100 cm (P =0.038).

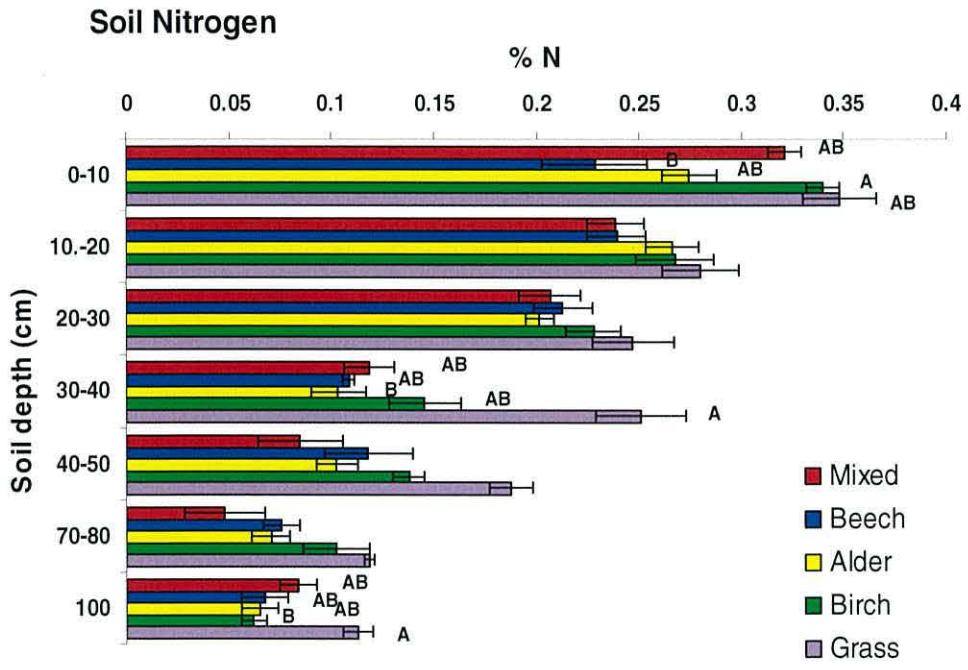


Figure 6.5. Nitrogen concentration of different tree species and grassland soils, estimated using a CHN analyser. Bars equal means, SE. Bars without the same indices are significantly different (P =0.05).

C: N ratio in soils: Overall, the C:N ratio of soils were gradually decreased with increasing soil depths under all tree species types and the highest C:N ratio was found in top soils (0-10 cm) of birch followed by tree mixture, alder, grassland and beech soils (Figure 6.6).

C: N ratio did not vary significantly among soils of tree species (P=0.07) ranging from 10.1 to 8.3 in the plantation blocks compared with 9.0 in the abandoned grassland. In deep soil layers (40-100 cm) the reduction in grassland C: N ratio was more pronounced compare to the soil under trees, although the lowest C: N ratio was found in the birch soil at 100 cm depth.

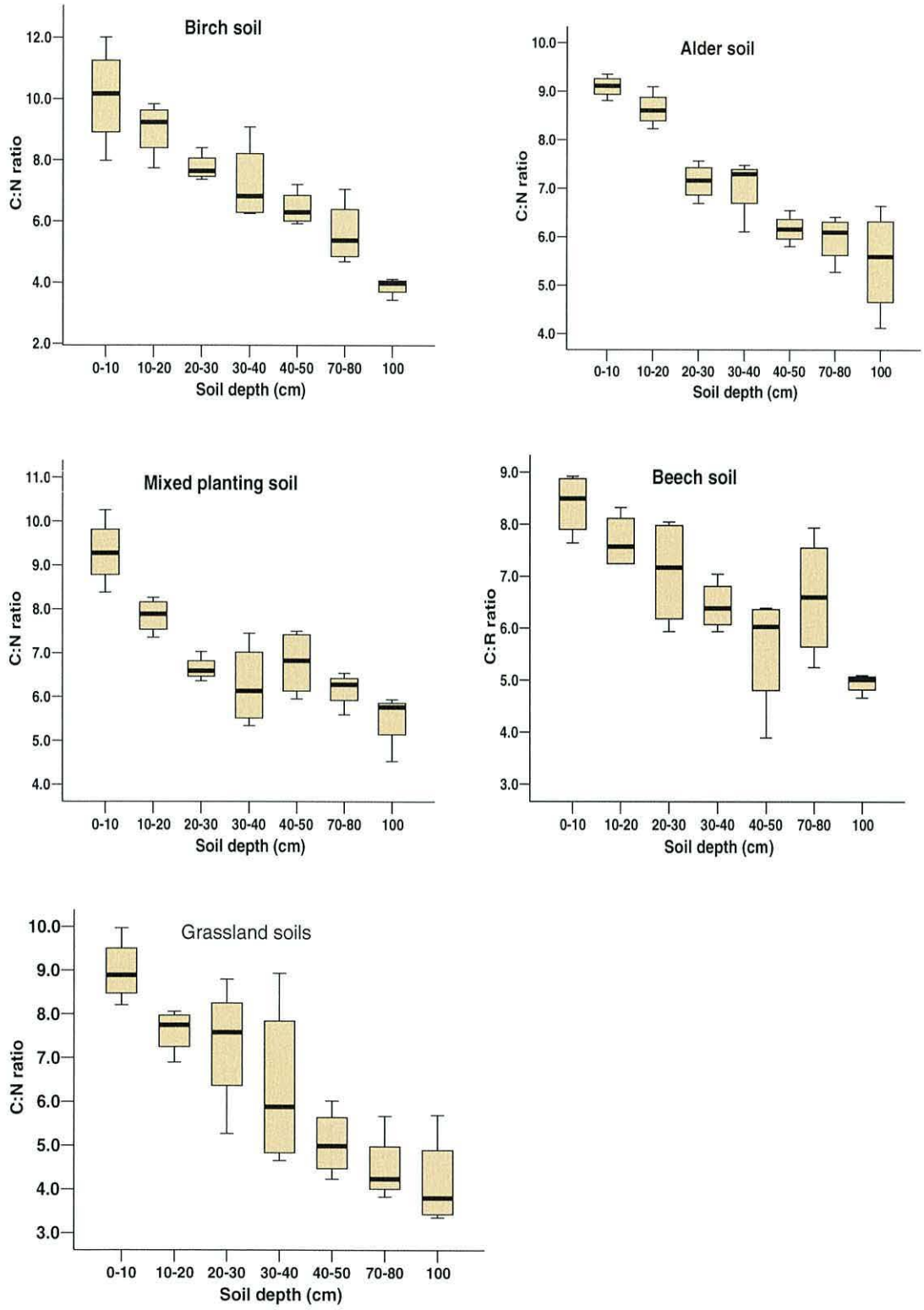


Figure 6.6 Vertical changes in soil C: N ratio under birch, alder, beech, mixed species plants, and grassland. The top and bottom horizontal bars of each box showed the highest and lowest value of C: N ratio at each depth intervals. Each box indicate value of middle 50 % data with upper and lower edges of the tinted box are upper and lower quartiles and the thicker horizontal line inside the box indicate median value of the data.

6.3.4 Organic carbon storage in soil

In absence of a distinctive forest floor, the mineral soil (0-10cm) including some forest floor materials was analyzed together for the 4 years old forest stands. Overall, slight variation with no clear influence of tree species types on C storage was observed (Figure 6.7). However, in the top 2 soil layers the differences in C stocks over species type were more pronounced than at the deeper layers. At 0-10 cm, the mean SOC stocks ranged between 2.78 kg C m^{-2} (birch soil) and 1.76 kg C m^{-2} (beech soil). The tree mixture (2.39 kg C m^{-2}) showed no significant effect on SOC storage. Relative to the grassland, soils under alder, mixture and beech stands stored a slightly lower amount of C at the topmost soils but not statistically significant. A more or less similar response of tree species on C accumulation was observed at 10-20 cm soil depth. The beech soil possessed the lowest C stocks over soil depth up to 1 m which was significantly lower than birch soils at 0-10 cm and 10-20cm (P =0.039 and 0.031 respectively). No significant variation in C storage between different tree species soils was observed at the deeper soil layers (30-100 cm) (Figure 6.7).

Overall, organic carbon stocks significantly declined over depth up to 1m for all tree species and grassland soils. The rates of decline in SOC stocks along vertical intervals were mostly same in all tree species. The cumulative soil C pools over soil depth followed a specific non linear pattern with a significant goodness of fit (Figure 6.8). The exponential model had the best fit to predict SOC pool in soil profile. The properties of the models are given in Table 6.3.

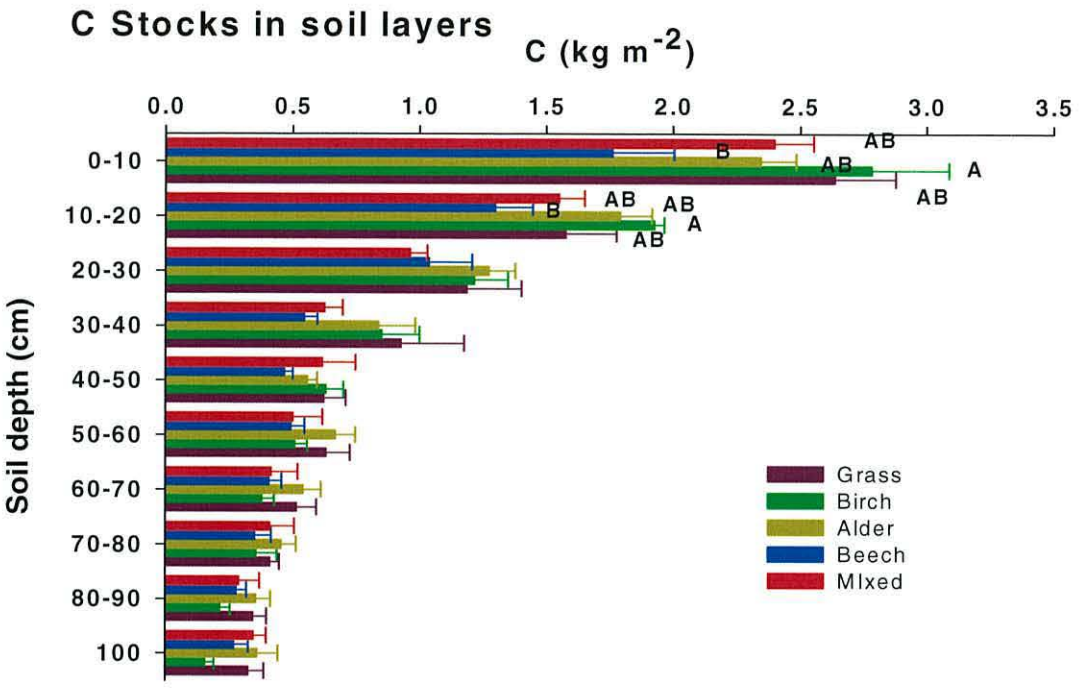


Figure 6.7 SOC storage pattern across depth in relation to tree species and grassland. Bars equal means, SE. Bars without the same indices are significantly different ($P < 0.05$).

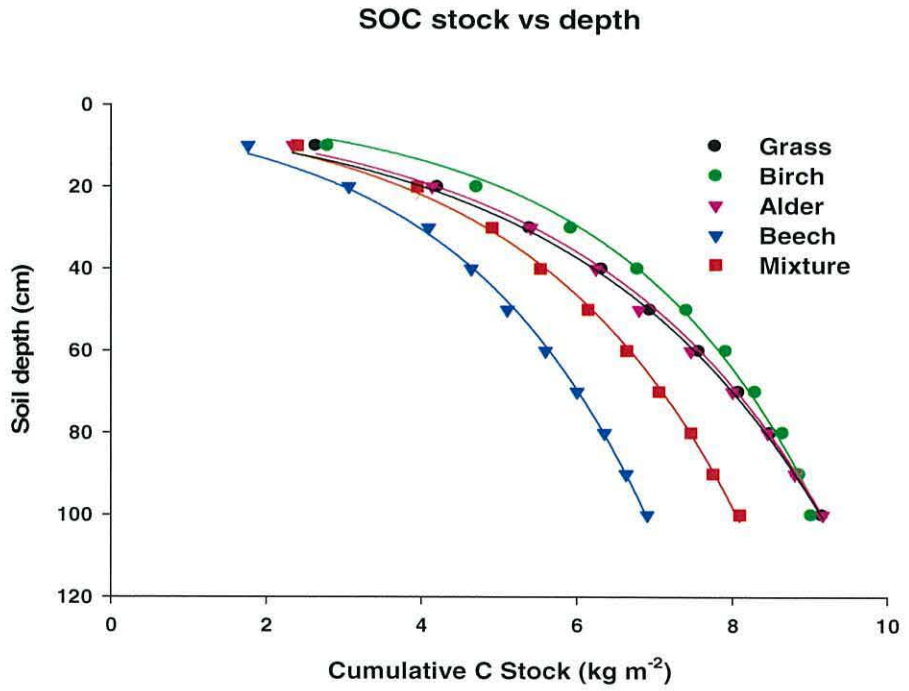


Figure 6.8 The relationship between soil depth and cumulative C stocks. Single exponential curves provide the best fit for different tree species and grassland soil (general model: $y = a*e^{b*x}$).

Table 6.3 Exponential, power and logarithm models to examine the relationship between SOC stock and soil depth under different tree species. y = Soil depth (cm), x = Cumulative stock of SOC (kg m^{-2}). Exponential model was found the best predictor of the relationship. Equations with different letters indicate statistically significant ($P < 0.05$).

	n	Equation	R^2	*P*
Grass	4	**$y = 5.1424\ e^{0.3240x}$ ab**	**0.9993**	**<0.0001**
		$y = 0.6168\ x^{2.2814}$	0.9922	<0.0001
		$y = -74.5699 + 69.9660 \ln x$	0.8266	0.0003
Birch	4	**$y = 2.8221\ e^{0.3807x}$ a**	**0.9944**	**<0.0001**
		$y = 0.1671\ x^{2.8763}$	0.9789	<0.0001
		$y = -80.9896 + 71.6279 \ln x$	0.7627	0.0010
Alder	4	**$y = 5.6618\ e^{0.3140x}$ ab**	**0.9986**	**<0.0001**
		$y = 0.7283\ x^{2.2089}$	0.9930	<0.0001
		$y = -62.4455 + 64.1110 \ln x$	0.7781	0.0005
Beech	4	**$y = 5.8123\ e^{0.4136x}$ b**	**0.9983**	**<0.0001**
		$y = 1.4335\ x^{2.1838}$	0.9932	<0.0001
		$y = -43.8245 + 64.0023 \ln x$	0.8062	0.0004
Mixture	4	**$y = 4.9384\ e^{0.3738x}$ ab**	**0.9982**	**<0.0001**
		$y = 0.7161\ x^{2.3539}$	0.9958	< 0.0001
		$y = -71.5951 + 72.9545 \ln x$	0.8222	0.0003

6.3.5 Total organic carbon pool

Four years after planting of birch, alder and beech and their mixture, small but non significant impacts on soil organic carbon pool size were detected. Alder plots contained the highest average organic C pool (8.0 kg m^{-2}) followed by birch (7.7 kg m^{-2}) and the beech plots (6.2 kg m^{-2}) (Figure 6.9). Mixed species plot contained 7.1 kg C m^{-2} on average with no significant difference compare to the single planting plots. The SOC pool size in grassland (8.02 kg m^{-2}) of the same age was same as in alder. The same order of magnitude

in SOC pool size in soils over vegetation types was observed without accounting for the stone volume.

Approximately 60-77 % of total C pool was located at the top 30cm of the soil profile (Figure 9). As the zone of maximum root activities, C pool size in this depth was partially influenced by root systems of the different tree species. At this zone, the highest proportion of total C pool (77 %) was observed in birch plots followed by alder (68 %), mixed (61 %) and beech and grassland (59 % each) soils

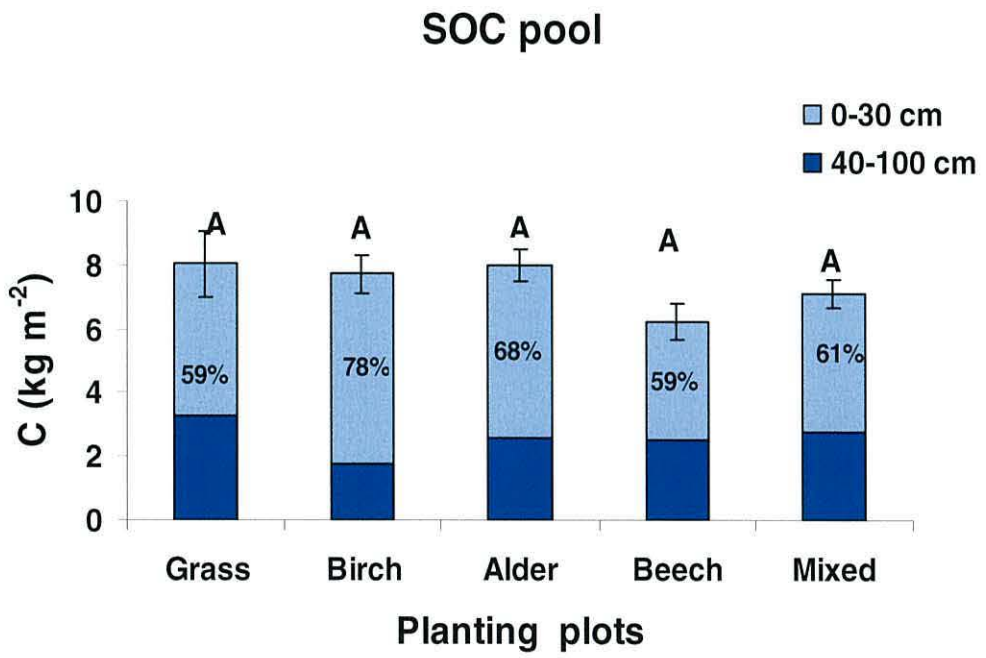


Figure 6. 9 Organic C pool size in top metre of soils, after afforested with birch, alder, beech and mixed stands with adjacent grassland after correction for stone volume in each block. Per cent values in each bar indicate proportion of total pool in the top 30 cm. Bars equal means, SE. Bars without the same indices are significantly different ($P < 0.05$).

6.4 Discussions

6.4.1 SOM and N under afforested soil

Organic matter in the forest soil is the largest C pool originating mainly from litterfall and root activities (Hagen-Thorn *et al.* 2004). However, the stability of soil organic matter further depends on the quality of litter input to soils. In general, young planting systems add SOM which may offset the losses occurred during establishment of the plantation (Jandl *et al.* 2007). Wilde (1964) investigated soil organic matter accumulation in red pine stands of 10-50 years age and found that a linear increase in SOM at the top 15 cm soils with stand age.

We estimated the highest organic matter content in birch soils compared to other tree species and species mixture. This might be because of two reasons: firstly, due to the quantity and quality of litterfall affecting the formation and stability of SOM (Paul et al, 2002). The litterfall on the birch plot was significantly higher than that of beech planting in our experimental sites. As a significant portion of this litter returned to atmosphere through decomposition, only the recalcitrant parts such as lignin remain in soils for the longer period. This might be attributed to a relatively larger amount of ligninecious materials at the upper layers of birch soils than others. Birch leaf litter contains higher lignin than the alder and lower than beech (Rosemary, 2007). In case of beech, we assumed that the quantity of litter played the major role. Secondly, the contributions of root decomposition, secretion of exudates and mychorrhizal turn over might be attributed to the higher SOM in birch plot. Kwasna *et al.* (2008) characterized fungal community in the tree root systems and reported higher fungal species dominancy in the silver birch (*Betula pendula*) than European beech (*Fagus sylvatica*) using pure culture isolation methods. Moreover, birch roots are host to a mychorrhizal community (ectomycorrhizas) which might be

contributing to the organic matter accumulation in soils (Neg *et al.* 2008; Smith and Read, 1997). Our result suggests a significant difference in SOM between grassland and woody plant soils at the upper part of soil profile. Again this might be due to the quality and quantity of the litter input. Although grassland accumulate substantial amounts of organic matter from grass litter and fine roots, the amount of recalcitrant materials produced by woody plants is higher than in grassland species (Post and Kwon, 2000).

The idea of incorporating the nitrogen fixing species in tree plantation especially in mixed plantation is basically as a potential soil resource management technique. Our analysis detected no influence of woody plant species including N fixing alder on N level in either monoculture or in the mixed culture soils. A similar observation was reported by Hoosbeek *et al.* (2011) in the same location that total soil N was not affected by nitrogen fixing *Alnus glutinosa*. This is contrary to the many previous findings that nitrogen fixing species increase soil nitrogen level in both pure and mixed plantings (Kaye, 2000). There is a general concept of higher N content and mineralization in mixed species compared to monospecific forests, however, this might be applicable only to N-limiting sites (Russell *et al.* 2007). However, Paschke *et al.* (1989) evaluated the effects of black alder (*Alnus glutinosa*) and autumn olive (*Elaegnus umbellata*) inter plantings with black walnut (*Juglans nigra*) and found significantly higher nitrogen and mineralization rates of litter, but there was no increase in soil total C and N 18 years after planting.

The nitrogen content was significantly higher in the grassland than the tree planting soils in some soil layers, but especially at the deeper soil layers. In general, litter quality is particularly low in lignin: N favours rapid decomposition and thus the large annual flux of nitrogen in grassland compare to forest soils (Hart *et al.* 1993). Nitrogen in the deep soil layers in grassland might be attributed to the higher leaching loss of dissolved organic nitrogen (DON) from the surface layers. Dijkstra *et al.* (2007) reported 64% of total

nitrogen loss as DON from grassland soils with high species richness in the Long Term Ecological research sites in Minnesota, USA.

6.4.2 Organic C in soils of pure and mixed stands

The higher organic C concentration in the upper layers (0-20cm) of birch soil compared to other species in our experiment could be attributed to slower decomposition of SOM and/or higher C accumulation through litter fall (Kaye et al, 2000). This agrees with the results from Howlett *et al.* (2010) who examined the SOC in gleyic Umbrisols (sandy loam) and found slightly higher (non significant) C under 13 years birch stands compared to pasture soils in Galicia, Spain. In addition to aboveground litter flux, we assumed that the activities and turn over of fine roots might have some contribution to C content at top soil layers. Larger specific root area and length, and the higher fine root morphological adaption in birch than alder was reported by Kuznetsova *et al.* (2010) in a comparative study between these two species in Estonia. The ectomycorrhizae of birch fine roots can grow a huge biomass with rapid turnover rate and thus may contribute to SOC in birch plots.

SOC in the alder plot was slightly lower than that of birch which might be because of site specific soil character. Consistent findings of no species effect on soil organic carbon in the same location was reported by Hoosbeek *et al.* (2011). Using the same plant species, they further observed no extra N was gained due to increasing supply of CO_2 to N fixing *Alnus glutinosa* in their experiment. This is in agreement with previous study in which consistently lower SOC and N concentration under nitrogen fixing tree *Pentaclethra* was reported after 15 years of plantings in tropical Costa Rica (Russell *et al.* 2007). Contrasting results were reported by some investigators that the higher accumulation of soil C was due to nitrogen fixing species through inhibiting decomposition of soil humus

(Fogg *et al.*, 1988; Berg and Matzner, 1997) and /or increasing NPP (Nadelhoffer *et al.* 1999). However, Neff *et al.* (2002) concluded that although the evidence showed a significant change in soil C, the net effect of elevated N on SOC storage is uncertain because increased productivity due to extra N supply can be offset by increasing decomposition of the light fraction of SOM carbon. Similar results of increase litter decomposition and N availability due to incorporation of N fixing species was reported by others (Mafongoya and Nair 1997).

The interactions among trees in the mixed-culture plantings can be additive (no effects), positive non-additive (synergistic) or negative non-additive (antagonistic) depending on the plant functional traits (Kaye *et al.* 2000; Korner, 2005). Our analysis showed no significant difference between actual and predicted SOC content in mixed species stands (Figure 6.3). This indicates additive effects of species in mixture, which suggests there is no beneficial effect of admixture regarding SOC content in soils. This finding may also be due to the nutrient rich soil of our experiment site (Hoosbeek *et al.* 2011). It has been suggested that site conditions such as nutrient level, can also influence the species-identity effect to such a magnitude that mixture effects could not be detected (Schulze *et al.* 2000).

6.4.3 SOC in grassland

No significant difference in SOC concentration was observed between grassland and woody plant soils. We postulated that root derived C accumulated at the upper layers of grassland soils resulted in a C concentration similar to that of woody plants. Grass species generally have a shallow root depth compared to tree species, and thus allocates the majority of the root biomass at the upmost soil layers (Haile *et al.* 2010). In deep soils layers of the grassland, C is presumably not of grass origin. In silvopastural sites, stable

isotope analysis showed that most of the SOC in deeper soil profiles originated from tree components (Haile, 2010). In our experiment, C in the deep grassland soil might be derived from previous land use of oak forest at the same location (Avery, 1980). A similar assumption was made by Silver *et al.* (2010) when working with annual grassland soils in California that C at 1m depth might be due to the presence of residual soil C from the historical presence of woody plants in the region. It is also possible that C was translocated down in the soil profile by earthworm or water as dissolve organic matter (Shuster *et al.* 2001; Mariani et al, 2007).

6.4.4 C:N ratio and Clay content

Our results showed that C:N ratio of soil organic matter decreased with increasing soil depth and the variations over depth were more pronounced than the influences of species which suggests accumulation of organic matter at similar decomposition level at the surface layers. Lal *et al.* (1995) suggested that a low C:N ratio in the deeper soil layers might be due to decomposition stage and age of humus. Batjes (1996) reported the lowest average C:N ratio of 7:1 at 100 cm depth in the xerosols and the highest average of 24:5.1 in podzols. Sakin *et al.* (2010) reported C :N ratio between 4.34 : 1 and 6.04 : 1 from 0-100cm depth of 16 soil series of entisols, vertisols, aridisols in Turkey. Although our results are consistent with these findings, C :N ratio generally reflects the location specific nature of organic compounds present in SOM. However the general trend is the highest C:N ratio in fresh organic matter, as decomposition proceeds easily degradable material disappears and N is immobilized by microbial community leaving behind more recalcitrant material of lower C:N ratio (Post *et al.*, 1985). The C: N of top soils in our study site was between 8.38 -10.08 indicating well decomposed and stabilized organic matter compared to fresh litter. The organic matter in subsoil layers is older and more

humified than that in topsoil layers, and thus a decreasing soil C: N ratio with soil depth is frequently observed (Callesen *et al.* 2007).

The positive exponential relationship between clay content and per cent SOC might be due to the formation of organo-mineral association in soils. The results are consistent with the findings of other investigators Gami *et al.* (2009) who reported exponential relationships between clay content and soil organic carbon in forest soils of Indo-Gangetic Plains. The vertical distribution of clay content in soil was similar in our planting site (Table 6.2). Decreasing soil clay content with increasing soil depth was observed in 4 representing soil profiles that were examined for soil particle size analysis. Strong positive correlation between clay content and SOC indicated formation of organo-mineral association in soils. The clay particles adsorved decomposed organic (especially humus) molecules is because of the large surface area of negatively charged clay particle and the electrical charges on the humic macromolecules which resulting an extremely stable irreversible bonding (Lukac and Godbold, 2011). This mechanism has great environmental significance by protecting soil organic matter physically and hence potential for long term C sequestration.

6.4.5 Soil C stock and species control

One of the challenging issues in estimating soil C storage may be that the observed differences are often very small relative to its large pool size (Rothe *et al.* 2002).

We estimated C stocks down the soil profile to 100 cm as most of the studies investigate standing stocks of SOM up to this depth (Carter *et al.* 1997). Various approaches regarding the sampling depth are followed according to the objectives of the studies. For an initial inventory or investigating responses of specific spatial variability, 0-100 cm should be preferred as a typical sampling depth. However, sequential monitoring the surface layer (0-

15 cm) is preferred as it is susceptible to most of the anthropogenic and environmental changes (Emmett *et al.* 2008). IPCC (2007) guidelines referred to 0-30 cm sampling depth for inventory and projections of greenhouse gas emission. Our study reveals that about 60-77% of storage C is confined within 0-30cm soil depth. Subhrajit *et al.* (2010) estimated 58 % of the storage C in the upper 50 cm of forest soils in India. This means that a substantial quantity of C exists at the deeper soil layers. Although top soil C is crucial due to pool size and functioning, C in deep soil is more important for its long term sequestration. Thus, for evaluating the C storage potential, extension of sampling depth up to 1m provides more realistic information.

No significance species difference in SOC storage was observed except between birch and beech soils at the surface layers (0-20 cm). This result was partially supported by Vesterdal *et al.* (2002) who reported no species variations in C content in three mineral soil layers (0-25 cm) in an experiment with oak and Norway spruce after 29 years of plantation in Denmark. This might be because species differences are more pronounced at the forest floor than mineral soils as no forest floor has been recognized at our experimental sites due to stand age (Dijkstra and Fitzhugh, 2003; Vesterdal *et al.* 2002). In our study, differences between SOC storage in birch and beech was clearly due to very much slower growth rate at the early stage of beech, which is reflected in the biomass production and stand size (Table 3.1).

Now it is interesting to examine the origin of storage C at the top two layers. Hagen-Thorn *et al.* (2004) hypothesized that if the variations in soil chemistry between 0-10 and 20-30cm among the species studied were mostly due to root turnover and exudates secretion, then root activities and other geochemical processes would be relatively similar in these two layers. As a result in a similar chemical composition, particularly in an abandoned arable land with forest plantation, where top 30cm plough layer is

homogeneous due to long cultivation and site preparation. In our studies, variations in SOC and consequently C storage among different species soils were more pronounced in the upper two layers (0-10 and 10-20 cm); however, SOC in the 0-10 cm layer was significantly higher than that of 20-30 cm layer, indicating differences were not due only to root activities. Therefore we assumed that both litter chemistry and root activities of the different species might be responsible for variation in SOC in the surface soils under different vegetation types.

We found a slightly higher C pool in grassland soil than tree planted soils. C storage in grassland differs with forest plantations in different ways. One of the most important mechanisms is rhizodeposition (Wood *et al.* 1991), the secretion of root exudates which are directly incorporated with soil particles and thus favoured to form a mineral coating for physical protection and long stabilization (Balesdent & Balabane 1996). Most of the organic matter in grassland soils is derived from grass root systems, and chemically the roots are high in lignin and polyphenolic compounds, which are recalcitrant to degradation and consequently store in soils for long time (Soussana *et al.* 2004). However, the woody plant species obviously produce higher quantity of litter input than grassland but still less effective in C storage might be due to favourable condition for decomposition on the forest floor (Brejda, 1997).

6.4.6 SOC storage in mixed species plantings

Our results showed that the response of mixed species stands on SOC storage was clearly additive, which means C stock in mixture was predictable from single stands plots. Consistent findings of no significant differences in mixed plantation of Chinese fir (*Cunninghamia lanceolata* (Lamb.) and broadleaf *Alnus cremastogyne* compared to monoculture was reported by Wang *et al.* (2009) in an afforested land after 15 years of planting in China. However, the contrasting results of higher SOC storage in mixed stands soil compared to single, especially when a nitrogen fixing species is included in the mixture, were observed by many investigators (Resh *et al.* 2002; Rothe and Binkley, 2001; Kaye *et al.* 2000). Roth and Binkley (2001) further commented that due to diverse interactions reported by many authors and lack of directly addressed research, the general conclusions were limited.

No effects of nitrogen fixing species (*Alnus glutinosa*) on SOC accumulation in mixed plots was observed in our experiments. The causes might be as follows: Firstly, the nitrogen level in our study site might be influenced the SOC storage in mixed species treatment. It has been suggested that increase in SOC due to species admixture was generally found to be pronounced only at oligotrophic sites (Rothe and Binkley, 2001), and may be because of high demand of nitrogen in the nutrient poor soils. Although our plantation site was former agricultural land still the soil was nutrient rich (Hoosbeek *et al*, 2011)). Therefore, the effects of nitrogen fixing tree and hence effect of mixed planting was not clear in our studies. It is clear from the above discussions that nitrogen fixing species may play the major role in C storage process in the mixed plantation system as some investigators suggested that plantations without nitrogen fixing species may or may not accumulate mineral soil C during afforestation (Richter *et al.* 1999, Post and Kwon 2000). Secondly, no clear effect of mixtures on C accumulation might be partially

attributed to the young stand age (Wang *et al.* 2009). Thirdly, the presence of overtopping species with opposing growth rate may suppress other species and consequently affects the benefit of mixture (Yanai, 1992). Thus the species with opposing growth rate might have some negative impacts on C storage potential in mixtures.

6.4.7 Carbon storage in deep soils

Vertical distribution of SOC storage is controlled by many biogeochemical and climatic factors, yet at identical soil and climatic conditions, vegetation and soil texture are likely to be more active factors (Jobbagy and Jackson 2000). We assumed that plant induced processes such as litter input and root activities might be the dominant factors in regulating the vertical C distribution in our study as particle size distributions are more or less similar under different plantation blocks. The vertical distribution of SOC stocks below 30 cm was similar for all tree species types. One of the major sources of deep soil C stock is the root system including the production of root necromass, release of exudates and turn over of the mycorrhizal hyphae network (Russell *et al.* 2007). Although these processes deal with a large quantity of carbon, their contribution to SOC storage is uncertain except the contribution of root necromass. However, C release from root exudates and mycorrhizal turnover may be potential route to soil C storage through the formation of soil micro aggregates or clay coatings (Lukac and Godbold, 2011). Another important process leading to the distribution of C storage at deep soils is bioturbation by earthworms some of which can reach to 1-2 m depth (Rumpel and Kogel-Knabner, 2010) and transport of fresh SOM into the burrows and mixed with mineral soils. Presence of earthworms has been noticed at our experiment sites but its population size or activity was not assessed. Hoosbeek *et al.* (2011) also confirmed the bioturbation process at the same sites.

We observed a strong positive exponential relationship between soil depth and cumulative soil C storage under different tree species. Lal *et al.* (2009) proposed a negative exponential relationship between C concentration and soil depth as soil C concentration decreased with increasing soil depth. Our analysis showed a relationship between soil depth and cumulative C stock which was increased with increasing soil depth. The strong relationship ($r^2 = 0.99$ for all species types) suggests the similar pattern of C stock over depth with the same inherent soil controls (Silver *et al.* 2010).

6.4.8 Organic C pool size

We estimated mean 7.26 kg C m^{-2} in top 1 m of tree planting blocks and 8.03 kg C m^{-2} in grassland areas of our study site. When the rock volume is excluded, the stock is 8.29 kg C m^{-2} and 9.16 kg C m^{-2} for plantation plots and grassland respectively. At the surface soil (0-10 cm) total C stock was 2.5 kg C m^{-2} which was more or less similar to a recent estimation of 2.8 kg C m^{-2} in the same plantation site (Hoosbeek, 2011). As the site was established on former agricultural land and very young stand age, we considered the study site as arable land category to compare with the previous studies and surveys. Our estimated value is smaller than the previous reports of 11 kg m^{-2} (Bradley *et al.* 2005) and 9.3 kgm^{-2} (Milne *et al.*, 2001) C density between 0-100 cm in arable land of Wales. However, more recently Emmett *et al.* (2007) estimated 5.29 kg m^{-2} C stocks in the arable land of Wales at 0-15 cm depth which is higher than our estimation of 4.26 kg C m^{-2} at 0-20 cm depth. Our estimates tended to be lower may be because of two reasons. Firstly due to spatial characteristics such as stony soils with shallow bedrocks at the site and the pool size is smaller than the average estimation for Wales. Secondly, due to change in land use, as abandoned agricultural lands are generally depleted in C specially in the surface layers

due to previous intensive agriculture and site preparation during plantation which need at least 30 years to replenish the depletion by new planting (Vesterdal *et al.* 2002).

6.5 Conclusion

Estimation of organic carbon reserve in forest soil is indispensible for quantification of size, distribution and changes in national carbon stocks in relation to UN climate change conventions. Although time consuming, field investigations is the most reliable and frequently used approach of SOC inventories. We estimated C stocks in the soil of broadleaved tree stands and grassland. Our results indicated some variations in upper soil layers under different plant stands, however, considering the SOC storage at top metre, no obvious species effects was observed. We estimated slightly lower C storage (7.3 kg C m^{-2}) compared to other studies which might be due to local soil conditions and changes in land use.

7. Labile and recalcitrant C pools in soil organic matter from single and mixed stands of 3 broadleaved trees.

7.1 Introduction

Soil is the largest reservoir of terrestrial carbon and acts as both sink and source of atmospheric carbon, which has been considered as main green house gas responsible for global climate change (Cheng and Kimble 2001). Atmospheric C once sequestrated into plant tissues through photosynthesis, is transferd to the soil as plant litter. Part of the carbon stored in soils is release to the atmosphere through heterotrophic respiration (Subke *et al.* 2009), and part is sequestrated as soil organic matter (SOM) for as long as a million years (Cheng *et al.* 2007). SOM ranges from fresh plant and microbial tissues to completely decomposed humic substances that vary not only in decay level but also in chemical composition from simple carbohydrates to complex lignin, resulting in a heterogeneous mixture of organic substances (Baldock and Skjemstad 2000). Thus as a consequences of C mineralization, organic compounds in the soil are in different stages of degradation, resulting in different C turnover time from days to years and even thousands of years (Davidson and Janssens 2006).

Soil organic matter can be divided into different pools on the basis of biodegradability depending on the chemical composition (Rovira and Vallejo 2007). Fractionation of soil organic C into different pools provides useful information in identifying structure, function and biodegradability of organic substances, especially in the identification of labile pools which are very active and sensitive to environmental change (Khanna *et al.* 2001). In models of SOM dynamics, soil organic matter is divided into

operational defined fractions composed of a range of substances. In the two- pool concept, soil organic matter is fractionated into rapidly decomposable *labile* pools and relatively stable *recalcitrant* pools (Kendra *et al.* 2004). The labile fraction can further divided into two labile pools derived from a two-step hydrolysis approach (Rovira and Vallejo, 2000). Various physical (Gregorich and Janzen 1996; Cambardella and Elliott 1992), chemical (Paul *et al.* 2001; Weil *et al.* 2003) and biological (Alvarez and Alvarez, 2000) techniques have been used to separate different organic fractions in soil, and the objectives of the study determine which approach should be deployed. Among different techniques, acid hydrolysis has been used widely for isolating labile and recalcitrant fractions of soil organic matter (Cheng *et al.* 2007).

Stability of organic carbon in soil mainly depends on the decomposition rate and turn over time of organic substances (Baldock and Skjemstad 2000). As the components of soil organic matter are complex and heterogeneous in terms of origin, chemical structure and bioavailability (Sollins *et al.* 1996), the period of time these components exist in soil varies widely and eventually affects the long term storage of C in soil (Pare *et al.* 2011). Therefore, simply estimating soil carbon content is not sufficient to study soil carbon storage in relation to eco-system carbon balance. It is necessary to characterize the quality of soil organic matter in relation to biodegradability.

Studies on C fractionation in relation to tree species and especially species mixture are seldom available. Many of the previous studies focused on methodological techniques, and biochemical characterization of fractions and its dynamics (Kendra *et al.* 2004; Rovira and Vallejo 2007; Zou *et al.* 2005). The present study focuses on C contents and fractionation of soils after afforestation using different native broadleaf species both single and in a mixture. This has been compared to a grassland soil in the same location to investigate the fractionation pattern between two contrasting land covers.

7.2 Materials and Methods

7.2.1 Analysis of soil physico-chemical properties

Soil: Soils from under single species planting blocks of birch (*Betula pendula*), alder (*Alnus glutinosa*) & beech (*Fagus sylvatica*) and mixed blocks of these three species as well as an adjacent grassland were used in this investigation. Each species and mixture had three replicated blocks; soil samples were collected from 12 plantation blocks (3 X 4 species types) at four different depths (viz. 0-10, 10-20, 40-50 and 100 cm) on July-Sempember, 2008 as mentioned in section 6.2.3. For comparison, soil samples from three grassland plots adjacent to the plantation blocks were sampled at the same time. Soils were air dried and passed through a 2 mm sieve and stored at 4° C temperature before analysis.

Total C and N in the soil: Total C and total N in the soil samples were measured using a CN analyser (TruSpec® CN). Gravimetric moisture content of the soil was determined before analysis by oven drying at 105 ^{O}C for 24 h.

Soil OM content and bulk density: Organic matter content in soils was determined by loss on ignition (LOI) methods using oven dry sample (105 ^{O}C) to ignite in muffle furnace at 450 ^{O}C over night and per cent organic matter was determined gravimetrically. Bulk density was determined by a core sampling method using 100 cm^3 steel cores and the soil volume was corrected for stones inside the core.

7.2.2 Soil microbial biomass

Microbial biomass was estimated following the fumigation-extraction method. Fresh soil samples were collected on July 2010, from the top soil layer (0-10 cm) of the birch, alder and beech plots and the analysis was done within 72 h. Air dried and sieved samples were (5 g dry weight equivalent) were extracted with 25 ml of 0.5 M K_2SO_4 in 50 ml centrifuge tubes. After shaking 1 hour at 200 rpm the extract was centrifuged for 5 mins at 4000 rpm, filtered through a Whatman no. 42 filter paper and preserved in 20 ml plastic scintillation vials. A second set of samples were used for fumigation with ethanol free chloroform in a vacuum desiccator. Ethanol was removed by washing 100 ml chloroform with 100 ml of 5 % H_2SO_4 using a separating funnel and finally the chloroform was washed three times with 100 ml deionised water (Witt *et al.* 2000). A 100ml glass beaker with approximately 40 ml of ethanol free chloroform and anti bumping granules was placed in the middle of glass scintillation vials containing soil samples in the desiccator. A vacuum pump was used to allow the chloroform to boil and the desiccator was placed in a dark cupboard for 24 hours. After fumigation the soil extraction with K_2SO_4 was collected as above. The C and N in both fumigated and non fumigated samples were extracted using Shimadzu TOC/TN analyzer.

7.2.3 Acid hydrolysis

Fresh mature leaves and fine roots (<2 mm diameter) were collected from birch, alder and beech and from a mixture of the grasses. The roots were washed and dried in the oven at 80 ^{O}C for 24 h with leaves and finally ground (fineness ~10μm) using mixer ball mill (Retsch Mixer Mill MM 200). Total C and N contents in the fresh plant materials were analyzed using CN analyser as mentioned earlier.

A two step acid hydrolysis method described by Hoosbeek *et al.* (2006) and Rovira & Vallejo (2002) was followed for complete release of labile and unhydrolyzed refractory C pools from soil samples. Approximately 500 mg (5 SEM) of air dry soil was transferred in a Pyrex glass tube and 15ml of 2.5 M H_2SO_4 added and thoroughly mixed. For each soil sample 3 replicates sub samples were taken for analysis. The mixture in the closed tube was warmed at 100 ^{O}C for 30 min in a digestion block. After cooling the hydrolysed solutions were transferred to centrifuge tubes and were centrifuged at 3500 rpm for 3 min and the clear solution decanted .The residue was washed twice with 15 ml of deionised water and the washings added to the hydrolysate and kept in glass bottle at 4 ^{O}C until analysis for C and N using TOC/TN analyser (Shimadzu TOC). This fraction constituted the labile pool I (LPI).

The unhydrolysed residues were transferred to Pyrex tubes, dried at 60 ^{O}C in the oven. After cooling, 2 ml of 13 M H_2SO_4 was added, agitated for 1 min by a vortex mixer and kept overnight at room temperature under continuous shaking. Thereafter, deionised water was added to dilute the acid to 1M and the residues were hydrolysed for 3 h at 100 ^{O}C with occasional shaking. The clear hydrolysate was decanted after centrifugation at 3500 rpm for 3 min. The residues were washed twice with distilled water and washings were added to the hydrolysate and kept at 4 ^{O}C, and analysed for C & N described above. This C fraction constituted the labile pool II (L-II). After washing twice with distilled water the residue was transferred to a ceramic crucible and dried at 60°C. Carbon in this fraction was taken as recalcitrant pool and calculated as the difference between the total C content in soil and the labile pools (LPI & LPII summed together) (Belay-Tedla et al 2009).

Hydrolysis of plant materials: Acid hydrolysis of fresh plant materials performed following the same protocol used for soils except that the sample size was 25 mg. The C: acid ratio was the same as that used in the soil hydrolysis. After each hydrolysis, residues were washed twice with distilled water and we used filter paper to separate extracts from un-hydrolyzed residues as plant residues do not settle down during washing. Both soil and plant samples were analysed in triplicate.

Estimation of labile and recalcitrant C pools in soil profiles: The absolute quantity of labile and recalcitrant C in different soil layers were estimated as area basis using bulk density data and the pool size in soil profile (0-100 cm) was calculated by summing amounts in individual layer intervals.

7.2.4 Statistical Analysis

The three replicates measurements were plotted to give a mean value. The homogeneity of variances was assessed by Levene's test and the homogeneity of variances was assumed. Total labile and recalcitrant C was compared across four depths and four species types separately using One-way ANOVA. To perform multiple comparisons (pairwise) among values of different depths of each species and among different species of each depth, Tukey HSD was used. All differences were considered significant when $p<0.05$ and all statistical analyses were done using SPSS 14.0

7.3 Results

7.3.1 Soil organic matter (SOM), organic C (SOC) and C: N

The per cent organic matter in different tree species and grassland soils followed the order: birch > alder> grass > tree mixture> beech, which was maintained in all soil layers except 10-20 and 100 cm where species mixture soils had higher organic matter than grass land (Table7.1). The soil organic matter content decreased with increasing sampling depths and at the top layers of different plantation soils the proportions were ranged 7.3-5.3% and dropped to less than half at 100 cm depth. The significant effects of plant species on SOM content were observed in the top two layers of soil profile ($P = 0.024$ and 0.038 in 0-10 and 10-20 cm layers respectively).

The mean content of soil organic carbon and nitrogen decreased significantly downward in the soil profiles from 0-100 cm irrespective of species types in the plots (Figure 7.1). The birch and alder soils tended to have the higher organic carbon (3.26-2.36%) than the grass and tree mixture soils (3.0-2.0%) in the upper two soil layers. In contrast, in the deeper segments (40-100 cm), soils of grass land and species mixture had higher C content (0.9-0.5%) than birch and alder soils (0.8-0.4%). Beech soils remained the lowest in C contents among all species types and grassland across the soil profile (2.0-0.4%). Interspecies variations in soil C content, however, were not statistically significant in a particular layer except between beech soil and birch and alder soils at 10-20 cm depth (P =0.023 and 0.036 respectively). The mean total C stocks varied across the species and mixture.

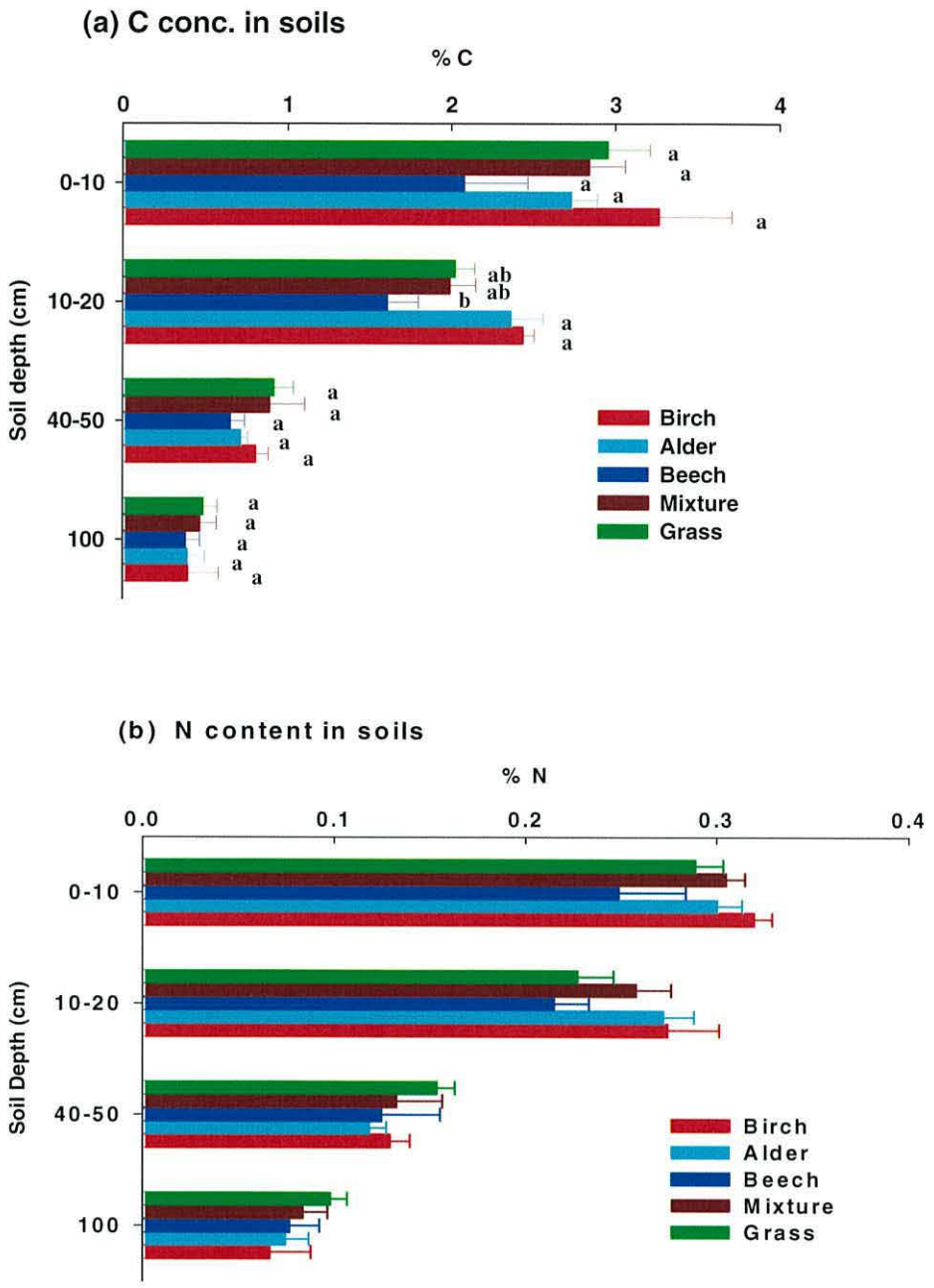
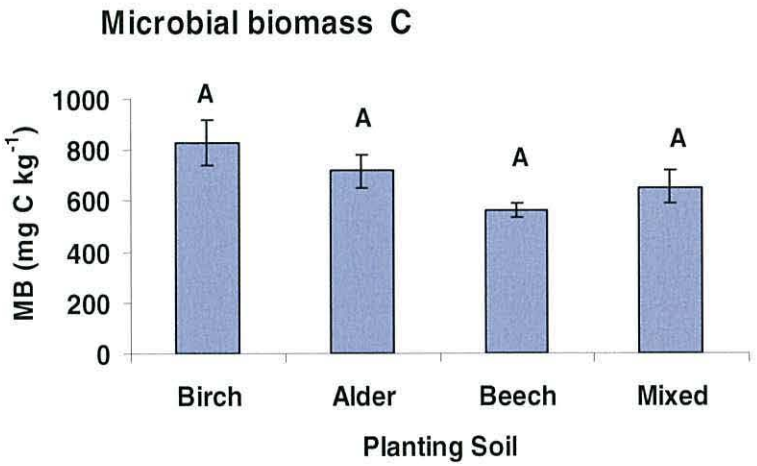


Figure 7.1 Vertical distributions of (a) soil organic C and (b) N concentrations under mono and mixculture stands of birch, alder and beech, and grassland. Bar equals means, SEM (n = 3). Bars with similar indices are not statistically significant ($P < 0.05$).

The C: N ratio of soil decreased with increasing soil depths under all plant species types and the highest C: N ratios were found in top soils (0-10 cm) of birch and grass plots followed by tree mixture, alder and beech (Table 7.1). In 10-20 cm layer , plant species were followed the similar order in C:N ratio values, except alder soil was higher than the mixture. In deeper soil layers (40-100 cm) the lowest C:N ratio was estimated in grass and beech soils whilst soils from plant mixture and birch plots showed the higher values than others.

7.3.2 Soil microbial biomass C and N

Small variations in soil microbial biomass C and N were observed in the surface soils of different plant species. In summer 2010 microbial biomass C in soils of birch, alder and beech plots ranged from 827 mg C kg^{-1} in birch to 562 mg C kg^{-1} in beech (Figure 7.2). Similar pattern of magnitude in microbial biomass N was observed over different plant species ranging between 127- 104 mg N kg^{-1}. However, in both cases the differences were non significant. The C: N ratio in the microbial biomass was between 6.1-6.8. On average, soil microbial C accounted for 2.3 % of the total and 5.3 % of the labile C of the surface soil (0-10 cm).



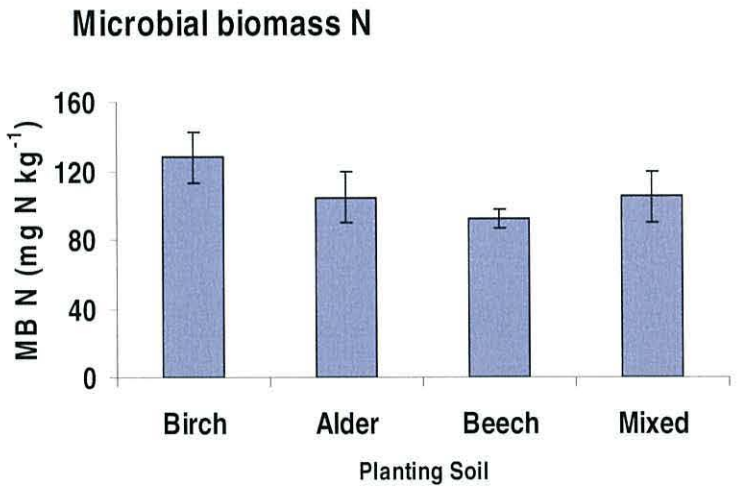


Figure7. 2 Microbial biomass (MB) C and N in soils of different tree species stands determined by fumigation-extraction method. Bars equal mean, SEM (n = 4). Bars with similar indices are not statistically significant ($P < 0.05$).

Table 7.1 Soil organic carbon (SOC) storage, organic matter (SOM) and C: N ratio of tree planting and grassland soils. Value equals mean, SEM in parentheses (n = 3). Values with similar indices are not statistically significant ($P < 0.05$).

Planting block	SOC storage (0-100 cm)	Sampling depth (cm)							
		0-10		10-20		40-50		100	
	kg m^{-2}	% SOM	C: N	% SOM	C: N	% SOM	C: N	% SOM	C: N
Birch	9.0 a (0.9)	7.3 (0.1)	10.2	6.4 (0.3)	8.9	3.9 (0.5)	6.2	3 (0.7)	5.8
Alder	8.7 a (0.8)	6.6 (0.3)	9.1	6.4 (0.4)	8.7	3.9 (0.4)	6	2.9 (0.5)	5.3
Beech	6.9 a (0.8)	5.3 (0.6)	8.3	4.8 (0.6)	7.5	2.8 (0.1)	5.2	2.2 (0.4)	4.9
Mixture	8.5 a (0.7)	6.2 (0.2)	9.3	5.3 (0.3)	7.7	3.6 (0.3)	6.7	2.8 (0.3)	5.5
Grass	10.2 a (0.9)	6.4 (0.2)	10.2	5.2 (0.2)	8.9	3.7 (0.4)	6	2.4 (0.2)	4.9

7.3.3 Proportion of labile (I & II) and recalcitrant C in soils:

Total labile C exceeded the recalcitrant fraction in all 3 single species soils of birch, alder, beech and grass soil except at 0-10 cm soil depth in birch and 100 cm soil depth in beech where the recalcitrant fraction of C was higher than the labile fraction (Table 7.2). In mixed species plots, higher labile C was determined in soils from 10-20 and 40-50 cm depths. In single and mixed species soils, the ratio of labile and recalcitrant C was the highest in the 40-50 cm layer than all other soil depths. However, in grassland soils, all layers except 0-10 cm layer had high labile to recalcitrant ratio.

In the upper two layers of soil (0-10 and 10-20 cm), the highest fraction of labile-I carbon was found in beech plots (36.3 and 38.7 %) and which were statistically significantly higher than in alder soils (P =0.012 and 0.002) (Figure 7.3). Birch and mixed species soils contained an intermediate level of labile-I C, which were not significantly different to alder or beech in 0-10 cm soil layer. However, at 10-20 cm depth, labile C in soils under both birch and the mixed species were significantly higher than alder (36.56%, p=0.006 and 33.28%, p=0.035 for birch and mixed respectively).

At the middle layers (40-50cm) the highest percentage of labile-I soil C was estimated in birch plots (43.1%) followed by beech, alder and mixed species plots, however, these variations were not statistically significant. A similar trend has been observed in the deepest layer (100 cm) of all 4 types of tree cover. In grassland soils, percentage of labile-I C found in upper two layers were similar to forest soils and not statistically significantly different but in the deeper two layers the values were higher

under grass (47.6 and 51.56% for 40-50cm and 100 cm respectively), and at 40-50 cm depth was significantly higher than that of the mixed species plots.

Between the 4 single and mixed species plots, alder soils had the highest percentage of labile-II carbon ranging from 37.7-29.0 %, which were significantly higher than the other 3 plantation soils in all layers except 100 cm. In birch, beech and mixed blocks, labile-II soil C ranged in between 11.4-24.4 % and were not statistically significant from each other. Grassland soils contained a significantly higher percentage of labile-II C than birch, beech and mixed species plot at all soil depths. In contrast, no significant difference was shown for the percentage of labile-II C in grassland soils compared to soils under alder at any soil depth.

7.3.4 Vertical distribution pattern of C fractions

Species composition did not significantly affect the relative concentrations of recalcitrant C in top 2 layers of soils, although they comprise the major portion of total C (55.8 %) in 0-10cm layer of birch and mixed plots, and was 49 % of total C in the 10-20 cm layer of the mixed plot (Figure 7.3). Alder and beech soils contained more or less similar proportions of recalcitrant C in these 2 layers ranging from 47.0-42.6 %. Further down the soil profile, significantly higher amounts of recalcitrant C was found in mixed blocks than in birch ($P = 0.014$) and alder ($P = 0.002$) soils at 40-50 cm; and in the 100 cm layer it was higher than under alder ($P = 0.013$). In both layers alder had the lowest proportion of recalcitrant C (27.3 and 33.9 % respectively). In grassland soils, percentage of recalcitrant C was significantly lower than in tree planting soils, except the top layer.

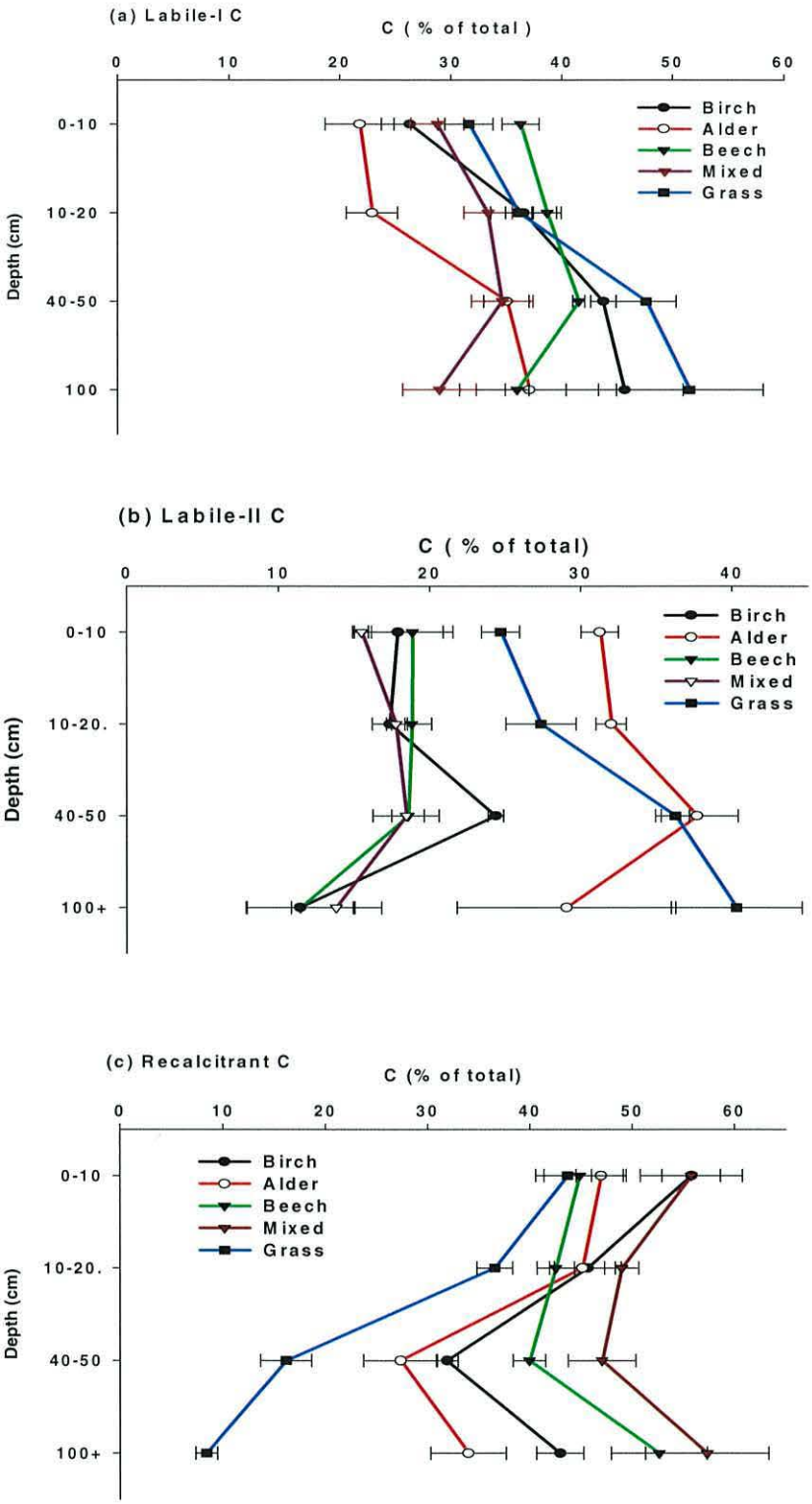


Figure 7. 3 Vertical distributions of labile (I & II) and recalcitrant C in the soils from tree species plots and grassland. Values shown are expresses as percentage of total C. Symbol points equal mean, SEM (n = 3).

Table 7.2 Relative amount of total labile (I & II) and recalcitrant C in the soils of single and mixed tree planting of birch (*Betula pendula*), alder (*Alnus glutinosa*), beech (*Fagus sylvatica*) and grassland. (Means, (SE), values with different letters indicate significant variations, a, b, c for comparison among species and xy for across depths).

		Plantation blocks									
		Birch	*P* <0.05	Alder	*P* <0.05	Beech	*P* <0.05	Mixed	*P* <0.05	Grass	*P* <0.05
Depth(cm)	C-fractions	% Soil C									
0-10	Total Labile	44.2		53.0		55.1		44.0		56.3	
	Recalcitrant	55.8		47.0		44.8		56.0		43.7	
		(5.0)	a x	(2.7)	a x	(4.3)	a x	(2.8)	a x	(2.3)	a x
10-20.	Total Labile	53.9		54.9		57.5		51.0		63.5	
	Recalcitrant	46.1		45.1		42.5		49.0		36.6	
		(3.7)	ab xy	(3.2)	ab x	(1.8)	ab x	(1.7)	a x	(1.7)	b x
40-50	Total Labile	68.1		72.7		60.1		53.0		83.9	
	Recalcitrant	31.9		27.3		39.9		47.0		16.1	
		(1.1)	ab y	(3.7)	abd y	(1.6)	bc x	(3.3)	c x	(2.5)	d y
100	Total Labile	57.1		66.0		47.4		42.7		91.8	
	Recalcitrant	42.9		33.9		52.6		57.3		8.1	
		(2.3)	ab xy	(3.7)	a xy	(4.7)	ab x	(6.0)	b x	(2.5)	c y

7.3.5 Carbon fractions in fresh plant materials

Extraction of tree and grass leaves and roots with the same chemical fractionation scheme showed that in both root and leaves materials of the tree, most of the C was in a residual form, i.e. poorly extractable (Figure 7.4). In leaves the fractions extracted by different strengths of acid hydrolysis were of similar levels. In contrast, in roots the labile-I fraction dominated the total labile C. In grass leaves and roots, the highest fraction was the labile-I and the amount of residual C was only 34.2 and 37.4 % of that found in the tree materials respectively.

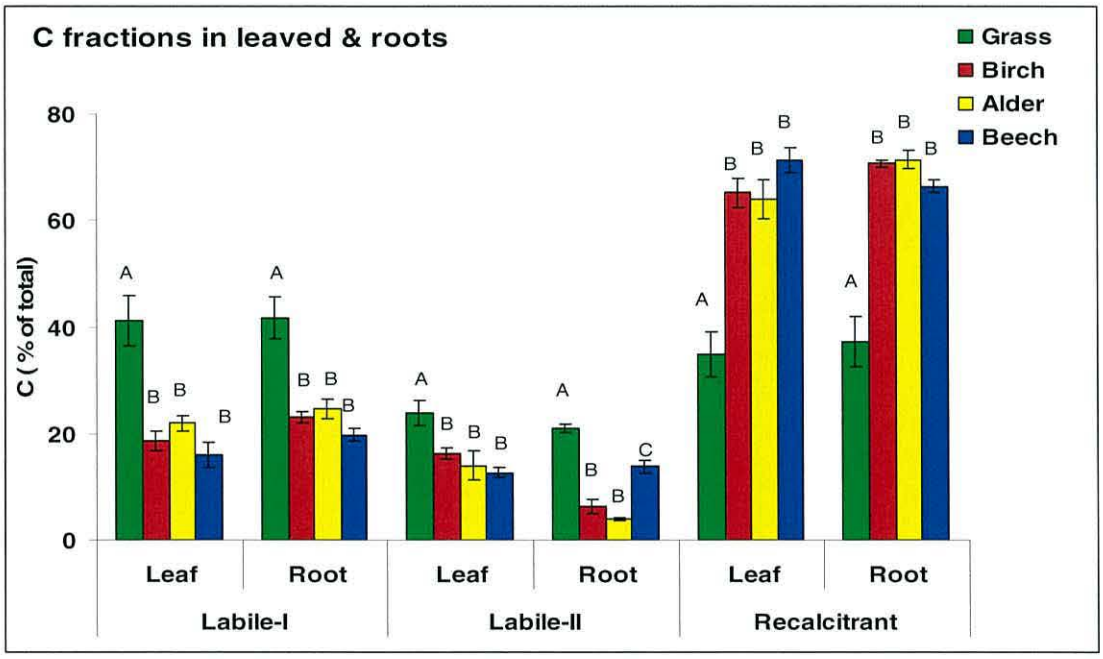


Figure 7.4 Labile and recalcitrant C in fresh leaves and roots of birch (*Betula pendula*), alder (*Alnus glutinosa*), beech (*Fagus sylvatica*) and mixed grass determined by acid hydrolysis method. Values shown are expressed as percentage of total C. Bar equal means, SEM (n = 4). Bars with same indices are not statistically significant ($P < 0.05$).

7.3.6 The absolute labile and recalcitrant C pools in soils

The vertical distribution of the absolute quantity of labile and recalcitrant C under the influence of tree species types is presented in Figure 7.5. A significant decrease in both labile and recalcitrant fractions of C was observed across the sampling depths. At 100 cm depth, the amount of labile C in grassland, birch, alder, beech and tree mixture soils decreased by 78, 92, 82, 85 and 85% to of that in the surface soil, respectively. Overall, the labile C pool size in the soil profile (0-100 cm) was grater in grassland soils > alder > birch > mixture > beech. One way ANOVA showed significant differences between species types in the labile C pool in soil profile (P = 0.009). Multiple comparisons using Tukey HSD revealed that the labile pool in beech and mixed species soils were significantly different to that of grassland soils. A similar vertical distribution pattern was observed in case of recalcitrant C across soil depth, but the influence of species type at a particular depth was not statistically significant. In the upper layers (0-40 cm) the quantity of recalcitrant C was higher in soil under birch > mixture > alder >grassland > beech. The recalcitrant C pool size in the soil profile (0-100 cm) was the highest in soil under the tree mixture (4.4 kg m-2) which was 51% of total C stock (Figure 7.6). The proportion of recalcitrant C pool in other soils was birch (45 %), alder (39 %) and beech (44 %). In contrast, grassland soil was lowest in recalcitrant C (29 %) compare to the other vegetation cover. However, the variations in recalcitrant C were not statistically significant (P= 0.986, 0.452, 0.192 and 0.144 when compared between mixed stand soil and that of birch, alder, beech and grass respectively).

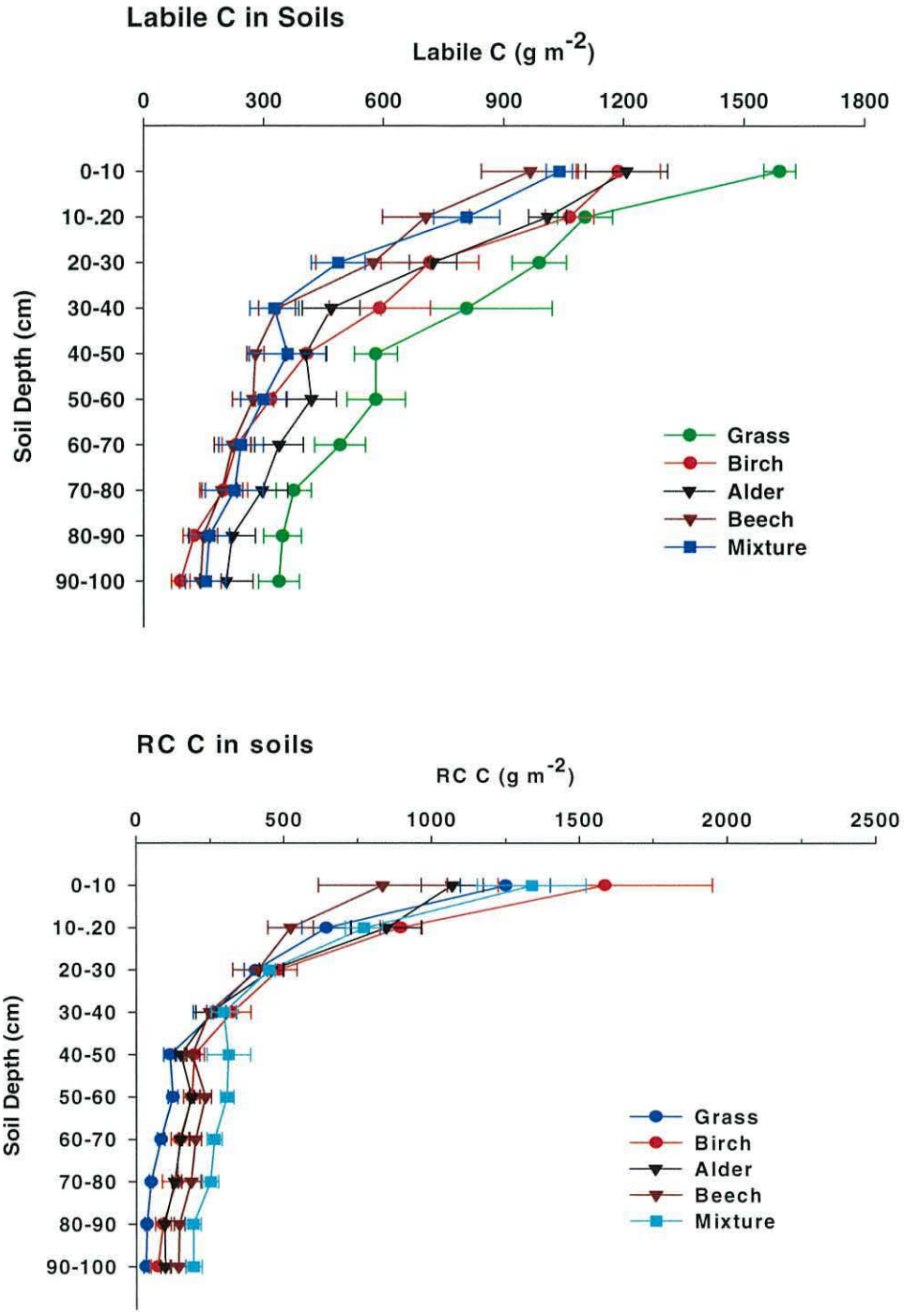


Figure 7.5 Absolute quantity of labile (I+II) and recalcitrant (RC) C in different layers of soil profile under different tree species stands and grassland. Symbol point equals mean, SEM (n = 3).

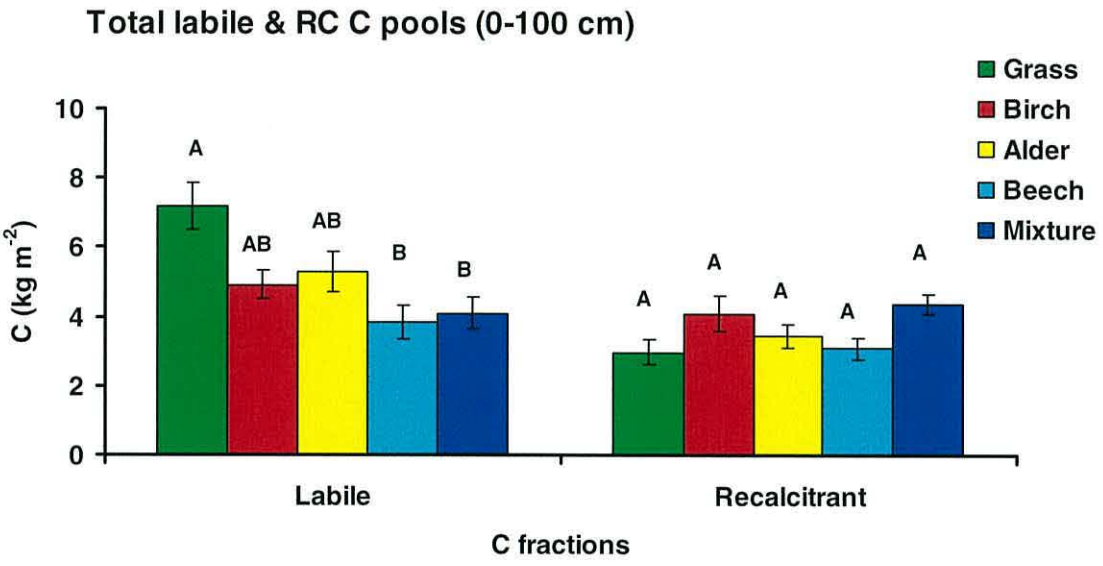


Figure 7.6 Total labile (I +II) and recalcitrant (RC) C pool in 1m soil profile from different tree species and grassland soils. Bar equal means, SEM (n = 3). Bars with same indices are not statistically significant ($P < 0.05$).

7.4 Discussion

7.4.1 Soil organic C stock under forest planting

The quantity and distribution of soil organic C are influenced by tree species through integrated effects of species-specific traits such as litter production, root turnover and secretion of exudates (Melillo *et al.* 1989; Stump and Binkley 1993). However the storage is primarily regulated by litter decomposition rate. Higher organic C concentration and storage in birch soil might be because of litter quality especially higher lignin content. C: N ratio of soil organic matter also indicates slow decomposition process in birch soil. Generally soil organic matter with a high C: N ratio decomposes more slowly than that of low C: N ratio. As faster growing N fixing species, alder produce high quality litter which favours the decay process and consequently less C storage than birch and mix species soils. As a late succession tree species beech growth and litter production was least among three plant species which might be attributed to the lowest C content in beech soils compare to birch and alder.

The C concentration in the grassland soil was slightly higher than tree planting soils. Declining soil organic carbon due to plantation of woody tree species has been reported by some investigators as woody plant differ with grassland in terms of rooting depth, nematode distribution , depth of soil nutrient uptake etc. (Jackson *et al.* 2002). Disturbance due to planting activities may be another cause of slightly low C content in planting soil compare to grassland (Turner and Lambert 2000).

Seasonal variations, forest types, age of the stand and pedo-climatic conditions are generally considered as the major factors affecting the microbial biomass in forest soils (Wardle, 1998). Litter quality and availability of substrate were reported as the cause of variation in soil microbial biomass under different plant species (Leckie *et al.* 2004). In the

present study, no significant difference in soil microbial biomass C and N was observed might be due to the very young age of trees (6 year). Similar results of no major variation in soil microbial biomass in the upper soil layer under 23-24 year Norway spruce, oak and beech stands was reported by Lejon *et al.* (2005) and postulated that the trees were too young to induce differences in soil microbial biomass.

7. 4.2 Carbon fractions in SOM of different plant species soils

Soil organic carbon fractionation by acid hydrolysis is mainly the separation of hydrolysable compounds from acid resistant materials and the fractionation process is regulated by chemical quality of the organic matter in soils (Rovia and Vallejo 2002). In our study we analyzed soils organic carbon originated from young plantations of birch, alder, beech and mixed of the 3, between tree species and especially between the tree species and grass soils. There were some significant differences in the amount and distribution of the different fractions. Labile-I C in alder soils, which mainly comprises non cellulosic polysaccharides and soluble sugars (Schnitzer and Preston, 1983), was significantly lower than in beech soils. This difference may reflect the faster decomposition of alder leaf litter in comparison to the slower decomposition of beech (Rovira and Ballejo, 2002; Lecerf and Chauvet, 2008). As soon as the decomposition process starts, the mineralized labile C in soil is leached down the soil profile as dissolved organic carbon (Currie and Aber,1997) and/or utilized by microbes and eventually release to atmosphere, and as a consequence labile C in soils decline. As most of the radio carbon studies have revealed that acid hydrolysable labile C is consistently younger than other residues (Leavitt *et al.* 1997; Paul *et al.* 1997), a high amount of labile-I carbon in beech soils is an indication of more fresh organic matter than in the alder soils. The labile-II fraction is considered to be comprised of cellulose polymers (Rovira and Vallejo, 2007).

The breakdown of these polymers feed into the labile-I fraction resulting the release of mono- and oligosaccharides to the non cellulosic pool (labile-I) (Rovira and Vallejo, 2007). In birch and beech as well as mixed species plots the labile-I fraction always exceeds the labile-II. However in alder soil labile-II exceeds labile-I except at 100 cm soil depth. This suggests that in the alder soils either transfer from labile-II (cellulose polymers) to labile-I (non cellulose polysaccharides and sugars) is blocked or that labile-I pool is more rapidly respired. Fluctuations in proportions of labile-I and labile-II in different plantation soils might be due to transfer of compound between these two pools (Rovira and Vallejo, 2002). The products of cellulose degradation are glucose and soluble cell-oligosaccharides and thus provide readily available C which is mediated by a complex of microbially derived enzymes cellulases (Sinsabaugh *et al.* 1981).

Relative proportion of recalcitrant C in upper soil layers was not influenced by tree species in our experiment. The consistent findings at the same location and plant species were reported by Hoosbeek *et al.* (2011) that no species effect on C stabilization process and C protection by micro-aggregate, when assessing by physical fractionation of POM (particulate organic matter) and micro-aggregate analysis techniques. Although recalcitrant C in lower layers of mixed plantation soils significantly differ with alder and birch but not with beech indicating intermediate position of mixed soils regarding labile and recalcitrant C pools which means no considerable effect of species mixture on variation of C fractions in these soils.

7.4.3 Distribution of labile C in soil profiles

Although no significant difference in labile-I and labile-II C has been found in 3 layers from 0-40 cm soils of different planting blocks except birch, there was a slight increase in labile-I C with increasing depth. Conversely, there was a decrease in

recalcitrant C fraction over depth down to middle of profiles with a significant decrease in the case of birch and alder. These results are consistent with the findings of some investigators working with beech *(Fagus sylvatica* L.) in forest soils (Joergensen and Mayer, 1990) who found that hydrolysable (using hot water and 12M H_2SO_4 extracts) sugar monomers increased with soil depth in comparison to litter layer, the non-hydrolysable carbon fraction decreased considerably in the C horizon (32-46 cm) than litter layer. Similar results of decreasing non hydrolysable organic C fraction with depth in 3 layers (0-5, 5-10 and 10-20 cm) of forest soil profile using acid hydrolysis and water stable aggregate fractionation techniques was reported by Tan *et al.* (2004). However contrasting results of decreasing labile C with increasing soil depth and non hydrolysable C increased with increasing depth have been reported by Cheng *et al.* (2007) in 60 cm deep soil profile in the sorghum (*Sorghum bicolour* (L) FACE experiment and control plots at the University of Arizona Maricopa Agricultural Farm. Hoosbeek *et al.* (2006) found that lower concentration of acid insoluble stable C at the subsurface soil layer (10-20 cm) than the surface soil of forest plantation of a former agricultural soil in Italy. Translocation of labile and recalcitrant C in soil profile might be one of the causes of decreasing C recalcitrance in this case because of easy downward movement of hydrolysable labile C than recalcitrant (Goh *et al.* 1984).

In forest humus layers lignin is considerably degraded during decomposition as a result of phenolic oxidation products. The recalcitrance of soil organic matter is largely due to the presence of lignin. Rumpel *et al.* (2002) estimated phenolic oxidation products (vanillyl-, syringyl-, and *p*-coumaryl- units) as the components of decomposed lignin in soil samples collected from soil profile in spruce forest (Dystric Cambisols) in Germany and found larger amounts of these products in mineral soils (A horizon and sub-soils) than the litter layers.A similar finding of increased oxidation of lignin in the deep soil layers of

temperate forest has been described by other researchers (Kogel 1986; Kogel-Knabner *et al.* 1991). In addition, oxidation of lignin contributes to release of hydrolysable phenolic compounds and thus can increase labile-C in the lower soil profile simultaneously (Rovira and Vallejo 2007).

7.4.4 Carbon fractions in deep soil layers

The changing pattern of total labile and recalcitrant C throughout the soil profiles remained consistent across all four plantation types down to a depth of 50 cm. Interestingly, at 100 cm depth the opposite trend in labile and recalcitrant C was observed, compared to the upper three soil layers, irrespective of species composition in the plots. Although changes between last two soil layers were not statistically significant, the pattern of change was consistent for all species type soils. Cheng *et al.* (2007) reported unexpected changes in labile and recalcitrant C at a depth of 80-100 cm in agricultural soils and postulated the contributions of deeper roots and leaching of soluble organic matter as the underlying causes. Substantial increase in decomposition of soil organic matter due to root-induced increase of the decomposition process (known as rhizosphere priming) has also been well documented (Dijkstra and Cheng, 2007; Cheng *et al.* 2003).
We assumed that the tree roots may have direct effects on increments of recalcitrant C in the deepest layers in the present study. The recalcitrant C from the lower layers of beech blocks were significantly higher than that found in alder soils might be due to functioning of root systems. Berger *et al.* (2002) reported that bulk of the beech roots in the upper 40 cm and more than 90 % of the beech root was found above 60 cm and occurred predominantly in deeper horizon compared to spruce. Curt and Prevosto (2003) observed higher concentration of birch roots in the uppermost soil horizon (0-15 cm) and in admixture with beech, birch is predominant in the top soil whilst beech colonizes in the

deeper soil layers. We assumed that higher recalcitrant C in the deep layers of beech and mix plots might be attributed to root turn-over.

7.4.5 Carbon fractions in grassland soils

Grassland soils differed strongly to the tree plots in distributions of labile and recalcitrant C through out the soil profiles. The grassland soils showed a uniform decrease in recalcitrant C with depth, which may be due to the high quality of grass litter especially lower lignin content than tree litter, and thus more easily decomposable substrates (Deschaseaux and Ponge, 2001). This is supported by the analyses of the grass leaf and root materials, which showed much higher extractable C compared to tree materials (Fig 7.4). It has been estimated that 70-75% of root biomass in grassland soils situated in the upper 15 cm of the soil profile (Gleixner *et al.* 2005) with few roots below 50 cm. The higher contribution of water soluble organic carbon as shown in the higher proportions of soil labile C may be due to a higher dissolved organic matter flux through the grass soil than forest soil due to low bulk densities at the upper layers of grassland soils. Translocation of dissolved organic carbon (DOC), originating from older soil organic carbon in the grassland soils down the profile was observed by some authors (Steinbeiss *et al.* 2007) with few roots below 40 cm depth.

7.4.6 The absolute pool size of C fractions in soil

Contribution of labile soil organic C plays an enormous role in the immediate nutrient release and ecosystem functioning, however, in long term C storage the recalcitrant pool plays the dominant role. Recalcitrant pools in the present study ranged between 30-51 % of total SOC stocks which was similar to findings of Falloon and Smith (2000) who reported that recalcitrant SOC fraction may constitute 15-50% of total SOC

pool. A slightly higher recalcitrant C pool was found in mixed species soils followed by birch and beech this might partially be due to higher lignin content in added birch and beech litter, as lignin in plant tissue has been considered as a potentially recalcitrant substance (de Leeuw and Largeau 1993; Kolattukudy 2001; de Leeuw *et al.* 2006). The role of lignin in soil recalcitrant pool might also be indirect. As some investigators suggested that the turnover time of 93 % lignin is < 1 year and only 7 % is 17 years old (Glaser 2005; Rasse *et al.* 2006). However, the lignin-derived aromatics are recognized as structural framework of humic substances, and thus played major role in stabilization of organic matter in soil (Stevenson, 1994).

The recalcitrance of SOM is attributed by the alkyl C chain of macromolecules of lipids, aromatics and phenolics compounds (Lorenz *et al.* 2007). Plant roots have high proportions of alkyl C and thus the plants with high root/shoot ratio can play important role in accumulation of recalcitrant C in soil (Lorenz and Lal 2005). Substantial amounts of non-hydrolysable substances from fresh roots of woody plants indicate the potential contribution of roots in the recalcitrant C pool in the present experiment site. In addition to inherent recalcitrance nature of root tissues, the role of root induced mechanisms of SOC stabilization was documented by some authors (Rasse *et al.* 2005; Lorenz *et al.* 2007).

Although the total C stock was higher in grassland soil than tree planting, the analytical results of the present study suggest that less than one third of the total C in grassland soil was recalcitrant. The acid hydrolysis of fresh plant tissues also revealed that grass root and leaf materials contain lower amounts of non-hydrolysable materials than tree materials which was supported by findings of Lorenz *et al.* (2007) that woody species tend to have higher proportion of alkyl C compared to agricultural crops. Although recalcitrant C pool in soil is also dynamic, considering its' longer residence time in soil, recalcitrant C can be used as an index of soil C storage potential under different land covers.

7.5 Conclusion

Fractionation for soil organic carbon into readily decomposable labile and relatively long lived recalcitrant pools can be used as an indicator of capability of soil to store C for long time periods. Our results indicated that 30-51 % of stored SOC under different tree stand and grassland was recalcitrant. Mixed tree stands showed higher potentiality of storing recalcitrant C, and this might be due to quantity of litter inputs from above and belowground. A higher quantity of recalcitrant C was observed in the soil organic matter from the tree stands than from grassland, suggesting potentiality of tree planting in long term C storage, over grassland.

7. General Discussion and Conclusion

8.1 General Discussion and Hypotheses

Forest ecosystem C dynamics is the part of global C cycles which involves many temporal pools and biogeochemical processes (Beedlow *et al.* 2004). In the present research project some major pools such as above and belowground biomass, soil organic matter and C, labile and recalcitrant C pools in SOM; and some processes such as litterfall and decomposition were investigated in relation to long term C storage in soils under three deciduous broadleaved tree species and their mixture (Figure 8.1). Two major factors influencing these systems considered in the present study were tree species traits and the interactive effects of mixture.

Many investigators observed distinctive soil environment and biotic communities under different tree species due to species traits (De Deyn *et al.* 2008; Ayres *et al.* 2009). Therefore species traits may have some control over the biogeochemical processes such as decomposition, respiration and storage of C. The mixture effects may be additive, synergistic or antagonistic depending on the individual species traits and their interactions. In the following sections, a general discussion is made on the major findings and on some critical issues of different experiments, and we relate the four hypotheses on mixture effects with the findings of four major experiments.

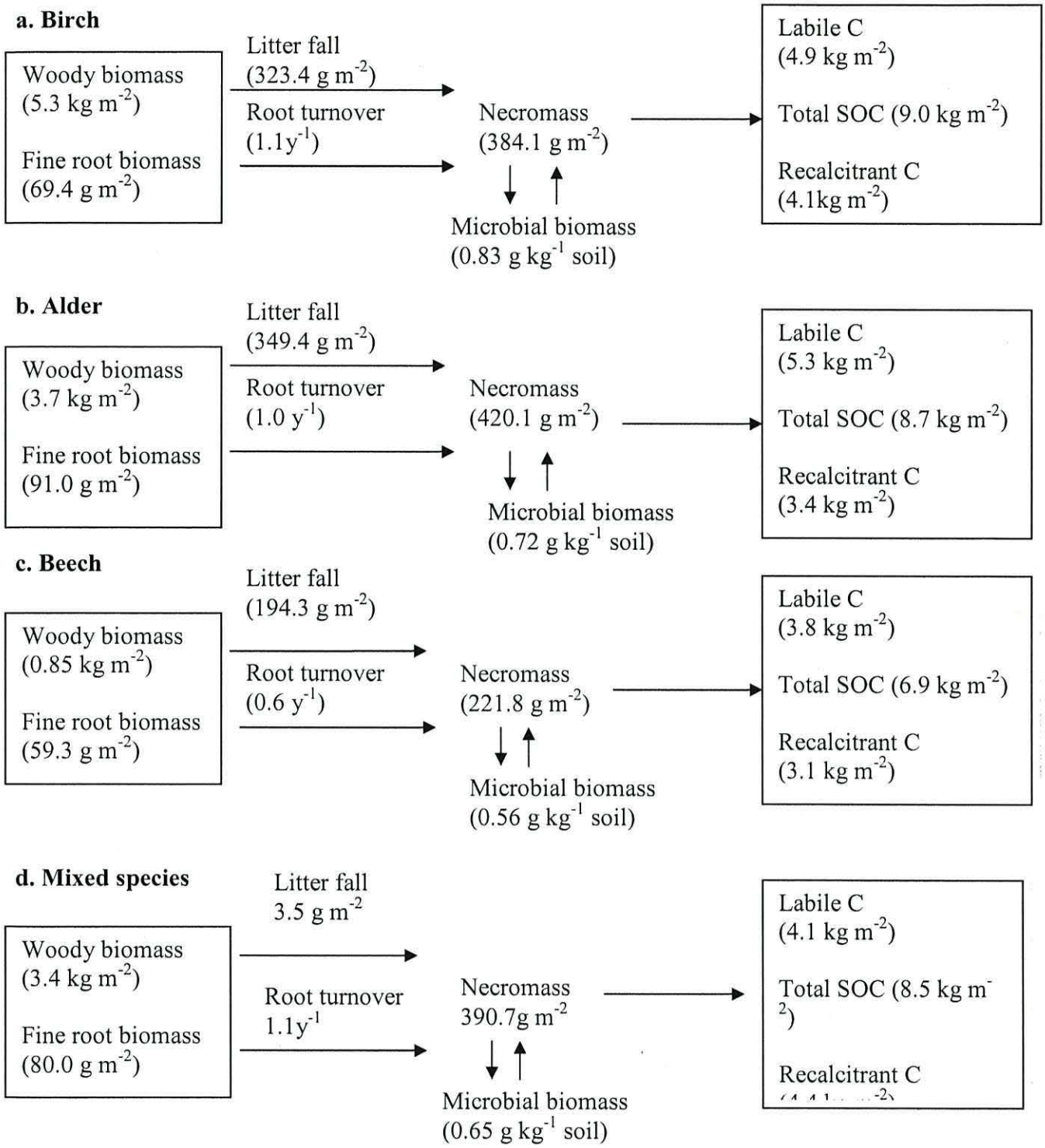


Figure 8.1 Ecosystem C pools under different plant species and mixture. Necromass pool was estimated by adding litterfall and root litter (fine root biomass × root turnover).

8.1.1 Above and belowground biomass and C dynamics

C dynamics in the forest ecosystem begins with the processes of carbohydrates synthesis and subsequent production of plant biomass. Analysis of aboveground woody biomass and the estimation of annual litterfall revealed that birch accumulated the highest woody biomass, but alder had the highest leaf litter fall. From a carbon sequestration point of view, woody biomass and foliar biomass make a difference in their C storage potentials. Although, leaf allometry was not used in this study to estimate the foliar biomass of different stands, the annual collection of leaf litterfall provided an estimation of foliar biomass (Adams 2008). The species-specific allometric equations, developed in the present studies fitted well when basal diameter was used for birch and beech, and DBH was used for alder. The equations predicted accurately the aboveground woody biomass, and can be used for assessment of woody biomass in similar climatic conditions. One interesting finding was that the higher woody biomass in birch than in alder, although the average height and DBH was higher in alder. The higher woody biomass in birch was attributed to higher proportion of branches in birch (25.5% of total dry wt. in birch and 17.5 % in alder). Therefore birch allocates higher portion of C in woody biomass but alder contributes a greater C to the forest floor as leaf litter. As a late successional tree, beech biomass was lowest but after 6 year of growth its yearly production rate was the highest among the three species. Alder had higher fine root biomass and fine root production, however in terms of fine root turnover rate birch was slightly higher than alder. The proportions of above and belowground biomass can be explained by the resource allocation strategy of the tree species (Gower, 1994; Helmisaari, 1995). The life span of fine roots depends on the efficiency of nutrient uptake, as long as soil nutrients are available fine roots are supplied with carbohydrates from aboveground part to keep them alive (Lukac and Godbold, 2011).

Roots die due to lack of available nutrients and plant allocates resources to elsewhere to develop new roots (Lukac and Godbold, 2011).

Fine root (< 2mm diameter) turnover rate indicated that, in terms of below ground litter input, the contributions of birch was the highest followed by the mixed stand, alder and beech. This might be the most significant finding in relation to soil C storage. In forest ecosystems, fine root production and turnover represent a considerable proportion annual net primary productivity which transfers to soil organic matter pool through root decomposition and rhizodeposition (Matamala *et al.* 2003; Norby *et al.* 2004). In a decomposition experiment of fresh leaf and root litter from Norway spruce (*Picea abies*), Hansson *et al.* (2010) found that roots decompose more slowly than needles due to litter quality, especially higher lignin content in spruce root (35-37 %) compare with needles (15 %), suggesting significant contribution of root derived C to soil organic carbon (OC) storage. A higher contribution of root C to the soil C pool than leaves was reported by many investigators (Ostertag *et al.* 2008; Johnson *et al.* 2007; Kalyn and Van Rees 2006). The results of our fractionation experiment indicated similar order of magnitude in the recalcitrant C pool in soil as fine root turnover, suggesting the possibilities of dominant role of fine roots in soil C stock than leaves. Root decomposition was not included in the present study, however, the very intense decomposition of leaf litter of birch, alder and beech in our experiment, suggest rapid mineralization of leaf derived C in our experiment site.

Close interaction of the surface of fine roots to the surface of soil minerals can enhance the stability of root derived exudates, which are generally readily decomposable, by formation of organo-clay complexes (Balesdent and Balabane 1996). The process may be particularly important in forest ecosystems where the absence of tillage or other mechanical operations prevents the admixture of aboveground litter with the mineral soil.

Therefore physical protection of soluble organic matter by soil mineral may be more likely in case of root exudates.

Hypothesis-1

Mixed species stand of birch, alder and beech has higher aboveground and fine root standing biomass, fine root biomass production compared to monoculture stands under similar pedo-climatic conditions

The results on effect of mixture on above and belowground standing biomass revealed that the production of biomass in mixture was additive, which means no facilitation or decrease in biomass in mixed culture plantings compared to the monocultures. However, at the tree level a statistically non-significant synergistic trend in alder and antagonistic trend in birch were observed. In the mixture, the growth of faster growing nitrogen-fixing species alder was higher than monoculture. In contrast, the growth of lower canopy species birch was higher in monoculture than in mixture, which indicates competitive relationship between nitrogen-fixing species and the companion species. Significantly lower biomass production of beech in mixture than monoculture suggests that beech was suppressed in mixture. Therefore, over all no positive effect of the mixture on growth or biomass production was observed.

The results suggest a similar mixture effect on standing fine root biomass with no clear mixture effects in compare to the monoculture. However, both standing biomass production of fine roots and fine root turnover rates showed increasing trends in mixture. Root interactions in mixtures can be competitive or facultative but are more complex to assess than aboveground biomass. The facultative interactions may happen between N fixing and the companion species through the release of N-rich compounds (Da Silva *et al.*

2009). The results of both above and belowground biomass do not support our first hypothesis.

8.1.2 Leaf litter decomposition experiments

The results of mass loss and subsequent decay rates were varied among the species, possibly due to variability in leaf traits particularly litter quality. The results of the laboratory incubation experiment showed very high dissolved inorganic nitrogen (DIN) from alder leaf litters indicating rapid decomposition and subsequent nitrification process in alder litter. On the other hand, birch leaf litter released the highest quantity of water soluble phenolics suggesting possibilities of slow decay rates in birch leaf litter. The highest decay rate was observed in alder leaf litter followed by birch and beech. The higher quality of alder leaf litter caused faster decay rate, whilst the higher quantity of lignin and phenolics in birch and beech caused the decay rate to be slower. The biphasic decomposition pattern in three species in our experiment may also due to chemical quality of the leaf litter. The C:N ratio decreased with increasing time, indicating formation of more stable substances as decay process proceeded. Plant functional traits are responsible for interspecific differences in organic matter dynamics (Violle *et al.* 2007; Westoby & Wright, 2006). Although interspecific variation in different traits is well established, some investigators reported intraspecific variability in leaf traits and decomposition rates among co-occurring plants (Lecerf and Chauvet, 2008).

The consequences of litter decomposition are the immediate release of easy decomposable C to the atmosphere and retention of relatively recalcitrant compounds in the soil, which has a potential for long time C storage. The alder leaf litter had the highest decomposition rate, therefore less contribution to soil organic carbon pool from foliar litter.

Similar to alder, substantial quantity of mass loss from birch and beech within 6 months indicates a small contribution of the three plant species to long term C storage in soils.

Role of plant secondary metabolites in decomposition especially lignin and other polyphenolic compound is well documented in many previous studies. The control of litter quality over the decomposition pattern was observed in our leaf litter decomposition experiment. Although it is recognized that decomposition is regulated by lignin concentration of litter at the late phase, the length of phase varies from species to species (Kalbitz *et al.* 2006). For example, Berg and Staaf (1980) found a mass loss of 26 to 36% during the shift from early phase to late phase in Scots pine needles. Berg and McClaugherty (2008) found that decomposition of Norway spruce and oak leaf litter related to lignin content from an early stage in the decay process. However, in the present study we estimated 71-90 % mass loss in the initial stage of birch, alder and beech leaf litter decomposition. Generally, there is a positive relationship between initial decay rate and major nutrient elements such as N, P, S or water soluble organic substances. C: N ratio and lignin:N ratio are also recognized as a regulator of decay process at initial stage of decomposition. N concentration in the substrate is generally used as an index of degradability in relation to microbial demand. Aber *et al.* (1984) reported that about 26-38 % of total N was associated with lignin in leaf litter of 6 hardwood tree species, suggesting the possibilities of overestimating the available form of N during decomposition. Our results showed significantly slower decay rate in beech litter at the beginning, and the same trend with narrow variation at the late phase of the experiment compared with birch and alder, suggesting lignin as a rate regulating factor from the initial phase of decomposition process. A similar finding of the best negative relationship between lignin concentration and the initial decay rate was reported by Davey *et al.* (2007) in a decomposition study with oak leaf litter for 2.5 year in an identical climate to Wales, UK. Results of our

incubation experiment with leaf litter revealed that higher amounts of water soluble phenolics were released from birch litter. Experimental evidence support that polyphenol can influence the decomposition rate by inhibiting the activities of nitrifiers during decomposition (Rice and Pancholy1973; Plam and Sanchez, 1990). Therefore, the overall slowest decay rate in birch leaf litter might be due to combined effects of plant secondary metabolites on decomposition dynamics.

Hypothesis-2

In mixed stands of birch, alder and beech, decomposition rate of mixed leaf litter is faster compared to the decomposition rate of pure leaf litter in their respective stands.

Mixed leaf litter of birch, alder and beech showed negative interactions in decomposition, in terms of mass loss and decay rate. The effect was antagonistic; however after the initial stage of decomposition the decay rate was synergistic. Decomposition in mixed litter is suggested to be regulated mainly by differences in litter quality among the component species (Wardle *et al.* 1997). Generally it has been recognized that diversified litter quality and decomposer abundance enhance the decay process in mixtures (Kaneko and Salamanca, 1999). However, the presence of inhibitory compounds can slow the decay rate. In the present study, the decay processes in mixture was partially inhibited due to high lignin content in birch and beech litter and the phenolic compounds of birch leaf litter. Analysis of decay rates at different time intervals revealed that the mixture effect was not consistent over the entire decay period. Initially the mixture effect was antagonistic but after decomposing initial compounds the positive interactions in decay rate was observed compare to single species litter. The findings of mixed litter decomposition in both laboratory and field conditions indicate decrease of decay rate in leaf mixture and consequently do not support the second hypothesis.

8.1.3 Carbon storage in tree stands and grassland soils

Soils under ifferent plant species exhibited variations in concentrations of organic matter and organic carbon especially in the upper layers of the soil profile. The quality and quantity of litter inputs from both above and belowground, and the magnitude of subsequent decomposition were the major determinants for accumulations of OC in soils. The estimated soil organic C stock was 7.3 and 8.0 kg m^{-2} C in the top 1m soils of tree planted and grassland soils respectively, at our experiment site. The vertical distribution of soil OC revealed that considerable amount of carbon accumulated in the deeper soil layers. Although no clear indication about the vegetation control on deep layer soil was found in the present study, the role of clay content was obvious. However, the control of site specific pedological processes and the supply of fresh carbon on deep soil C have been proposed by some authors (Rumple and Kogel-Knabner, 2010; Fontaine *et al.* 2007). From C sequestration point of view, the deep soil C needs to be considered in the assessment of soil C stock (Hobely and Willgoose, 2010). Slightly lower C stocks in the tree plantation soil than the grassland of same location indicating slightly loss of C due to planting disturbance.

Hypothesis-3

Soil organic carbon storage in deciduous tree stands is higher than the adjacent grassland; and in mixed stand of birch, alder and beech C stock is higher than the single stand of component species.

Grassland soil has slightly higher soil organic C stocks compared to tree planting plots which might be due to loss of C during the land preparation or an initial transient phenomenon following land use change (Vesterdal *et al.* 2002; Carlyle 1993). The experimental results of present studies demonstrate the additive effect of species mixture on soil C storage. The C stock in mixed species plots can be predicted from storage of pure

stands of component species. Soil C storage is influenced by species diversity indirectly through many ecosystem processes some of which we addressed in the present study. Conceptually, soil C storage can be estimated from the balance between total C input and output in the system. In practice *in situ* measurement is the best approach. From the results of mixed species plots, it is clear that species mixture used in the current study, did not enhance or stimulate the production, transformation and decomposition of litter inputs in soil compare to single species plots, which was reflected in the soil C storage. Although increased fine root production and antagonistic mixture effect of decomposition process favoured the C input in mixed plot, it was not strong enough to influence the total C storage in 1 m soil profile. Thus the results of the present experiment rejected both part of the hypothesis that deciduous tree stand contains higher SOC storage than grassland and tree mixture causes higher SOC stock than monoculture of component species.

8.1.4 Carbon fractions in pure and mixed tree stands

Fractionation of C in soil organic matter into active labile and relatively less reactive recalcitrant pools is important for two ecosystem processes. Firstly, the instant supply of plant nutrients for primary producer, and secondly for long term C storage in soils. In the present study, the C fraction discussion was focussed to the second aspect. The distribution of labile and recalcitrant C varied across both plant species and the depth of soil profile. The results indicated that a higher recalcitrant C pool in soil under birch but the vertical distribution was mainly species-specific. The plantation soils differed in distribution pattern of labile and recalcitrant C compared to the grassland soil. The inconsistent finding in this experiment was the substantial quantity of labile C in the deep soil layers. Production and leaching of dissolved organic carbon (DOC) especially from root systems plays an important role in vertical distribution of labile and recalcitrant C in soil systems.

Uselman *et al.* (2007) found that both senesced fine roots and fine root exudation produced DOC (30 and ~60 % of total DOC respectively) which can be lost through leaching, however DOC from exudates are more labile than DOC produced by freshly senesced roots. Rumpel and Kogel-Knabner (2010) proposed that the preferential flow acts as transport pathways for young dissolved DOM into deep soil horizons. The loamy textured soil of the grassland in the present experiment site, we assume favours the transport of DOM into deeper soil horizons, as DOM leaching was reportedly strongly dependent on soil texture and homogeneity of plant cover (Chevallier *et al.* 2000).

Soil organic carbon stock and stability of storage C is of great environmental significant in connection to ecosystem C sequestration. We assumed that clay-organic C association played a vital role in over all stability of C in our experiment site. Carbohydrates are readily degradable by micro-organisms but can exist in soil systems for long period if they form an association on the clay surface or physically protected by clay coatings (Cheshire, 1992). The process is particularly important in rhizosphere where root tissues release soluble exudates at the root-soil interface. Farrar *et al.* (2003) reported the secretion of water soluble exudates such as sugars and various organic acids with negatively charged ion components (viz. acetate, oxalate, malate, citrate etc.). Although these compounds are highly labile in nature, Jones (1998) suggested that due to their negative charge, these compounds can rapidly adsorb on the surface of minerals through ion bonding. The large surface area of Fe and Al oxides and hydroxides of clays can act as an effective sorbants for soluble organic matter and thus inhibits their degradation particularly in subsoil horizons where the Fe and Al oxy-hydroxides are generally abundant (van Hees *et al.* 2003; Jones and Edwards 1998; Kaiser and Zech 1998). In our studies, the substantial amount of OC in the subsoil layers and exponential relationship between clay content and SOC might be attributed to clay-mineral association.

It is now well established that leaching of dissolved organic carbon (DOC) from forest litter plays an important role in transport and stabilization of C in soil especially in deciduous woodland (Jones *et al.* 2002; Kalbitz *et al.* 2000; Kuiters and Mulder 1993). DOC and other undecomposed organic fragments can contribute as binding material in formation of macro and micro aggregates and thus become protected from microbial attack. Golchin *et al.* (1994) reported that the availability of OC for microbial decomposition is limited when it is inside soil aggregates. Physically protected OC may not be recalcitrant in nature as some biologically labile C may be protected in the process mentioned above.

Hypothesis-4

Mixed species stands of birch, alder and beech has higher recalcitrant C pool in soil compare to mono species stands.

Relatively higher amount of recalcitrant C in mixed plots compare to monoculture indicates longer residence time of storage C in mixtures. The mixture effect on the formation of labile and recalcitrant C in soil is governed by mainly the quality of litter in mixed stands and subsequent decay rate. The high concentration of lignin in birch and beech litter can accumulate higher recalcitrant materials in mixed soil compare to monoculture. The antagonistic effect of mixed leaf litter on decomposition can have an influence on labile and recalcitrant C pools in soil. The higher root turnover rate in mixed stand than alder and beech enhanced the recalcitrant C pool in the soil. The results of C fractionation of mixed planting soil indicates that the mixed tree planting can serve as a potential tool for long term C storage in soil. The hypothesis that mixed species stands of birch, alder and beech contains higher recalcitrant C in soil was supported by our results following slow decomposition and high root turnover rates and hence we accept the hypothesis.

8.2 Overall conclusion

C storage and stability in forest soil are the integral part of ecosystem C dynamics and thus need an integrated approach to study the responses of any attribute. High above ground woody biomass, slow decomposition rate and relatively higher root turnover rate in birch favour the long term soil C storage among the three deciduous tree species. Although plant species is the single factor that regulates and initiates C functioning in the ecosystem, our results suggest no strong influence of species on soil C stock.

Species mixture can be a potential management option to achieve more benefits from ecosystem C dynamics. The results from mixed stands demonstrated the slow decomposition rate, high accumulation of recalcitrant C along with high root turnover rate suggesting the potential of plant mixture to enhance stability of SOC. The influence of plant species on long term C storage in soil is not straightforward, rather the collective interactions of different tree functions such as above and belowground biomass production, litter flux, litter decomposition, root turnover and formation of recalcitrant soil organic matter etc., act as a determinant for C storage. Some other findings of our experiment were that C storage was 6.2-8.0 kg C m^{-2} in top metre of soil of our experiment site, of which 30-51 % was recalcitrant C, disturbance due to establishment of the plantation was low, as SOC pool was similar in tree planting and grassland soils, and potentiality of root derived C was a major contribution to SOC.

The original contribution of this research to knowledge is that the responses of native tree species mixture on ecosystem C pools and processes such as, long term C fixation in woody biomass, biodegradation of leafy biomass, fine root production and turnover, and accumulation of recalcitrant C in soil etc., differ from that of single species stands. The integrated effects of these factors eventually made mixed planting of birch, alder and beech

as more advantageous regarding SOC stocks, compared to single species plantations. Selection of species for mixed plantation is crucial as individual species identity, their synergistic or antagonistic interactions or combination of both affect the biological flow of C in the woodland, which was obvious from the present studies. However, due to the young stage of tree plantation, the variations in interspecific responses to ecosystem processes may be small.

Comprehensive long term research is necessary especially to monitor the production and turnover rates of above and belowground biomass for a deeper mechanistic understanding of the ecosystem processes. In addition, the methodological shortcomings particularly to study the belowground activities need to be addressed for better understanding of these regulatory processes.

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