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Tree Species Effects on Soil C Dynamics in Temperate Forests

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Tree species effects on soil C dynamics in temperate forests

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Abstract

Terrestrial ecosystems account for two-fifths of the total exchange of CO_2 between the earth and the atmosphere, with forests contributing 80% of that exchange. Forest biomass and forest soils are particularly important carbon (C) sinks, however forest soil C stocks can vary widely, depending on the dominant tree species. Species-specific differences in the quality and quantity of plant litter inputs can influence soil C dynamics and storage because they control decomposition processes, altering soil respiration and soil properties. However, our knowledge of how tree species identity influences the interactions between decomposition processes, soil C dynamics and soil C storage is still deficient. Resolving this knowledge gap is important to determine how tree species selection for afforestation might help us increase soil C sequestration and mitigate the effects of climate change. Using microcosm experiments and *in situ* mesocosms, I studied interactions between litter quality and soil properties for different temperate tree species in the UK. I measured key litter properties, and quantified the effect of litter quality and quantity on soil CO_2 efflux and soil properties. My results show that litter quality, represented by nitrogen and lignin content, plays a major role in regulating soil C dynamics via litter decomposition. Litter quality also modified changes in soil CO_2 efflux in response to altered litter inputs but the effect varied strongly by species. Using reciprocal transplant experiments in single-species stands of alder, oak, and pine, I demonstrate variable influences of litter quality and the 'home-field advantage' on decomposition and soil CO_2 efflux. The present work provides an insight into the linkages between litter quality, decomposition and soil respiration in temperate forests. My results represent an important first step in identifying the future role of different tree species on soil C dynamics under climate change, which could inform forestry rotation and reforestation practices.

1. Introduction

A large proportion of Earth's land surface has changed as a result of human activity, causing alterations to the functioning of ecosystems on a global scale (Vitousek et al, 1997). Land-use change plays a key role in this global transformation, where the increase of land area for food production and forestry are important drivers of the exaggerated loss of biological diversity (Barnosky et al, 2011). Interactions among several global change phenomena, such as the increase in nitrogen deposition, climate changes, and species invasion and extinction have caused global concern and raised awareness for the importance of understanding what drives these phenomena and their environmental consequences (Bardgett et al, 2010).

Climate change is arguably the global phenomenon that has captured the most attention (Wardle, 2002). There are numerous lines of evidence for the effects of climate change on processes at different levels, from individuals to ecosystems, and with diverse geographical distribution (Cox et al., 2000; Jobbagy et al., 2000; Schlesinger et al., 2000). However, there are still gaps in our understanding of the effects of climate change for ecological processes at a global and regional scale, and little we know about how future climate scenarios will affect ecological interactions (Dixon et al., 1994). These poorly understood interactions reduce the accuracy in our attempts to mitigate climate change and its effect on the functioning of ecosystems.

Carbon dioxide (CO_2) has received much attention as an atmospheric gas with rapidly increasing concentrations (Figure 1), which are responsible for *c.* 60% of the observed global warming (Houghton et al, 1996; Grace 2004). Since the beginning of the industrial era, the concentrations of CO_2 in the atmosphere have increased from 277 ppm in 1750 to 395.31 ppm in 2013 (Le Quere, 2014; Figure 1.1). Consequently, each of the last three decades has been successively warmer than any preceding decade since 1850 (IPCC,

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2014). Therefore, it is important to understand the mechanisms regulating the increase of atmospheric CO_2 concentrations and its ecological effects, as it will help us to better describe climate change and predict future scenarios (Reichstein et al., 2013).

1.1. Forest and C dynamics

Terrestrial ecosystems play an important role in the global C cycle under climate change, which has given rise to a substantial body of research into terrestrial C dynamics (Schlesinger, 1997).

Forests play a particularly important role in the global C cycle, as they cover *c.* 40% of the total land surface area (Jobbagy & Jackson 2000), making them the largest terrestrial store of C (Malhi et al. 1999). Estimated C stocks in forests and forest soils is *c.* 2150 GtC, which is three times greater than the atmospheric C pool (IPCC, 2000). Moreover, studies estimate that forest standing biomass constitutes *c.* 82-86% of all aboveground C worldwide (Ritcher et all 1999), representing a substantial part of the global C budget. At the same time, forests are particularly sensitive to climate change because of their inability to rapidly adapt to environmental changes, which means that there is a high potential for alterations to forest functioning in response to the independent or combined effect of several climate change factors, such as the rise in temperature, droughts and floods (Lindner, 2014).

Forest ecosystems include producer and decomposer subsystems, which interact and depend upon each other, whereby the producer subsystem is the primary source of organic C to decomposers, and the decomposer subsystem breaks down, releases and cycles nutrients for producers (Wardle, 2002). These feedbacks can cause changes in biotic or abiotic soil properties, which affect the establishment, growth and/or reproduction of plant species (Wardle, 2004). Additionally, positive and/or negative feedbacks can promote coexistence, by diminishing fitness differences between species (negative

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feedbacks), or by generating multiple steady states and promoting coexistence via species partitioning in space and/or time (positive feedbacks; Barot, 2004; Pacala, 1997). Such interactions and feedbacks between subsystems and the species within them need to be taken into account to assess the potential effects of environmental change on ecosystem function, as they might lead to changes in C sequestration and emissions.

The majority of net primary productivity in forests enters the system via organic C inputs from plant aboveground biomass (producers) into the soil, providing substrate for microbial decomposers (McNaughton et al. 1989). Accordingly, plant litter is crucial for ecosystem function, as it is a key component within the processes controlling ecosystem productivity, gas fluxes and C sequestration (Swift et al. 1979; Wardle et al, 2004). Thus, the quality and quantity of litter inputs have the potential to affect the feedbacks between producer and decomposer subsystems and modify ecosystem carbon and nutrient dynamics (Prescott et al, 2013).

Research on forest C dynamics has become a useful tool to assess the role of forest vegetation and soils under climate change (Shibata et al, 2005), and forms a fundamental component of ecosystem ecology, particularly because of the close relationship between forest C dynamics and productivity (Cole and Rapp 1981). However, despite the global importance of forest C dynamics, our understanding of the interactions between aboveground and belowground subsystems remains deficient.

Approximately 80% to 90% of total plant production (e.g. root exudates, leaves, roots) enters the soil food web, returning C to the soil (Zhu et al., 1996). These organic materials, which are an important source of labile C, are broken down by the soil biota and eventually decomposed by soil microbes. During this process, a large proportion of the C is released back to the atmosphere as CO_2 as a consequence of microbial respiration (Bardgett and Wardle, 2010).
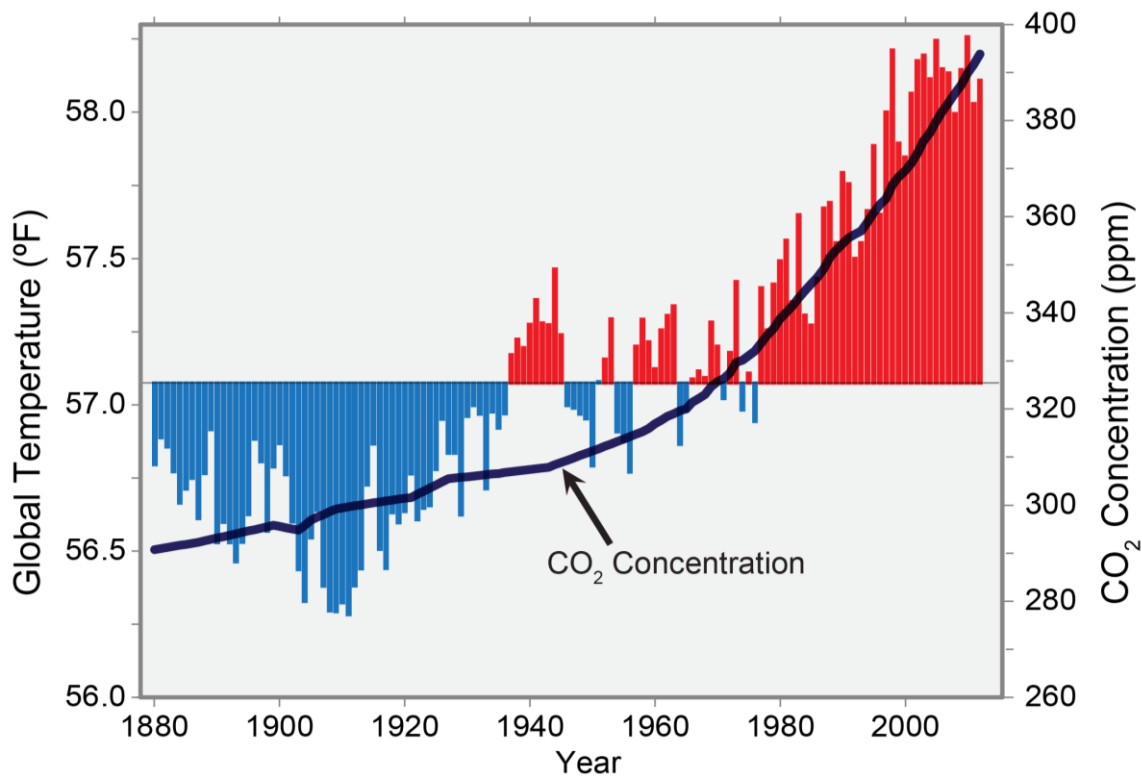
Figure 1.1. Long-term temperature averages and changes in global temperature and CO_2 concentrations over time. Red bars show temperatures above the long-term average, and blue bars indicate temperatures below the long-term average. The black line shows atmospheric carbon dioxide (CO_2) concentration in parts per million (ppm). Figure reproduced from Melillo et al. (2014).

It is therefore important to acknowledge that an important portion of the forest C cycle occurs belowground, and it will be altered by changes in the biotic and abiotic variables associated with it, such as tree species composition and changes in climate.

1.2. Tree species effects on C dynamics

Tree species presence and distribution can regulate the resources entering the forest soil, as these resources vary according to species-specific differences in the quality and quantity of leaf litter and root inputs (Wardle, 2002). In forests, variations in the chemical properties of leaf litter and differences in tree functional traits (e.g. deciduous, conifer, nitrogen fixing) can been important soil forming factors, shaping C cycling in forests

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(Hobbie, 1992; Mitchell et al., 2010). This is particularly important for countries in the European Union, because their forest landscape (which comprises 35% of the total area) contains up to 49% of the soil organic C stock (Smith et al 2002) and is likely to be the result of past forest management decisions (Vesterdal et al, 2013). In this context, the informed selection of tree species for managed afforested land is of great importance, and the variable influence of different tree species on C dynamics becomes a key factor for consideration. In particular, studies have demonstrated the crucial role of trees in shaping forest ecosystem by generating species-specific effects on soil properties and soil communities (Vesterdal et al, 2008; Lucas-Borja, 2012; Vesterdal et al, 2012; Vesterdal et al, 2013), affecting litter decomposition and soil respiration. However, the mechanisms behind these interactions and how they affect forest soil processes are still unclear (Prescott, 2013).

1.3. Litter quality and quantity

The quantity and quality of litter both play important but distinct roles in forest soil C dynamics (Westoby and Wright, 2006) but whereas litter quantity can be easily measured, the selection of leaf litter properties to determine "litter quality" varies among studies (Couteaux et al., 1995; Aerts, 1997, Prescott, 2010). Previous work has shown the importance of total C and nitrogen (N) content, the C:N ratio, lignin content, cellulose content and the ratio of lignin to nitrogen to represent litter quality, particularly because of their importance in litter decomposition and soil C dynamics (Schulps et al., 2008). Litter decomposition involves two simultaneous sets of processes: i) the mineralization and humification of lignin, cellulose and other compounds by the soil microbial community; and ii) the leaching into the soil of nutrients and C, which are subsequently progressively mineralized or immobilized (Wardle, 2002). Decomposition processes are also controlled by abiotic factors, such as climate, and by biotic factors such as soil macro and micro

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fauna (Couteaux, 1995), all of which affect belowground microbial activity and the consequent release of CO_2 by respiration (Scherer-Lorenzen, 2008). In addition, leaf litter properties can affect decomposition rates by promoting or inhibiting soil microbial activity as a consequence of levels of chemical compounds present in leaves (Sariyildiz and Anderson, 2003). Moreover, the content of nutrients and C in leaf litter also have a direct impact on microbial activity, as they are the main energy source for decomposers (Freschet et al., 2013). A number of studies show how the presence of different tree species can affect soil pH, soil C and N content, and other soil properties (Hobbie, 1992; McNamara, 2008), but the influence of species-specific litter properties on C dynamics under climate change is poorly characterised.

Variations in the quantity of litter inputs are an important driver of soil C dynamics (Raich and Tufekciogul 2000; Xu et al. 2013) because changes in litter inputs directly influence the amount of resources entering the soil. Litter manipulation experiments are a useful tool for research into the importance of litterfall in forest ecosystems (Sayer 2006). For instance, long-term litter removal treatments cause nutrient depletion, which can alter other soil processes and reduce soil quality (Sayer 2006). Litter removal also causes a reduction in soil respiration because it removes organic substrates for microbial soil biota (Xu et al. 2013). On the other hand, the effects of litter addition can be less evident, as big pulses of litter inputs commonly occur naturally, such as at the beginning of autumn in temperate forests, which can account for a large proportion of the annual litterfall (Fenn, 2014). Litter inputs are likely to increase in response to elevated concentration of atmospheric CO_2 and patterns of litterfall could also be affected by changes in climate and species composition.

The body of research presented in this thesis aimed to determine the effects of different tree species on soil C dynamics via their litter inputs. In particular, I conducted a series of experiments, including a microcosm study in the lab, and *in situ* mesocosm experiments

within an established tree growth trial, to assess the relative influence of litter and soil properties on decomposition processes and soil respiration (CO_2 efflux).

- In Chapter 2, I present a detailed account of the methodology used to conduct these experiments, including descriptions of my field sites, and laboratory and field procedures I used to answer key research questions about forest soil C dynamics.

- In Chapter 3, titled **"Litter quality affects the dynamics and storage of soil carbon under different tree species in a temperate woodland"**, I present the results from a soil microcosm study, which used a factorial design to assess the influence of litter from ash, oak, sycamore and a species mixture on the C dynamics of soils collected from under the same species. For this study I used litter and soil collected from a naturally established temperate woodland near Wytham, UK.

- In Chapter 4, titled **"Litter quality controls the response of soil carbon dynamics to altered litter inputs in a managed temperate woodland"**, I explored the effects of litter addition and litter removal treatments in single-species plots of alder, oak or pine, and all possible two-species mixtures, in a forestry growth trial at Gisburn Forest, UK. I measured litter quality, and quantified litter decomposition for each species and mixture to interpret changes in soil respiration in response to litter treatments and assess the potential release of soil C through 'priming effects'.

- Finally, in Chapter 5, titled "**Tree species identity and litter quality regulate soil carbon dynamics in a managed temperate woodland**", I present evidence for the influence of litter from different tree species on soil C dynamics using a reciprocal litter transplant experiment, within the Gisburn Forest plots. I quantified litter decomposition and soil respiration in response to "home" or "foreign" litter in single-species plots of oak, alder or pine, measured changes in soil properties in response to litter addition and assessed the occurrence of 'home field advantage'.

Taken together, the results of each study provide important insights into the linkages between litter quality, decomposition and soil respiration in temperate forests. Thus, the work presented in this thesis represents an advance in research into the influence of different tree species on soil C dynamics under climate change, which could inform forestry rotation and reforestation practices in future.

2. Methods

2.1.Introduction

Temperate forest soils are a key global carbon (C) repository. Several factors, both biotic and abiotic, have an effect on C dynamics in temperate forest soils. Tree species identity and distribution influence soil processes via the quantity and quality of litter inputs to the soil, which suggests that differences in tree species can also influence soil C storage and its release as CO_2.

The research I present in this thesis investigates differences in the litter quality of temperate tree species and how these differences affect C dynamics in forest soils. Furthermore, I explore the implications of how tree species identity could modify soil carbon dynamics under climate change. To achieve this, I conducted a series of studies that include lab incubations and field experiments. Each experiment was designed to answer a specific set of questions, however most of my experiments follow the same lab and field procedures.

The aim of this chapter is to present the methodology used to conduct my experiments. I describe in detail my experimental designs, sample collections, measurements, and the laboratory procedures I used to analyse litter and soil samples.

2.2. Field sites

I established field experiments in two different locations in the UK; both are temperate woodlands but they differ in site history and dominant tree species.

2.2.1. Wytham Woods

Wytham Woods (henceforth "Wytham"), to the west of Oxford, UK (1º19´W 51º46´N; Kirby and Thomas 2000; Fig 2.1), is owned by the University of Oxford and commonly used for

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environmental research. Wytham comprises of 390 ha with a variety of habitats including ancient semi-natural woodland, secondary woodland and plantations as well as calcareous grasslands. According to the SSSI citation, Wytham has an exceptionally rich flora and fauna, with over 500 species of vascular plants. It is also one of the most researched areas of woodland in the world, with important long-term biological monitoring that includes climate change data for the last 18 years. My field experiment was established in an area of 100-year old, naturally established mixed deciduous woodland. The canopy is dominated by ash (*Fraxinus excelsior L.*), sycamore (*Acer pseudoplatanus L.*), and oak (*Quercus robur L.;* Fenn, 2015) and I therefore chose these as focal species for my experiments.

2.2.1.1. Experimental design.

I established 20 plots, measuring 4-m x 4-m each, in naturally occurring stands dominated by ash, oak or sycamore, and mixed stands with all three species. Plots were chosen based on two criteria: (a) the canopy above each plot was composed exclusively of the relevant focal species, and (b) plots were surrounded by at least three mature individuals of the focal species, with the exception of oak. For oak, plots were established at 2-m distance from the trunk and beneath the canopy of at least one adult tree because adult oak trees did not occur in clusters at the study site. Following these two criteria I ensured that the soil in each plot was mainly influenced by the focal species. In total, I selected five replicate plots for each individual species and five for the tree-species mixtures. I used the 20 plots to conduct two experiments:

Experiment 1. **"Litter quality affects the dynamics and storage of soil carbon under different tree species in a temperate woodland"** (see Chapter 3)

I collected soil samples from each block to conduct a lab microcosm experiment. The aim of this experiment was to assess the influence of tree species on soil properties and CO_2

efflux. I collected litter and soils from the 20 plots at Wytham and established microcosms in a fully factorial design with all possible combinations of litter species and soil types.

2.2.2. Gisburn Forest

I conducted studies within a long-term tree growth trial established by Forest Research, located in Gisburn Forest, northwest England (henceforth "Gisburn"; Fig. 2.1). The site is *c.* 35 km inland from the coast (54° 1′ N; 2° 22′ W), with elevation ranging from 260 to 290 m a.s.l., and the site slopes slightly to the south-west.

The first rotation of the growth trial was planted in 1955. The trial is currently in its second rotation, which was planted in April 1991 following the same ploughing, planting pattern and spacing as the first rotation. Consequently, at the time of my study, the soil in each plot had been under the influence of the same tree species or mixture of species for 60 years. The experiment includes single-species plots of alder (*Alnus glutinosa L.*), oak (*Quercus petraea L.*), Scots pine (*Pinus sylvestris L.*), Norway spruce (*Picea abies L.*) and Sitka spruce (*Picea sitchensis L.*).

The growth trial consists of a total of 36 square 0.2-ha plots with a core plot of 0.1 ha, which is used for all tree growth measurements. Each species was planted in monoculture and in all possible two-way species combinations in a 50:50 mixture. Thus, there are 15 single-species and mixed-species treatments in three replicate blocks, as well as unplanted control plots (for details, see Mason and Connolly 2013). The layout of the species mixtures consisted of a 'checkerboard' pattern of alternating groups of 18 plants of each species in a six by three plant layout at 1.5-m x 1.5-m spacing. This layout was used to reduce the risk of a faster growing species suppressing slower growing trees and to ensure longer-term continuity of the mixed plots. For my experiments, I selected tree species based on functional group differences. I included all plots with monocultures of

alder (nitrogen-fixer), oak (broadleaf deciduous), and scots pine (evergreen) and all plots with the paired combinations of these species, giving six treatments and a total of 18 plots.

2.2.2.1. Experimental design.

I conducted two field experiments at Gisburn Forest.

Experiment 2. **"Litter quality controls the response of soil carbon dynamics to altered litter inputs in a managed temperate woodland"** (see Chapter 4)

I used a reciprocal litter transplantation experiment to determine how the "home-field advantage" (Ayres et al., 2009) affects soil C dynamics. I established three *in situ* mesocosms per single-species plot; two received a 'foreign' litter input from each of the other two species and the third one received litter from the 'home' species. Litter addition followed a factorial design with all litter types decomposing within mesocosms on each soil type. I analysed initial and final soil properties, measured litter decomposition rates and performed monthly field soil CO_2 efflux measurements during 18 months.

Experiment 3. **"Tree species identity and litter quality regulate soil carbon dynamics in response to inputs on 'foreign' litter in a managed temperate woodland"** (see Chapter 5)

The second experiment at Gisburn explored the effects of increased litter inputs on soil C dynamics, particularly how different tree species and mixtures contribute to priming effects. I used litter removal and litter addition treatments within *in situ* mesocosms to assess the effect of altered litter inputs on soil properties over time. I measured soil CO_2 efflux monthly during 18 months and determined litter decomposition rates for each species using litterbags (see section 2.3.3.).

Figure 2.1. Aerial photographs of Wytham Woods, Oxfordshire, UK. a) Wytham Woods location, Oxfordshire b) Specific location of the experimental plots at Wytham Woods. Image b representa a 16-ha area, with red square showing the approximate location of the study area at Wytham woods (Images were obtained from: Wytham Woods-Imagery ©2017 Infoterra Ltd & Bluesky, Digital Globe, Map Data ©2017 Google; and Gisburn Forest-Imagery ©2017 Getmapping plc, Map Data ©2017 Google).

2.3. Field measurements

2.3.1. Soil respiration measurements

Soil respiration (soil CO_2 efflux) measurements were taken monthly from *in situ* mesocosms installed at both field sites. Mesocosms were installed to standardize the soil surface area and to delimit treatment area. Mesocosms represent an important tool in ecological research (Stewart et al. 2013) and I used them for my experiments to contain treatment effects within a discrete area and avoid disrupting long-term measurements at the sites. All mesocosms were installed at least one month before the start of data collection (Fig 2). Mesocosms were polyvinyl chloride (PVC) tubes (20-cm diameter and13-cm height) sunk 3-cm deep into the soil leaving a 10-cm length aboveground, aiming for a flat and visually undisturbed soil surface inside the mesocosm.

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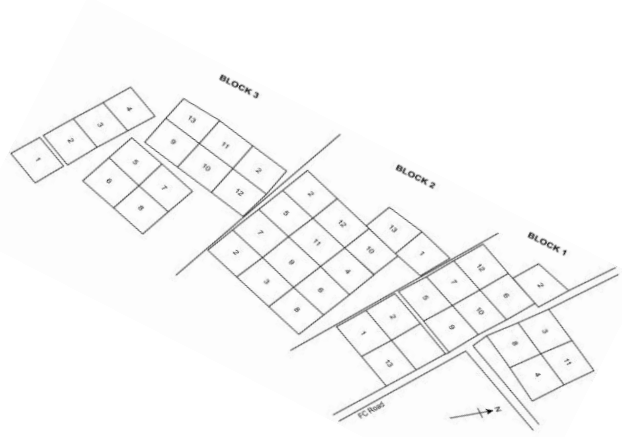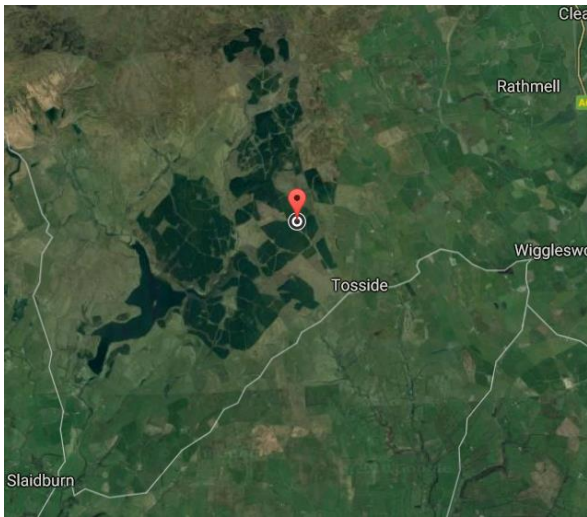
Figure 2.2. Aerial photographs of Gisburn Forest, UK.: a) Gisburn Forest, Lancashire, UK. b) Diagram representing the distribution of plots and experimental set up at Gisburn Forest. (Images were obtained from: Wytham Woods-Imagery ©2017 Infoterra Ltd & Bluesky, Digital Globe, Map Data ©2017 Google; and Gisburn Forest-Imagery ©2017 Getmapping plc, Map Data ©2017 Google).

All mesocosms were installed manually at a minimum distance from tree trunks, as tree proximity can influence soil respiration rates (Hanson et al., 2000). All mesocosms were placed at 2-m from the nearest tree trunk at Wytham Woods. At Gisburn Forest, collars were placed outside of the core measurement plot and at least 1-m from the nearest tree trunk. All vegetation and litter within the collars was removed one month prior to soil CO_2 efflux measurements and checked monthly to avoid plant growth and unwanted litter.

To avoid naturally falling litter entering the mesocosms, wire mesh 'hats' were placed on top of each collar. Each hat was made from a 40-cm square of 1-cm aperture wire mesh, which was cut and folded to create a cone that fit over the mesocosm (Fig. 2a). The aperture in the hats was enough to permit water and light to transfer freely. Hats were pinned to the soil using commercial tent pegs; they were removed before taking respiration measurements and replaced immediately afterwards.

Soil CO_2 efflux measurements were taken using a LiCor L1-8100A soil survey system (LiCor BioSciences, Lincoln, Nebraska, USA; Fig. 2.2), a closed-system infra-red gas analyser with a soil chamber that fits on top of the mesocosms. The IRGA measures the concentration of CO_2 in the headspace over time and calculates efflux rates from the accumulation of CO_2 within the chamber. To eliminate the effects of turbulence from chamber closure, a 15-sec post-purge period and a 15-sec dead band period were set for each measurement.

2.3.2 Soil and litter collection

Soil collection.

Soil samples were collected from both field sites at 0-10 cm depth using a 2.5-cm diameter soil corer. At Wytham Woods, soil was collected from inside the experimental plots, avoiding trees closer than 2-m. At Gisburn Forest, soils were collected from the core plot, avoiding trees closer than 1-m. The number of cores taken varied depending on the quantity of soil needed for analysis and/or lab incubations. To determine initial soil properties, soils from each plot were collected and mixed to create a composite sample per replicate. To determine treatment effects, soils were collected from within mesocosms at the end of experiments. All samples were sealed in plastic bags and brought back to the lab. Soil samples were then either processed fresh (within 24 h after collection) or oven-dried for further analysis.

Litter collection.

Freshly fallen litter of all species included in my experiments was collected from the study sites. Only litter on the top layer of the forest floor was selected and to ensure its freshness, I made sure the base of petioles was still green.

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Figure 2.3. Photographs of mesocosms in the field and field CO_2 efflux measurements. a) Mesocosm in an oak stand, showing the wire-mesh hat to prevent natural litter inputs. b) The LiCor L1-8100A survey system during a soil CO_2 efflux measurement.

2.3.3 Leaf litter decomposition (Litterbag experiment)

To measure leaf litter decomposition rates for different tree species in the field, I made 10-cm x 10-cm litterbags using 1-mm nylon mesh containing 3-g of dry leaf litter that was chopped and sieved to 1 cm. Litterbags were placed next to mesocosms in the field, removing all litter and vegetation to ensure direct contact with the soil, and the bags were fixed using metal pegs. Sets of litterbags were collected after two and four months to calculate litter decomposition rates.

2.4. Sample processing and laboratory procedures

2.4.1. Litter processing and analysis

Leaves and litter samples were brought back to the lab and oven dried (60 ⁰C). Litter used in field experiments was left intact, litter used in soil incubation was knife-milled (Retsch GM300, Hann, Germany) and sieved (2 mm), and litter used for litterbag experiments was manually chopped and sieved (1 cm). The remaining litter was ground using a ball mill

(Retsch MM400, Hann, Germany) to perform further analyses and chemical analyses were performed on three replicate composite samples per species.

2.4.2. Litter chemical properties

I determined the total C and N content and conducted fibre analysis of leaf litter samples. I measured the C and N content of the leaf litter on 0.30-g subsamples using a Vario ELIII Element Analyser (Elementar, Hessia, Germany) and calculated ratios of C to N (C:N ratio). Fibre analysis was performed to determine lignin and cellulose content following the Van Soest method (Van Soest, 1963), which uses a series of digestions to calculate fiber content from plant material by mass loss. Acid detergent lignin (ADL) and cellulose were determined on 1-g of oven-dried (60 $^\circ$C) and knife-milled (1 mm) litter. I performed the extractions using a Fibertec^{TM} 1020 hot extraction unit (Foss, Hilleroed, Denmark). Each sample was placed in a glass crucible and 1-g of celite was added as a filtration aid. Total acid detergent fibre (ADF) was obtained after washing the samples with boiling acid detergent solution (0.5 M H_2SO_4 + CTAB(Cetyl trimethylammonium bromide)) for 1 h, followed by a 5-min acetone soak. The resulting ADF samples were drained and oven-dried at 105 $^\circ$C for 5 h. Cellulose was then solubilized by soaking the ADF samples in 70% H_2SO_4 for 3 h, followed by washing with hot deionized H_2O (until acid-free, determined using pH indicator paper). The remaining sample was dried for 2 h at 130 $^\circ$C and then ashed in a furnace for 3 h at 525 $^\circ$C. Cellulose content was calculated from the mass loss after solubilization and lignin content was calculated from the mass of the residue pre ashing.

2.4.2. Soil analysis

2.4.2.1. Soil moisture, water holding capacity, and pH

Gravimetric soil water content was determined by oven-drying 20-g of fresh soil to constant weight at 105 ºC. Soil water holding capacity (WHC) was measured by placing 100-g of oven-dried soil in a plastic container. The base of the container had small holes to allow drainage. Soil-filled containers were placed in a water bath for 24 h to allow the soil to absorb water, and removed from the water bath for another 24 h, allowing water to drain freely. Subsamples were then weighed to calculate water-holding (field) capacity by mass difference.

Soil pH was measured in a 1:3 slurry of soil to deionised water. The slurry was made by mixing 3-g of fresh soil in 9-g of distilled water in a small plastic cup and shaking the mixture for 1 h. Soil pH was then measured using a S220 Seven Compact pH meter (Mettler Toledo, Columbus OH, USA). The pH meter was calibrated at the beginning of measurements and the electrode was rinsed in between measurements with distilled water.

2.4.2.2. Soil microbial C

Soil microbial biomass was determined on paired subsamples (8-g fresh weight) by chloroform fumigation extraction (Vance et al, 1987), with modifications (Jones & Willet, 2006) within 24 h of sample collection from the field. One of the subsamples from each pair was fumigated by placing the soil samples in a desiccator with a 250-ml beaker containing 40-ml of ethanol-free chloroform, and another smaller beaker with 20-g of soda lime. To keep the samples from dehydrating, I included damp commercial paper towel. The desiccator was then closed and evacuated using a suction pump until the chloroform boiled. After 5 minutes, the pump was turned off (making sure the desiccator was completely sealed) and left for 24 h. After this period, the stopcock on the desiccator was opened and all air was pumped out three times to make sure samples were free of chloroform. The fumigated and unfumigated sub-samples were then placed in 50-ml plastic falcon tubes with 40-ml 0.5M K_2SO_4. The samples were shaken at 200 rpm for 1 h,

centrifuged at 3000 rpm for 5 minutes and filtered (Whatman 42 filter paper). The extracts were refrigerated until total C analysis was performed (see section 2.4.2.3. below). Soil microbial biomass was estimated by the difference in total C between fumigated and unfumigated subsamples.

2.4.2.3. Soil carbon and nutrient concentrations

I measured total soil C and N content on 0.15 g oven-dried (105 ºC), ground soil using a Vario ELIII Element Analyser (Elementar, Hessia, Germany). This procedure also calculate C:N ratios.

I extracted ammonium-N and nitrate-N from soil samples using a KCl solution. As the conversion of ammonium to nitrate can occur rapidly after sampling, the extraction process started during sampling in the field. The day before collection, I filled a 50-ml plastic falcon tube with 20 ml 2M KCl solution for each sample. The tubes were refrigerated overnight and taken to the field for soil collection. Soil cores were collected and placed in bags to create a composite sample (see section 2.3.2. above), from which 2 g were subsampled and added to each tube. All tubes were kept cool until the samples were brought back to the lab. Within 24 h, all samples were shaken at 200 rpm for 1 h, allowed to settle for 30 min and filtered (Whatman 42 filter paper). Samples where then refrigerated until analysis for NH_4^+-N and NO_3^--N using an Autoanalyser (Bran and Luebbe AA 3; Seal Analytical, Southampton, UK).

2.5. Soil microcosm experiments

Soil microcosms are commonly used to assess soil processes under controlled lab conditions. However, vessel size, soil quantity, and C source differ among studies that suggest different approaches to soil incubations (Alef, 1995). Differences in the microcosm design can influence the accumulation of CO_2, evaporation of water from the soil, and the appropriate duration of the experiment, which in turn can affect the results of soil

microcosm experiments. Measurements of soil CO_2 efflux are also influenced by ambient CO_2 concentrations, and the build-up of CO_2 within incubation jars should be avoided to maintain similar CO_2 efflux to field conditions (Shoji and Komatsu, 2006). At the same time, soil moisture is critical for microbial activity and decomposition rates, which in turn affect soil respiration measurements (Borken et al, 2003). Given the need to limit CO_2 accumulation while minimising water loss, I conducted a pilot study to determine the optimal microcosm design for my experiment. Soils for this pilot study were collected from Wytham Woods in November 2013, oven dried (38 °C), sieved (2 mm) and manually homogenized to remove roots and stones.

2.5.1. Pilot studies to determine microcosm design.

To test the influence of microcosm design on soil water content and soil CO_2 efflux, I conducted three pilot studies looking at (a) the effect of incubation size on soil water loss and CO_2 efflux, (b) the effect of different lid designs on CO_2 efflux and water loss and c) the effect of litter processing on CO_2 efflux.

In all three studies, I measured soil CO_2 efflux using an infra-red gas analyser with an eight-channel multiplexer adapted to incubation jars (LI-8100 and LI-8150, LiCor Biosciences, Lincoln, Nebraska, USA). Soil CO_2 efflux from each microcosm was measured during 2 minutes with a dead-band period of 15 s to account for turbulence at the beginning of measurements.

(a) The effect of incubation size on soil water loss and soil CO_2 efflux. For this study, I used Kilner™ jars of three different volumes (0.25 L, 0.5 L and 1 L). For each jar size, I established two replicates of three different soil quantities (Table 1) for a total of nine treatments and 18 microcosms. Soils in all microcosms were brought to 50% WHC and left uncovered to measure water loss over time. I recorded water loss by mass difference and measured CO_2 efflux every hour for the first 6 h and 18 h later for a 24 h total observation

time. Results of these observations showed that microcosms with less soil lost more water (Fig. 2.3a).

Table 2.1. Different combinations of jar size and soil mass used in a pilot study of microcosm designs, testing for water loss, lid design and their effect on soil CO_2 efflux. I used three different jar sizes: small (0.25L), medium (0.5L) and large (1L), and each jar size was tested with three different soil quantities.

Jar size (L)		Soil quantity (g)
0.25	small	10
	medium	20
	large	50
0.5	small	20
	medium	50
	large	100
1	small	50
	medium	100
	large	200

The study also demonstrated that water loss affected soil CO_2 efflux, as soil respiration rates declined with the decrease in WHC (Fig. 2.3b). Of all tested microcosms, those consisting of 0.5-L jars and 50-g of soil had the lowest rate of water loss and the smallest fluctuations in CO_2 efflux.

b) *The effect of different lid designs on CO_2 accumulation in microcosms*. To reduce CO_2 accumulation within and water loss from the mesocosms, I tested three different lid designs: 1) 'open', with no lid, 2) 'vented' a 1-cm opening in the centre of the lid, and 3) 'closed' with standard lids. I used these lids in combination with all nine microcosm sizes (nine microcosm sizes x three lid designs x two replicates; 54 microcosms in total). The soils in this experiment were incubated at 50% WHC, observations were made during four days, and CO_2 efflux was measured at 48 h and 96 h. To ensure constant WHC, the microcosms were weighed 1 h prior to respiration measurements and the weight was

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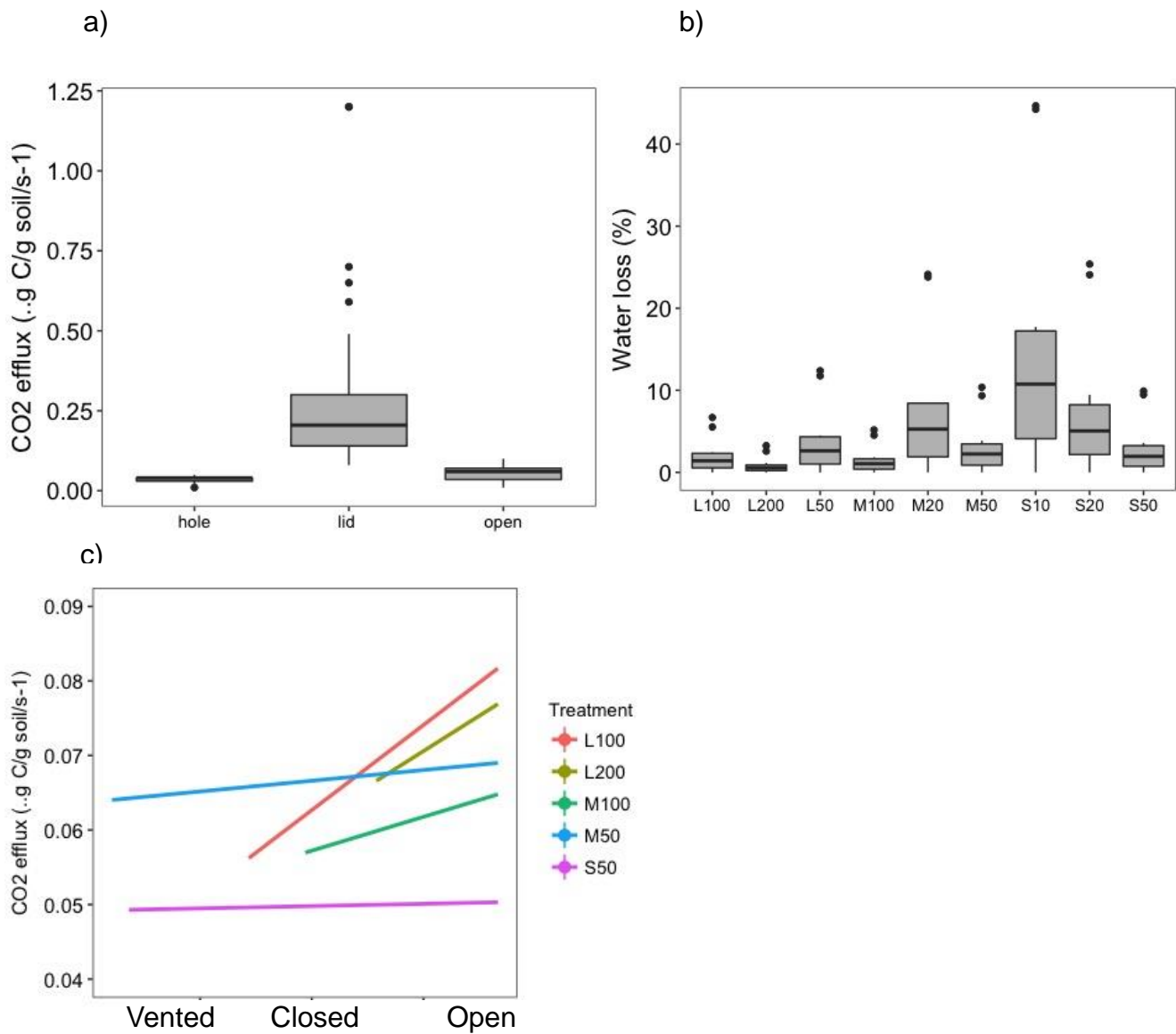
Figure 2.4. Figures show results from pilot experiments exploring the effect of different microcosm designs (treatment) on soil water loss and CO_2 efflux. a) Evaluation of the effect of soil water content on CO_2 efflux among the best 5 microcosms designs; the treatments show the size of the jars (L= 1L, M = 0.5 L and S = 0.25 L) and the quantity of soil. b) Graph shows soil water loss on each treatment; the treatments show the size of the jars (L= 1L, M = 0.5 L and S = 0.25 L) and the quantity of soil; b) c) Differences in soil CO_2 efflux during a test of lid design of incubation jars; where 'open' = without a lid, 'vented' = lid with a 1-cm opening in the centre and 'closed' = completely closed.

readjusted by water addition. All lids were removed 30 min before the start of respiration measurements. Results of these observations (Fig. 2.3c) showed that accumulation of CO2 in 'closed' mesocosms was still evident during measurements. However, CO_2 efflux in 'vented' microcosms had low variation and was similar to 'open' microcosms, but with only a modest decrease in water content. Based on these results, the microcosm design used for my next experiment was a 0.5-L Kilner™ jar containing 50-g of soil and a vented lid.

c) *Effect of litter processing on soil CO_2 efflux in microcosms.* Litter for this test was collected from Wytham Woods. A 50:50 mixture of oak and sycamore leaf litter was used and the quantity added to each microcosm (0.3 g) was estimated using litterfall data from experimental plots in Wytham Woods. Litter was oven dried at 60 ºC and either chopped into 1-cm^2 pieces or ground using a ball mill. The litter was then either thoroughly mixed with the soil (chopped litter only) or placed on the soil surface resulting in three different litter treatments: chopped-mixed, chopped-top, ground-top, as well as a control treatment with no litter addition (Fig 2.4). Following the results of the previous pilot study, microcosms consisted of 0.5-L Kilner™ jars with 50-g of soil and a vented lid (five microcosms per litter treatment; 15 total).

The soils were incubated at 50% WHC for three weeks and soil CO_2 efflux measurements were taken every two days. Soils were brought up to 50% WHC 1 h prior to respiration measurements as described above. Results showed a difference in the strength of the response among treatments, with ground litter placed on the soil surface showing higher CO_2 efflux rates compared to the other litter treatments. Based on these results, the selected incubation design for my microcosm experiment consisted of a 0.5-L Kilner jar™ with a vented lid containing 50-g of dry soil and ground leaf litter added on top of the soil as the experimental C input.

a)   b)   c)
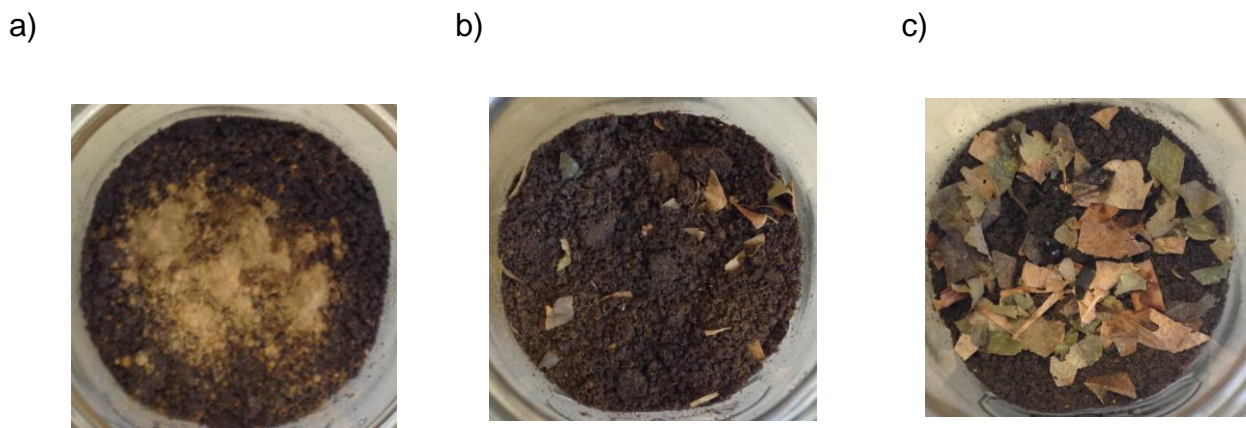
Figure 2.5. Figure shows different litter treatments and results for my experiment studying the effect of litter fragment size on soil CO_2 efflux. Pictures show microcosms with different litter treatments: a) ground litter placed on the surface, b) chopped litter mixed with soil, and c) chopped litter placed on the surface. Results show that ground litter treatment evoke the highest respiration rates.

2.5.2. Soil incubations for microcosm experiments

Soil samples for the microcosm experiments were brought back to the lab immediately after collection and oven-dried to constant weight at 38 °C. Samples were sieved (2 mm) and 50-g of soil were placed within 0.5-L glass Kilner™ jars with a vented lid (Fig. 2.5). To allow soil CO_2 efflux to stabilise before treatment addition, soils were pre-incubated at constant room temperature and 50% WHC for 13 days. Microcosms were brought up to 50% WHC 1 h before each respiration measurement.

I measured soil CO_2 efflux using an infra-red gas analyser with an eight-channel multiplexer adapted to incubation jars (LI-8100 and LI-8150, LiCor Biosciences, Lincoln, Nebraska, USA). Once CO_2 efflux from the microcosms had stabilized, I performed one single litte addition of 0.3-g of ground litter, corresponding to double the annual litterfall at Wytham Woods. The litter was placed carefully on the soil surface to create an evenly

distributed litter layer. After litter addition, I measured soil CO_2 efflux three times a week during four weeks and then once a week for another two weeks (14 observations in total). I terminated the experiment 43 days after the start of litter treatments and sampled the soil inside the microcosms for soil chemical analysis.

Figure 2.6. Photo of the microcosm design used to study the effects of different tree species litter on soil C dynamics. I used vented Kilner™ jars (0.5 L) containing 50-g of dried, sieved soil, and ground litter.

Chapter 3. Litter quality affects the dynamics and storage of soil carbon under different tree species in a temperate woodland.

Abstract

Forest soil carbon (C) stocks vary widely depending on the dominant tree species, and differences in the quality of leaf litter also affects soil carbon dynamics during decomposition. However, less is known about how the interaction between soil properties and litter quality will influence carbon dynamics in future., In this chapter, the interactive effects of litter quality and soil properties from stands of different tree species in mixed temperate woodland in the UK were studied, using a microcosm experiment. I measured key properties of ash, oak, sycamore, and mixed-species litter, and their effect on soil CO_2 efflux and soil properties were quantified during a four week incubation. Pre-treatment soil CO_2 efflux varied by species and was linked to soil pH and the ratio of C to nitrogen (N) in the soil, whereby CO_2 efflux was highest for mixed-species and lowest for oak soils. However, changes in soil CO_2 efflux and microbial biomass after litter additions revealed interactions between litter quality and initial soil properties. Peak and cumulative soil CO_2 efflux from microcosms was related to litter quality. Sycamore litter had the highest N and lowest lignin content; whereas the reverse was observed for oak litter. Accordingly, sycamore induced the highest peak CO_2 efflux, regardless of soil type, with respiration rates up to 57.1% higher than soils to which oak litter was added. However, the magnitude of this response was modified by soil type. In particular, soil type largely explained changes in soil microbial biomass carbon (MBC) and pH after litter addition, regardless of litter type. My results give an insight into the potential contribution of different tree species to soil C storage to mitigate rising atmospheric levels of CO_2.

3.1. Introduction

Forests play a key role in global carbon (C) cycling and sequestration (Dixon et al., 1994; Peng et al., 2008, Schimel and Gulledge, 2001). Forests represent *c.* 80% and 40% of global terrestrial aboveground and belowground C stocks respectively, with an estimated total C stock of 2150 Gt C, making them one of the world's major C stores (Kirschbaum et al., 1996). Accordingly, forests are particularly important in the efforts to reduce atmospheric concentrations of CO_2 because their rate of CO_2 exchange with the atmosphere ($\approx$ 50 Pg C yr^{-1}) is seven times larger than anthropogenic emissions (Brown et al., 1995). However, despite the number of studies assessing forest C pools (Batjes, 1996; Jobbágy and Jackson, 2000; Six et al., 2002), our understanding of soil C dynamics in different forest types needs to be improved.

In many temperate forests, the soil contains more than twice as much C as the aboveground biomass (Eswaran et al., 1993; Goodale et al., 2002). A large quantity of C enters the soil as leaf and root litter (McNaughton et al., 1989), which is estimated to contribute about 70% to the annual C flux, with leaf litter decomposition as the primary source of soil C and nutrients (Warembourg and Paul, 1977). During leaf litter decomposition, nutrients from organic materials are mineralised by microorganisms and made available for plant uptake, whereas C is either stabilised and stored in the soil or released back to the atmosphere as CO_2 (Aber and Melillo, 1991; Schlesinger, 1997; Gartner and Cardon, 2004). The turnover, storage and release of C and nutrients during decomposition is the result of many complex interactions, which are influenced by soil type, decomposer communities, climate conditions and tree species (Vesterdal and Raulund-Rasmussen, 1998; Côté et al., 2000; Callesen et al., 2003; Jandl et al., 2007; Prescott, 2010). Nonetheless, there is still a particular need for a better understanding of how plant - soil interactions will affect temperate forests in a changing environment.

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Changes in climate conditions are likely to affect temperate forests (Reichstein et al., 2013). This is particularly important because temperate forests are essential for ecosystem C sequestration above and belowground. However, belowground C stocks are likely to be more stable over the long term than C sequestered in aboveground biomass (Batjes, 1998). This makes soils an important sink for C, which could help stem the rise in atmospheric CO_2 (Vesterdal, 2012). However, our current knowledge of the mechanisms underlying temperate forest soil C dynamics, and how these mechanisms may be affected by climate change, is still deficient.

A particular aspect that remains poorly explored, is how different tree species affect soil C dynamics in forest. Forest vegetation is known to affect soil C dynamics (Folster et al, 2001), and studies in temperate forest have demonstrated that soil C concentrations vary with tree species (Ahmed et al, 2016), but it is still unclear how the litter of different tree species influences soil processes. This lack of knowledge becomes critical in the face of climate change, as tree species are likely to have different responses that affect important aspects such as tree growth, seedling emergence, and survival rate (Jiang et al, 2014; Fisichelli et al, 2014), which in turn could affect species composition. Additionally, variations in rainfall patterns and the likelihood of more frequent extreme events like storms and droughts (IPCC 2013; Clark et al, 2014) underlines the importance of understanding how such events affect tree species and their consequent effect on forests role in C assimilation and sequestration. This information should be incorporated in projections of the role of forests in mitigating CO_2 emissions, which would help countries achieve greater accuracy for reports under the Climate Convention and the Kyoto Protocol (Peltoniemi et al., 2007).

3.1.1. Tree species variation in litter quality and decomposition

Litter quality can be defined as a set of physical and chemical characteristics that regulate rates of mineralization by decomposers (Paustian et al., 1997). Such characteristics include concentrations of C, N, lignin and polyphenols (Gentile et al., 2011; Hattenschwiler, 2005), control turnover rates of organic matter and essential mineral elements in the soil (Melillo *et al.,* 1982; McClaugherty and Berg, 1987; Berg and Ekbohm*,* 1993) via leaf litter decomposition. Hence, variation in these characteristics among tree species is particularly important in forests nutrient cycling (Couteaux, 1995; Gehrke, 1995; Yang, 2014).

The decomposition of plant material is central to ecosystem functioning because it underpins the cycling of C and nutrients (Swift et al. 1979, Cadish & Giller 1997), which in turn influence plant growth and C storage (Wardle 2002, Bardgett 2005). However, the rate of litter decomposition is governed by both the physical and chemical traits of leaf litter, which determine the quality of substrate available to decomposer organisms and the available habitat space in the forest floor (Berg et al. 1993, Perez-Harguindeguy et al. 2000). Therefore, it is expected that tree species that vary in litter quality will also vary in decomposition rates, thus having different effects in C and nutrient cycling.

In general, leaf litter with high N and P concentrations relative to C concentration decomposes faster than leaf litter with low relative concentration of N and P (Webster & Benfield, 1986; Enriquez et al., 1993). Other indicator of leaf litter decomposition rates is the concentrations of soluble polysaccharides, which are labile C sources, and thus are easily degraded and consumed by microbes. In contrast, more complex C compounds in leaf litter, such as lignin or tannins, are recalcitrant C resources, and thus metabolically more costly to be used by microbes (Sinsabaugh et al., 1993), and expected to slow down decomposition rates (Schindler and Gessner, 2009).

Lignin to nitrogen ratios (Lignin:N ratio) play a key role in decomposition (Sinsabaugh et al., 1993). Lignin's' recalcitrance helps to slow down microbial decomposition because only specialized biota (predominantly fungi) are able to synthesize extracellular enzymes that break down lignin into biologically usable forms (Swift et al., 1979). At the same time, Lignin:N ratios are expected to vary with species. In a recent study conducted by Osono and Takeda in 2004, the Lignin:N ratios of 14 broadleaf tree species in a Japanese temperate forest were compared. In these tree species, litter Lignin:N ratios ranged from

10.3 to 80.0 and had a significant effect over litter decomposition, as they helped predict the mobilisation and immobilisation of N.

In addition, litter N is hypothesized to control rates of decomposition by alleviating N limitation of litter C degradation (Berg and Staaf, 1980). Hence, specific litter chemical properties can be a better predictor of soil processes than plant species composition, plant species richness, and litter chemical diversity (Meier and Bowman, 2008). Experimental evidence also shows that differences in leaf properties among species, particularly C, N and lignin concentrations will affect the rate of organic matter inputs to the soil (Binkley and Giardina, 1998; Schulp, 2008). In this case, litter quality appears to be the most important control on soil C and N cycling rates, with litter chemical diversity related to soil respiration and net N mineralization rates (Meier and Bowman, 2008). At the same time, litter quality can be affected by the soil (Lutz and Chandler, 1946), and although there is evidence for a relationship between litter and soil nutrient concentrations (Kost and Boerner, 1985), this is not consistent across similar studies (Leyton, 1948; Staaf, 1982) possibly due to factors such as differences in nutrient re-translocation. Despite much previous research on how differences in plant litter, particularly litter quality, affect ecosystem dynamics (Aerts, 1997; Berg, 2000; Facelli and Pickett, 1991) there are still many open questions about how different tree species affect C dynamics in temperate forest soils.

3.1.1. Tree species effects on forest soil C

European forests (which includes the forests in the landmass between the Atlantic Ocean and the Ural, excluding Turkey and the Mediterranean isles) absorb 7 to 12% of Europe's CO_2 emissions (Janssens el al., 2003). This promotes the efforts made by European countries to increase forest coverage, as afforested land functions as an important C offset (Janssens el al., 2003). Tree species selection is an important criterion for such reforestation efforts, as the appropriate choice can increase total C stocks by up to 18%,

with most of the initial accumulation occurring in the forest soil (Jandl et al., 2007; Guo and Gifford, 2002).

Tree species effects on soil properties have been acknowledged for more than half a century (Zinke, 1962), with studies exploring their contribution to ecosystem processes and productivity, gas fluxes, and C sequestration (Binkley and Giardina, 1998; Raich and Tufekcioglu, 2000; Wardle et al., 2004; Schulp et al., 2008; Prescott and Grayston, 2013). Common garden studies have demonstrated that tree species composition affects soil organic carbon (SOC) pools (Vesterdal et al., 2008), whereby SOC concentrations are determined by the balance between inputs (e.g. tree litter) and outputs of C (heterotrophic respiration). Tree species identity can play important role in SOC formation, because this balance depends on the quality and quantity of litter inputs, (Borken et al., 2002; Vesterdal, 2008).

Recent studies demonstrate that forest floor concentrations of C, N and C:N ratios differ with litter quality (Vesterdal et al., 2008). Additionally, tree species with similar litter fall rates can differ in forest floor litter accumulation, which is influenced by differences in litter decomposition (Hansen et al., 2009). This suggests that litter quality can influence soil C stocks by regulating C input rates despite tree species differences in litter quantity (Vesterdal et al, 2012). Furthermore, topsoil C concentrations can be affected by dissolved organic carbon (DOC) leaching from the litter, which also varies with litter quality (Kleja et al., 2008, Fröberg et al., 2011). Finally, differences in litter quality influence soil C turnover and storage, directly affecting the rate by which soils release CO_2 back to the atmosphere (Diaz-Pines et al., 2014). It follows that tree species identity and differences in their litter quality is important for C dynamics in forest soils. Despite this, knowledge of the mechanisms underlying the tree species effects on soil C and nutrient dynamics are still understudied, particularly when assessing the role of plant-soil feedbacks in the global C budget (Jandl et al., 2007; Vesterdal, 2008).

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The aim of this study was to quantify differences in tree species litter quality and explore how such differences affect forest soil CO_2 efflux. I used a microcosm experiment to explore how leaf litter and soil properties interact and tested how differences in litter quality promote changes in soil properties and affect soil CO_2 efflux. I hypothesised that:

H1) Soil properties and CO_2 efflux vary with tree species, whereas litter with a high lignin content will promote lower respiration rates; and

H2) Litter with high C content will evoke higher respiration rates and increase soil microbial carbon (MBC).

3.2. Methods

3.2.1. Study site

I collected soil and leaf litter samples from a 100 year old, naturally established mixed deciduous woodland at Wytham Woods, Oxfordshire, UK (1º19´W 51º46´N; Kirby and Thomas, 2000) in May 2014. I selected ash (*Fraxinus excelsior* L.), sycamore (*Acer pseudoplatanus* L.), and oak (*Quercus robur* L.) as my focal species as they were the dominant species at the site (Lopez-Sangil et al. 2017). A tree survey close to the study site gives adult tree canopy coverage of 17% for ash, 70% for sycamore and 5% for oak (Fenn, 2015).

I selected five 4-m x 4-m sampling areas in naturally occurring stands dominated by ash, sycamore or oak and mixed stands with all three species, giving a total of 20 sampling sites. Site selection was based on two criteria. (A) the canopy above each sampling area was composed exclusively of the relevant focal species to ensure that the soil was mainly influenced by a given specie. (B) The sampling area was surrounded by at least three mature individuals of the focal species, with the exception of oak, where sampling areas were established at 2-m distance from the trunk and beneath the canopy of at least one adult tree (adult oak trees did not occur in clusters at the study site).

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3.2.2. Soil and litter collection and processing.

From each sampling site, I collected six soil cores to create a composite soil sample (0-10 cm depth using a 2.5-cm diameter soil corer). The soils were oven-dried at 38 °C for two days and sieved (2-mm mesh) to remove stones and debris. The soils were stored in the lab at room temperature until the start of the microcosm experiments. Soils from individual sampling sites were considered as replicates throughout the experiment, giving $n = 5$ per species and mixture.

I collected freshly fallen litter of all three species from the surface of the forest soil floor during the autumn of 2014 (October to November). Leaf litter was collected around the sampling sites and I ensured that only recently fallen litter was collected by checking that the base of the petioles was still green. The litter samples were brought back to the lab, oven-dried at 60 °C, shredded with a knife mill (Retsch GM300, Hann, Germany) and sieved (2-mm mesh).

3.2.3. Microcosm experiment

To determine the influence of litter and soil type on soil C dynamics, I established microcosms consisting of 0.5-L glass Kilner^{TM} jars containing 50-g of soil (dry weight). I applied four soil treatments and four litter treatments in a factorial design so that each litter type (ash, sycamore, oak or mixed) was incubated with each soil type in five replicate jars, giving a total of 16 treatments and 80 microcosms. Soils were pre-incubated at constant room temperature (*c.* 20°C) and 50% water holding capacity. To prevent excessive water loss and CO_2 accumulation within the microcosm, jars were closed using a vented lid design (with a 1-cm diameter hole in the centre, *see Chapter 2 section 2.5*). Throughout the experiment, the soil water content was maintained by weighing the jars and adding the corresponding amount of deionised water at least 1 h prior to CO_2 measurements.

I measured soil CO_2 efflux using an infra-red gas analyser with an eight-channel multiplexer adapted to incubation jars (LI-8100 and LI-8150, LiCor Biosciences, Lincoln, Nebraska, USA). I measured soil CO_2 efflux in pre-incubated soils until it stabilised (13 days), and then I added 3-g of litter, corresponding to the mean annual litterfall at Wytham Woods (Lopez-Sangil; *unpublished data*). The litter was placed carefully on the soil surface to create an evenly distributed litter layer. After litter addition, I measured soil CO_2 efflux three times a week during four weeks and then once a week for another two weeks (14 observations in total). I terminated the experiment 43 days after the start of litter treatments and sampled the soil inside the microcosms.

3.2.4. Soil analyses

To assess the influence of litter type on soil properties, I performed all analyses on soil samples at two time points: after collection (20 soil samples) and at the end of experiment (32 soil samples).

3.2.4.1. Soil water content, water holding capacity and pH

I determined gravimetric soil water content using 20-g subsamples of fresh soil. I measured the fresh weight of the soils within 24 h of collection and then the samples were dried at 105 ºC for 48 h to calculate soil water content. To determine soil water holding capacity, I placed 100-g of dried soil in a container with small holes in the base to allow drainage and placed then the containers were placed in a water bath for 24 h until saturated. I allowed the soil to drain freely for another 24 h and then calculated the water holding (field) capacity from the difference in weight. I measured soil pH on a slurry of soil in deionised water (1:3 ratio) using a S220 Seven Compact pH meter (Mettler Toledo, Columbus OH, USA).

3.2.4.2. Total soil carbon, nitrogen and microbial biomass

I measured the total C and N content of the soil on ground subsamples of oven-dried soil using a Vario ELIII Element Analyser (Elementar, Hessia, Germany). To determine soil microbial biomass carbon (MBC), I performed the chloroform fumigation extraction method (Vance et al, 1987) with modifications (Jones & Willet, 2006) on paired subsamples (8-g dry weight equivalent) of fresh soil. Briefly, one subsample was fumigated with ethanol-free chloroform for 24 h and both subsamples were extracted in 40 ml 0.5M K_2SO_4, shaken at 200 rpm for 1 h, centrifuged at 3000 rpm, and filtered. The extracts were refrigerated for ~10 days until analysis for total C analysis on a TOC-L combustion analyser coupled with a TNM-L unit (Shimadzu Corp, Kyoto, Japan). Soil MBC was estimated by the difference in C content between fumigated and unfumigated subsamples, without correction for extraction efficiency.

3.2.5. Litter properties

To assess litter quality, I analysed leaf litter from composite samples of each study species (three replicates per species, 12 samples). I determined the total C and N content and conducted fibre analysis of the litter samples were determined following the Van Soest method (Van Soest 1963). I measured the C and N content of the leaf litter on oven-dried (60 ºC) ground subsamples using a Vario ELIII Element Analyser (Elementar, Hessia, Germany). Acid detergent lignin (ADL) and cellulose were determined on 1 g oven-dried (60 ºC) knife-milled (1 mm) litter samples. I performed the extractions using a Fibertec™ 1020 hot extraction unit (Foss, Hilleroed, Denmark). Briefly, each sample was placed in a glass crucible and 1-g of celite was added as a filtration aid. Total acid detergent fibre (ADF) was obtained after washing the samples with boiling acid detergent solution (0.5 M H_2SO_4 + CTAB) for 1 h, followed by a 5-min acetone soak; the resulting ADF samples were drained and oven-dried at 105 ºC for 5 h. Cellulose was then solubilized by soaking the

ADF samples in H_2SO_4 for 3 h, followed by washing with hot deionised H_2O (until acid-free). The remaining sample was dried for 2 h at 130 ºC and then ashed in a furnace for 3 h at 525 ºC. Cellulose content was calculated from the mass loss after solubilization and lignin content was calculated from the mass of the residue.

3.2.5. Statistical analysis

All statistical analyses were performed in R version 3.2.4 (R Core Team, 2016). I used Principle Components Analysis (PCA; *rda* function in the vegan package; Oksanen et al., 2017) to explore the initial differences in soil properties among the four soil types. I included all soil variables measured (C, N, C:N ratio, pH, ammonium-N and nitrate-N) and scaled them to allow direct comparisons. The scores of the first two ordination axes (PC1 and PC2) were included as explanatory variables in linear models (*lm function*) to investigate the effect of initial soil properties on baseline soil CO_2 efflux, whereby CO_2 efflux was modelled as a function of PC1, PC2 and their interaction as explanatory variables. The best model was identified by comparing nested models using AICs and *p*-values to check for model improvement (Pinheiro and Bates, 2000).

 I used linear mixed effects models (*lmer* function in the lme4 package; Bates et al., 2015) to determine the influence of soil type and litter type on total and peak soil respiration. Total respiration was calculated by summing all respiration measurements for each microcosm after litter addition, whereas peak respiration was the highest respiration value after litter addition. I included litter type, soil type and their interaction as fixed effects and sampling site as a random effect. To determine the effect of soil type and litter treatment on the differences between initial and final soil properties, I calculated response ratios for C, MBC, pH and N. I used response ratio as a way of measuring treatment effect on soil variable using the equation: *RR = ln (t / c)*; where *'ln'* is natural logarithm; *'t'* is the value at the end of the experiment, accounting for treatment effects; and *'c'* is the initial value, used

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as a control. I then used linear mixed effect models to model the response ratios as a function of soil type and litter type (fixed affects), using sampling site as a random effect. The best model was identified as described above and the fit of all final models were inspected with diagnostic plots.

3.3. Results

3.3.1. Litter quality

Litter properties varied among species (Table 3.1.): oak had the highest C content, C:N ratio, and lignin content. By contrast, sycamore had the lowest C concentration, C:N ratio, and lignin content. Based on species differences in lignin:N ratios, I categorized litter quality from high to low; where sycamore litter as 'high quality', followed by ash, mix as 'medium quality' and oak as 'low quality'.

3.3.2. Initial soil differences and respiration

Ordination showed that initial soil properties (Table 3.1.) varied with tree species (Figure 3.1.). Sycamore and oak soils differed the most, with oak soils having the lowest C:N ratio and pH compared to sycamore, whereas mixed soils showed the greatest variability among samples. Accordingly, the first ordination axis (PC1) explained a significant proportion of the variation in pre-treatment respiration rates ($R^2 = 0.42$, $p = 0.001$).

Additionally, soils under the influence of mixed species showed higher pre-treatment average CO_2 efflux. Compared to mixed-species soils, CO_2 efflux was 16%, 24% lower in soils influenced by ash and oak respectively, and 39% lower in sycamore soils (Figure 3.2.).

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Table 3.1. Leaf litter properties of different tree species and soil properties of soils influenced by different tree species used in an incubation study. Soils and litter were collected at Wytham woods, UK. Litter properties show carbon (C), nitrogen (N), lignin (L) and cellulose content, and C:N and L:N ratios for freshly fallen leaf litter of ash, oak, sycamore, and a litter mixture containing an equal mass of litter from each species for n = 3 analytical replicates. Soil properties show total carbon (C), total nitrogen (N), microbial biomass carbon (MBC), carbon to nitrogen ratios (C:N ratio), soil pH (pH), ammonium-N (NH_4) and nitrate-N (NO_3) for n = 5 plots per species. Soil was collected from patches of the soil dominated by adult trees of ash, oak, sycamore, or a mixture of species respectively.

		Total C (%)	Total N (%)	C:N ratio	Lignin (%)	Cellulose (%)	L:N ratio	
	Ash	43.44	1.18	36.79	8.2	13.61	6.95	
Litter	Oak	47.01	1.19	39.64	10.96	12.46	9.21	
	Sycamore	44.39	1.6	27.76	6.84	9.83	4.28	
	Mix	44.01	1.22	36.19	8.67	11.97	7.11	
		Total C (%)	Total N (%)	MBC	C:N ratio	pH	NH_4-N	NO_3-N
	Ash	5.51	0.45	129.78	12.56	6.85	0.04	.003
Soil	Oak	6.49	0.49	90.34	13.44	5.82	0.04	.004
	Sycamore	3.53	0.22	82.03	16.20	6.22	0.03	.002
	Mix	6.82	0.49	83.83	13.87	7.06	0.03	.002
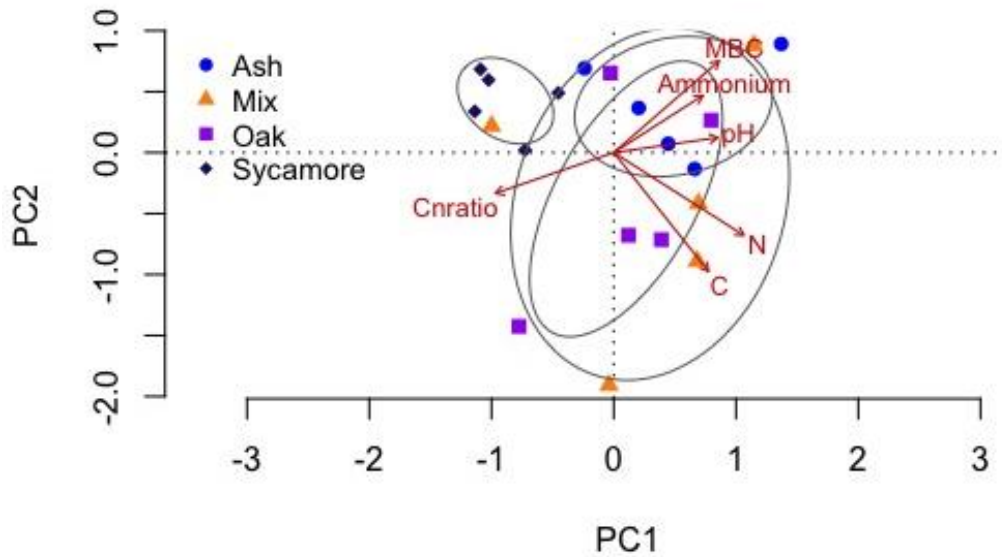
Figure 3.1. PCA ordination plot of initial differences in soil properties for each soil type (ash, sycamore, oak and 'mix' with all three species), showing a clear separation of sycamore soils from all other soil types; vectors show the relative influence of each soil property on the distribution of samples in ordination space, where C is carbon, N is nitrogen, MBC is microbial biomass carbon, pH is soil pH and Ammonium is ammonium-N.

3.3.3. The effect of different litter type on soil CO_2 efflux.

Soil CO_2 efflux increased markedly in all soils after litter addition. All treatments showed a similar pattern in soil CO_2 efflux, with peak respiration rates 12 days after litter addition (Figure 3). Results from mixed effect models show a significant effect of litter type on peak respiration (χ^2 = 228.2, p <0.001; Figure 3) whereby microcosms with oak litter had the lowest peak of soil CO_2 efflux, while microcosms with sycamore litter had the highest. Mean peak respiration in soils receiving sycamore litter was 57.1% higher than in soils receiving oak litter.
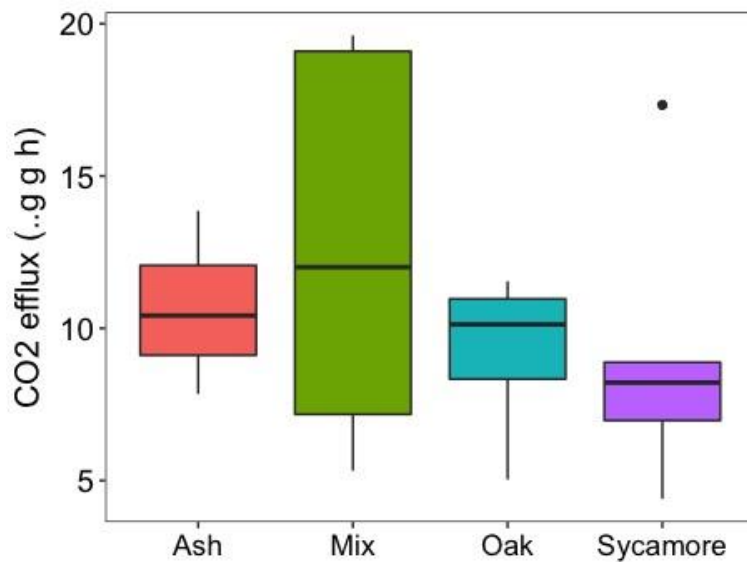
Figure 3.2. Baseline mean CO_2 efflux from each soil type at the start of a 4 week incubation experiment using soils and litter collected at Wytham woods, UK. Boxes indicate upper and lower quartiles and median lines for n = 5 microcosms per soil type using soils collected from patches in the forest under the influence of ash, oak, sycamore or a mixture of species.

Additionally, results from mixed effect models show a significant effect of litter type on cumulative respiration (χ^2 = 200.2, p <0.001; Figure 4), with microcosms that received ash, sycamore and mixed litter having the highest total respiration in all soil types, whereas microcosms with oak litter respired the lowest.
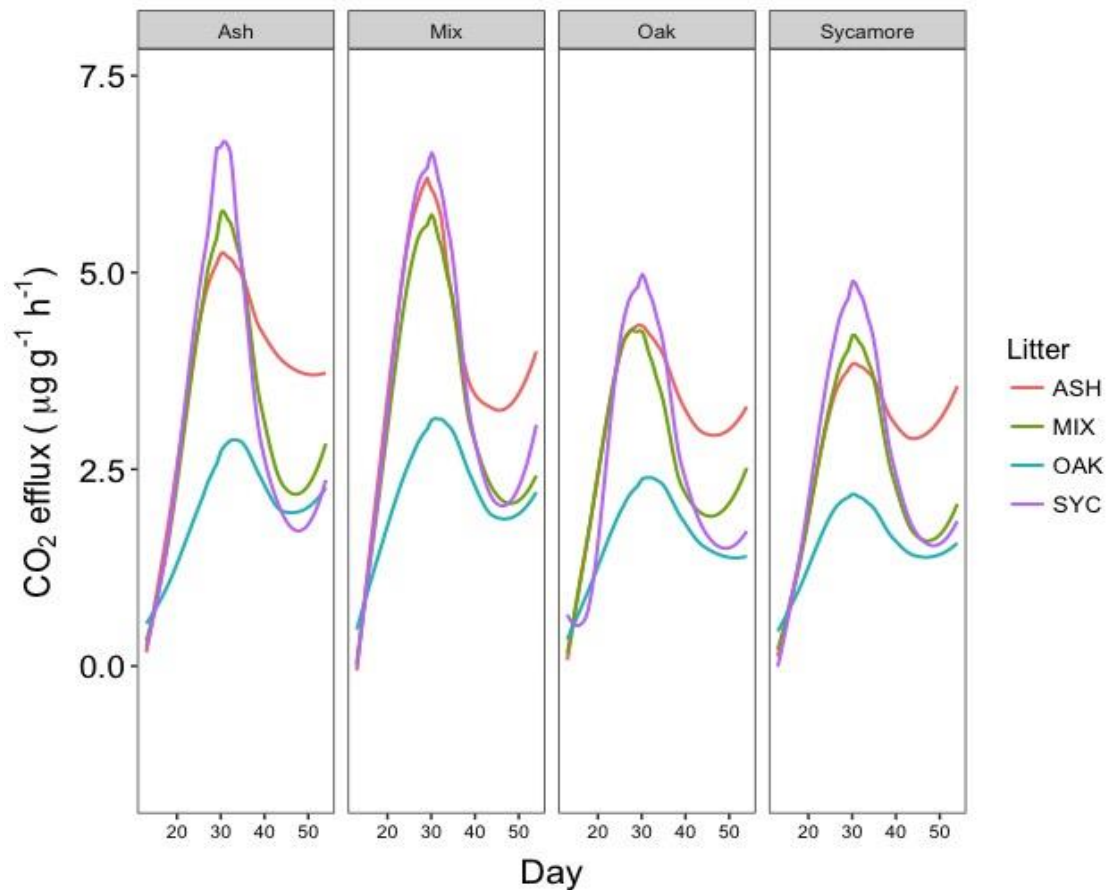
Figure 3.3. Mean CO_2 efflux from soils collected under ash, oak, sycamore or mixed species after the addition of litter from the same species in a factorial design. Observations were made during a 43 day incubation experiment in laboratory microcosms (n = 16 soil and litter combination), showing the pattern of soil respiration over the duration of the experiment and the clear peak in respiration rates 12 days after the start of litter treatments on day 13.

3.3.4. The effect of different litter on soil properties

Initial soil properties changed after litter addition but each soil type (with minor exceptions) responded similarly despite the type of litter added (Figure 3.5.). Accordingly, there was no significant effect of soil or litter type on response ratios for C and N. In contrast, for MBC and pH, the models including soil type were the best fit (x^2 = 11.63, p = 0.008; x^2 = 36.13, p = 0.001). In general, MBC declined following litter addition in ash, oak and mixed soils

while increasing in sycamore soils. The pH in all soil types increased after litter addition, with sycamore soils showing the bigger increase and ash and mix soils differing the most.
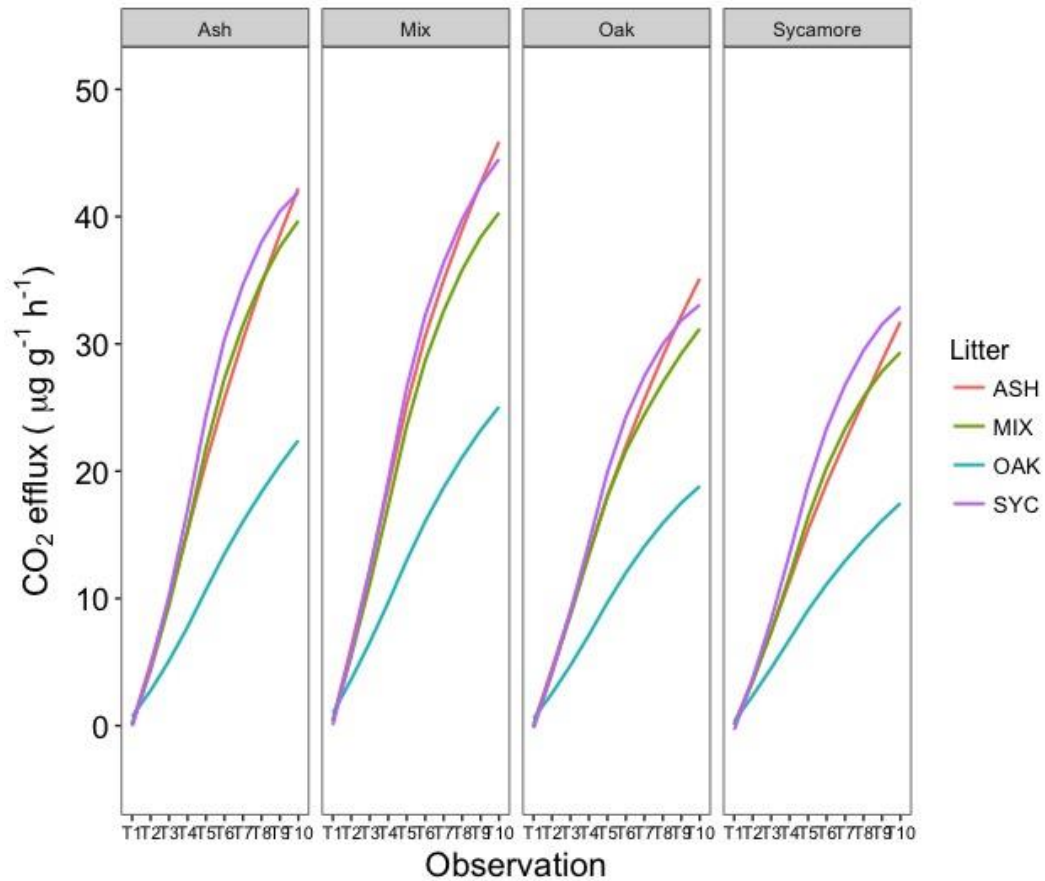
Figure 3.4. Cumulative CO_2 efflux from soils collected under ash, oak, sycamore or mixed species after the addition of litter from the same species in a factorial design. Observations were made during a 43 day incubation experiment in laboratory microcosms (n = 16 soil and litter combination), showing the additive effect of soil respiration measurements during the duration of the experiment.
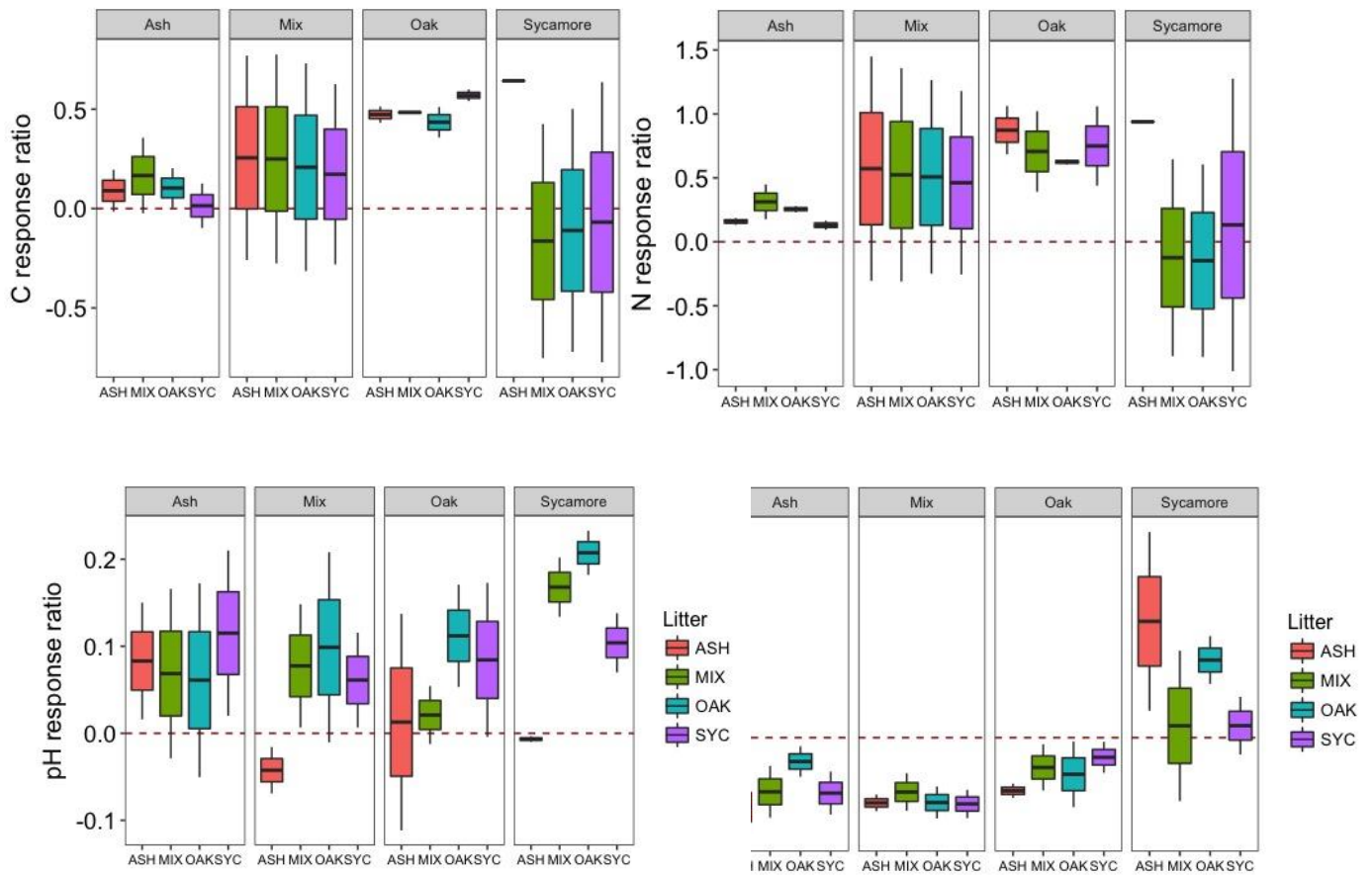
Figure 3.5. Response ratios of soil properties at the end of an incubation experiment in response to the addition of different species of litter. The experiment used freshly fallen litter from ash, oak, sycamore and mixed-species, and soils collected under patches of the forest dominated by the same species in a factorial design (n = 16 soil and litter combinations). The graphs show response ratios for carbon (C), nitrogen (N), microbial biomass carbon (MBC) and soil pH (pH) in all treatments compared to initial values of the respective property. Boxes indicate upper and lower quartiles and median lines per treatment.

3.4. Discussion

My experiment assessed how different tree species litter affect C dynamics in temperate forest soils. Specifically, it provided an insight into how differences in litter quality affect soil properties and soil C dynamics.

My experiment assessed how different tree species litter affect C dynamics in temperate forest soils. Specifically, it provided an insight into short term soil responses to differences in litter quality, focusing on the effect of litter addition in soil properties and soil respiration.

Initial soil properties and basal soil respiration

Soils influenced by mixed tree species had higher basal respiration rates than soils influenced by single species (Figure 3.2.). This suggests that, under natural conditions, microbial activity in patches of the forest dominated by a mixture of species is higher than those dominated by ash, oak or sycamore. Mixed soils also had a higher pH before treatment addition. This difference in soil pH could be important, as soil pH is determined by the concentration of cations in soil water (Brady, 1974) and influences the input, release and availability of nutrients and C (Sayer, 2006). However, microbial decomposition of organic matter can also affect soil pH as a consequence of the production of organic acids (Anderson and Domsch, 1993). Hence, increased microbial activity in mixed soils in this study could result in the slightly higher pH and explain the high respiration rates despite lower MBC compared to the single-species soils.

Interestingly, soils influenced by sycamore had the lowest CO_2 efflux and the lowest C concentration, despite the 'high quality' of sycamore litter (Figure 3.2., Table 4.1). The quality of litter and its decomposition play an integral role in determining the rate by which C enters the soil (Reynolds and Hunter, 2001; Subke et al., 2004), and it is likely that the rapid decomposition of sycamore litter releases much of the labile carbon as CO_2, which

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results in lower carbon inputs to the soil and reduces the quality and quantity of resources for soil microbes (Franzluebbers, 2002). This suggests that species with rapidly decomposing litter, such as sycamore, do not promote soil C storage in this temperate forest.

Differences in litter quality and their effect on soil respiration

Litter type modified the dynamics of soil CO_2 efflux, whereby microcosms containing sycamore litter had the highest peak CO_2 efflux after litter addition, and microcosms containing oak litter had the lowest, regardless of basal respiration rates. Peak respiration is likely to be linked to litter quality, as sycamore and oak also had the highest and lowest litter quality, respectively (Table 4.1.). 'High quality' litter is likely to promote higher soil respiration as it decomposes rapidly (Bardgett and Wardle, 2010). Hence, variation in tree species litter quality is tightly linked to soil C storage and soil respiration via its regulatory effect on decomposition rates (Berg, 2000). However, controlled lab experiments such as those used in this study exclude or standardize several biotic (e.g. soil fauna) and abiotic (e.g. temperature, soil moisture) factors affecting decomposition (Riutta et al., 2012). They are also limited to short-term responses, which can underestimate long-term effects and limit the implications of these results for processes *in situ*. Nonetheless, the results of my lab experiments help to explain the variation in soil C content in stands dominated by different species.

Nonetheless, my study suggests a potential long-term effect of litter quality on soil C dynamics because the different soil types regulated the magnitude of soil CO_2 efflux. Soils collected from ash, mixed species and oak stands had higher CO_2 efflux throughout the experiment than those influenced by sycamore trees, irrespective of the type of litter added (Figure 3.3.). This suggests that differences in soil properties under the long-term influence

of specific tree species have a stronger influence on soil CO_2 efflux than short-term inputs of differing litter quality. Soil properties are likely to be linked to differences in litter chemical properties among tree species, which in turn regulate decomposition rates and the activity of decomposer organisms (Swift et al., 1979, Berg and McClaugherty 2003). For instance, plant species can influence the taxa of decomposers present in the soil, thus favouring the decomposition of their own litter (Ayres et al., 2009). Additionally, the rates of processes that occur in the soil often vary strongly according to litter quality of the plant species present (Binkley and Giardina, 1998). Hence, it is likely that the microbial decomposer communities associated with soils under specific tree species have a greater influence on CO_2 efflux during decomposition than the nature of the organic material being decomposed.

Litter quality effect on soil properties.

By the end of the experiment, soil MBC and pH had changed as a result of the interaction between litter and soil type. Although sycamore soils had the lowest initial MBC, they also showed the largest increase in MBC after litter addition (Figure 3.5.), suggesting that the availability of C in the soil may limit microbial growth (de Graff et al., 2006). The low CO_2 efflux from sycamore soils (Figure 3.3.) could indicate that the microbial community is adapted to labile C inputs and less efficient at breaking down recalcitrant substrates. It is therefore likely that the additional C from leaf litter was invested in microbial growth, rather than turnover, hence increasing soil total C (Lopez-Sangil et al. 2017). Additionally, oak soils showed the greatest increase in total soil C, irrespectively of the litter type added. At the same time, the soil CO_2 response to litter inputs was low in oak soils (Figure 3.3.) and oak litter had the highest lignin and lowest N content (Table 3.1.). Due to the long-term inputs of low-quality litter, it is likely that oak soils are dominated by slow-growing microbial

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taxa capable of breaking down more recalcitrant organic material (de Graff et al., 2006). Consequently, the slow decomposition rates of oak litter and the long-term effect of low-quality litter on soil properties and C dynamics could promote soil C storage.

My results provide evidence for the influence of tree species litter on the dynamics of C in temperate forest soils. Litter quality influences soil C dynamics over the short-term via decomposition rates but tree species can also influence soil C storage over the long-term by modifying key soil properties, which modulate the response of the soils to different types of litter inputs.

3.5. Conclusion

In the face of climate change, many countries aim to increase forest coverage as a measure to increase soil C sequestration and help reduce atmospheric CO_2 levels (Campbell-Arvai et al., 2017). Although C is stored rapidly in the above-ground biomass of fast-growing trees, the influence of tree species on soil C dynamics and storage is often overlooked. My experiment demonstrates the variable influence of different tree species on soil C dynamics and soil properties. The interaction between tree litter inputs and soil properties will affect soil C storage and CO_2 release in temperate forests and hence, it is important to select appropriate tree species for afforestation purposes. My results suggest that soil C storage could be promoted by slow-growing species such as oak, but that rapidly growing species with high-quality litter, such as sycamore, are less beneficial for increasing belowground C stocks. However, replacing sycamore with species such as ash, that grow rapidly but have lower quality litter, could alter soil C dynamics and increase soil C storage in the future.

Chapter 4. Litter quality controls soil carbon dynamics to altered litter quantity inputs in a temperate woodland.

Abstract

Forest productivity is likely to increase in response to elevated concentrations of atmospheric CO_2, altering the inputs of leaf litter into forest soils. Increased litterfall is expected to affect soil carbon (C) stocks, but such effects will vary with tree species because species-specific variations in the quality of leaf litter regulates litter decomposition and the input of C into the soil. To determine the influence from different single and mixed-species on soil C dynamics, I experimentally altered litter inputs during a 15-month field study in a managed woodland near Gisburn, UK. I established *in situ* mesocosms in single-species plots of alder, oak and pine as well as in mixed-species plots of all possible two-species mixtures. I measured differences in litter properties and initial soil properties for each species and mixture; and manipulated the quantity of litter inputs to quantify the effects of increased litterfall on soil CO_2 efflux (soil respiration). Soil C to N (C:N) ratios affected pre-treatment soil respiration, whereby soil respiration was higher in plots planted with alder, which had lower C:N ratios due to N fixation. Additionally, the distinct litter decomposition rates among species and mixtures largely predicted soil respiration, whereas alder litter (high-quality) decomposed rapidly and promoted high soil respiration, and pine litter (low quality litter) had the opposite effect. Litter removal (LR) decreased soil respiration in single-species plots of alder and oak, but not in pine or mixed-species plots. Litter addition (LA) treatments had no consistent effect, but there was a temporary increase in soil respiration in single-species plots, which lasted longer in alder and oak compared to pine and occurred in different periods of time for each species.

My study provides an insight into the variable effects of increased litterfall from different tree species on soil C dynamics. In particular, I show clear links between litter quality, decomposition rates and the l response of soil respiration to altered litter inputs.

4.1 Introduction

The concentration of CO_2 in the atmosphere has increased from 277 ppm in 1750, at the beginning of the industrial era, to 395 ppm in 2013 (Joos and Spahni, 2008). Nonetheless, terrestrial ecosystems are capable of absorbing a significant portion of anthropogenic CO_2 emissions, with much of this uptake occurring via carbon accumulation in forest biomass and soils (Le Quéré et al, 2009; Reichstein et at., 2013). In this context, forest ecosystems have received especial attention as key components in the efforts to mitigate CO_2 emissions because of their ability to storage large quantities of C. Forests cover approximately 3.8 billion ha globally (Pan et al., 2011), storing about 82 to 86% of all aboveground C (Richter et al., 1999; Six et al., 2002). At the same time, forest soils contain about 70 to 73% of all soil organic carbon (SOC; Birdsey et al., 1993; Six et al., 2002), making them a significant component of the forest C budget (Ussiri, 2017). Furthermore, the exchange of CO_2 between forests and the atmosphere via photosynthesis and respiration is ≈50 Pg C/yr annually, which is seven times greater than anthropogenic C emissions. At the same time, forest soils contain about 70 to 73% of all SOC (Birdsey et al., 1993; Six et al., 2002), making them a significant component of the forest C budget (Ussiri, 2017).

Forests vegetation and soils are key contributors to the global C budget because of their potential in mitigating the raise of atmospheric CO_2 via C sequestration (Schlesinger and Andrews, 2000; Seidl *et al.*, 2011). However, most research has focused on exploring aboveground C dynamics, underestimating soil processes and limiting our understanding

of the C pools in forest soils (Kusyakov and Domanski, 2000). These is particularly important under climate change, as forest productivity is likely to increase in response to the rise in atmospheric CO_2 levels, altering aboveground inputs into forests soils. These reinforces the importance of expanding our knowledge of belowground C dynamics, particularly because changes in forest soil respiration would alter total CO_2 emissions from forest ecosystems, affecting the considerable contribution of forests to the global C budget (Ussiri, 2017).

4.1.2. Afforestation and tree species differences

Many countries worldwide are attempting to offset C emissions and increase C storage by increasing forest coverage. (Linder et al., 2004). This has promoted the afforestation of former agricultural land as it helps increase the C pool in the aboveground biomass, and replenishes the soil C pool (Jandl et al, 2006). Research within reforestation projects has contributed to our knowledge of soil C dynamics in forests (Peltoniemi et al., 2007). Afforestation can also increase total C stocks by 18%, with the initial C accumulation occurring in the forest floor (Guo and Gifford, 2002). However, afforestation strategies aimed at simultaneously maximizing above and belowground C sequestration are scarce (Brown et al., 1996) and little is known about how the selected tree species (Batjes, 2014). Tree species is an important factor in regulating soil C dynamics in forests because of their strong influence on soil chemical properties (Vesterdal and Raulund-Rasmussen, 1998; Six et al., 2002a). For instance, many deciduous tree species (with high wood density) accumulate more aboveground C than coniferous species (with low wood density). In contrast, some coniferous species tend to accumulate more SOM in the forest floor, but less in the mineral soil compared with deciduous trees (Jandl et al., 2006). However, the effect of tree species on soil processes across sites is still unclear,

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particularly because different species vary in the way they affect the storage of C (Stone, 1975; Augusto et al., 2002; Binkley and Menyailo, 2005) and they also vary in their effect on the balance between C input (via litterfall and rhizodeposition) and the release of C during decomposition (Jandl et al., 2006). These processes are often strongly related to the variation in the quantity and quality of litter inputs among tree species and are also affected by climate. Changes in the interactions between plant inputs and soil processes make it particularly difficult to predict soil C storage under climate change. One such plant-soil interaction, which is likely to occur more frequently as a consequence of increased litterfall, is the so-called "priming effect".

Priming effects are defined as an increase in soil organic C mineralisation following the input of a fresh organic matter (Bingeman et al.,1953). Priming effects therefore have the potential to release stored C from the soil as forest productivity increases under elevated CO_2 (Reichstein et al., 2013). However, the likelihood of whether a given tree species will promote priming effects is likely to vary according to the quality of litter inputs to the soil because laboratory studies have shown that priming effects vary with the quantity and quality of organic inputs (Kuzyakov et al. 2000). Priming effects have been observed in many types of laboratory and field studies and they commonly occur in as part of terrestrial ecosystem soil C dynamics (Fontaine et al., 2011).

The mechanisms underlying soil C release by priming effects remain poorly understood (Kuzyakov et al., 2010). However, one of the first proposed mechanisms suggests links between priming effects and the quality of organic matter. Soil organic matter represents a low-quality resource for soil microorganisms and this limits the rate at which soil organic matter is mineralized. Hence, inputs of high-quality fresh organic material to the soil will result in greater nutrient availability for the soil microbial community, which results in increased microbial activity and promotes the mineralisation of soil organic matter (Lohnis, 1926; Broadbent, 1947). However, there remain fundamental knowledge gaps about how

variations in organic inputs into the soil (e.g. species-specific variation in litter quality and quantity) will regulate the occurrence of priming. For instance, a microcosm study conducted by Nottingham in 2009 measured priming effects in response to different substrates that act as a source of C. His results demonstrate that variations in the availability of C for decomposers significantly affect priming effects. As substrate that in which the supply of C occurs rapidly evoke the fastest priming effects, calculated via soil CO_2 efflux. According to these results, we can predict that leaf litter from different tree species can potentially cause different priming responses, as different tree species vary in litter quality. Furthermore, little we know about how such differences in litter quality will affect soil process facing a an increase in aboveground productivity. (Ussiri, 2017).

4.1.3. Litter quality and quantity

Variations in the quality of litter of different tree species affect soil CO_2 efflux (Giardina and Ryan, 2000; Liski et al., 2003), as species litter can vary in labile C content (Hobbie et al., 2000, Forrester et al., 2013). Litter can also differ in N and lignin content, C/N ratio, and leaf area, which are highly related to decomposition rates (Peterken, 2001; Reich et al., 2005; Hobbie et al., 2006; Vesterdal et al., 2008). For instance, differences between litter quality from broadleaved and conifer species are the reason behind their difference in decomposition rates, with broadleaved species decomposing faster, thus regulating microbial processes (Johansson, 1995; Sugihara et al., 2014). Litter quality can also affect soil pH, which in turn can alter soil microbial activity and the decomposition of soil organic matter (Blagodatskaya and Anderson, 1998). These differences enhance the importance of a better understanding of species-specific effects on soil processes, as an informed species selection in afforested land will increase C sequestration.

To explore the effect of increase litterfall in plots with different tree species and the occurrence of priming effects, I conducted a litter manipulation experiment in the field. The aim of my study was to quantify how different litter quality and alterations in litter quantity interact to affect forest soil CO_2 efflux. I used a mesocosm experiment in which I manipulated litter inputs of different tree species to assess changes in soil properties, soil CO_2 efflux, and the occurrence of priming effects. I hypothesised that:

H1) Soil CO_2 efflux in plots planted with different tree species will mirror the pattern of litter decomposition of those species.

H2) The increase in soil respiration in response to litter addition will differ among tree species; with a greater increase after the addition of "high-quality" litter, characterised by high N and low lignin content.

H3) Priming effects are the result of higher microbial activity with additional labile C inputs, and therefore the likelihood of priming effects will be greater following the addition of "high-quality" litter.

4.2. Methods

4.2.1. Field site

I conducted my study within a long-term tree growth trial established by Forest Research in 1955, which was located in Gisburn Forest, northwest England (henceforth "Gisburn"; Figure 1). A storm in 1990 fell a large proportion of the trees and the site was consequently completely fell using a helicopter to remove tree trunks, thus assuring minimal soil disturbance. The trial is currently in its second rotation (planted in April 1991) with all plots following the same tree species arrangement as the first rotation. Consequently, the soil in each plot has been under the influence of the same tree species or mixture of species for

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over 60 years. For my study, I selected single-species plots (*c.* 45-m x 45-m each) of black alder (*Alnus glutinosa L.,* henceforth alder), sessile oak (*Quercus petraea L.,* henceforth oak), Scots pine (*Pinus sylvestris L.,* henceforth pine); and 50:50 mixed-species plots of alder-oak, alder-pine and oak-pine in three replicate blocks giving a total of 18 plots.

Figure 4.1. Aerial photographs of Gisburn Forest, UK.: a) Gisburn Forest, Lancashire, UK. b) Diagram representing the distribution of plots and experimental set up at Gisburn Forest. (Images were obtained from: Wytham Woods-Imagery ©2017 Infoterra Ltd & Bluesky, Digital Globe, Map Data ©2017 Google; and Gisburn Forest-Imagery ©2017 Getmapping plc, Map Data ©2017 Google).

4.2.2. Litter treatment addition

To determine how variation in litter quantity affects soil C dynamics, I conducted a litter manipulation experiment using mesocosms to contain treatments within a discrete area and avoid disrupting long-term measurements at the sites. In each plot, I installed three mesocosms, consisting of polypropylene tubes (20-cm inner diameter and 12-cm height) that were sunk into the soil to 3-cm depth and located at least 1-m from the nearest tree trunk. All

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mesocosms were installed in May 2014, and were left undisturbed for one month before measurements were taken. All vegetation and litter within the collars were carefully removed after installation and checked monthly to remove recently germinated seedlings.

To quantify pre-treatment differences in soil CO_2 efflux, I measured soil respiration during three months prior the addition of treatments. After this period, one mesocosm per plot was left as an undisturbed control with natural litter inputs (henceforth CT), in the second mesocosm, all litter was removed (henceforth LR) and added to the third mesocosm, effectively doubling litter inputs (henceforth LA). Litter manipulation was conducted monthly, starting in July 2015 and terminating after 15 months of observations in October 2016.

4.2.3. Soil respiration measurements

To determine if litter treatment affected soil respiration, I took monthly soil CO_2 efflux measurements of each mesocosm from August 2015 (one month after the start of treatments) to October 2016 using a soil CO_2 survey system (Li-8100A; LiCor BioSciences, Lincoln, Nebraska, USA). To eliminate the effects of turbulence from chamber closure, a 15-s post-purge period and a 15-s dead-band period was set for each measurement. No measurements were made in December 2015 and January 2016 because the soil was frozen.

4.2.4. Litter collection and processing

I collected freshly fallen litter of all species from two litter traps per plot during October and November 2015. Litter traps were constructed using polypropylene tubes and mesh (1-m above the soil surface, 1-m^2 area). Leaf litter samples were brought back to the lab and oven-dried at 60 ºC. Litter for decomposition experiments was manually chopped and sieved to 1 cm

and the remaining litter was ground using a ball mill (Retsch MM400, Hann, Germany) for chemical analyses.

To assess litter quality, I analysed three analytical replicates for each study species or species combination (24 samples total). I measured the C and N content of the leaf litter on oven-dried (60 °C) ground subsamples using a Vario EL III Element Analyser (Elementar, Hessia, Germany). I conducted fibre analysis of the litter samples following the Van Soest method (Van Soest 1963). Briefly, acid detergent lignin (ADL) and cellulose were determined on 1 g oven-dried (60 °C) knife-milled (1 mm) litter samples using a Fibertec^{TM} 1020 hot extraction unit (Foss, Hilleroed, Denmark). Each sample was placed in a glass crucible and 1-g of celite was added as a filtration aid. Total acid detergent fibre (ADF) was obtained after washing the samples with boiling acid detergent solution (0.5 M H_2SO_4 + CTAB) for 1 h, followed by a 5-min acetone soak; the resulting ADF samples were drained and oven-dried at 105 °C for 5 h. Cellulose was then solubilized by soaking the ADF samples in H_2SO_4 for 3 h, followed by washing with hot deionised H_2O (until acid-free). The remaining sample was dried for 2 h at 130 °C and then ashed in a furnace at 525 °C for 3 h. Cellulose content was calculated from the mass loss after solubilization and lignin content was calculated from the mass of the residue.

4.2.5. Litter decomposition experiment

I used litterbags to measure differences in decomposition rates for each litter type in my study, where each litter type decomposed in the plot of origin. The experiment was conducted in the field using litterbags (10-cm x 10-cm) made of nylon mesh (aperture 1 mm). Each bag was filled with 3 g of leaf litter of one of the three study species or two-species mixtures (six litter types in total), and four litterbags were placed in each of the three plots planted with the corresponding species (72 litterbags in total). Vegetation and litter was carefully removed from the soil surface in an area *c.* 1-m from the mesocosms,

and litterbags were pinned to the soil to ensure good contact. The experiment started in July 2015 and I collected two bags in September 2015 (after 3 months) and two bags in January 2016 (after 6 months). After collection, leaf litter was removed from the litterbags, oven dried (60 ⁰C) and weighed to calculate mass loss over time.

4.2.6. Soil collection and analysis

To measure initial soil properties in the study plots, I collected six soil samples at 0-10 cm depth using a 2.5-cm diameter soil corer and mixed them to create one composite sample per replicate plot. Following the same procedure, I collected three soil cores from within the mesocosms at the end of experiments to determine leaf litter effects on soil properties. All samples were sealed in plastic bags, brought back to the lab and either processed within 24 h of collection or oven-dried for further analysis.

4.2.7. Soil water content and pH

I determined gravimetric soil water content using 20-g subsamples of fresh soil. I measured the fresh weight of the soils within 24 h of collection and then dried the samples at 105 ⁰C for 48 h to calculate soil water content.I measured soil pH on a slurry of soil in deionised water (1:3 ratio) using a S220 Seven Compact pH meter (Mettler Toledo, Columbus OH, USA).

4.2.8. Soil total C and N; and microbial biomass C and N.

I measured the total C and N content of the soil on ground subsamples of oven-dried soil using a Vario ELIII Element Analyser (Elementar, Hessia, Germany). To determine soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN), I performed the chloroform fumigation extraction method (Vance et al, 1987) with modifications (Jones &

Willet, 2006) on paired subsamples (8-g dry weight equivalent) of fresh soil. Briefly, one subsample was fumigated with ethanol-free chloroform for 24 h and both subsamples were extracted in 40 ml 0.5M K_2SO_4, shaken at 200 rpm for 1 h, centrifuged at 3000 rpm, and filtered. The extracts were refrigerated for ~10 days until analysis for total C on a TOC-L combustion analyser coupled with a TNM-L unit (Shimadzu Corp, Kyoto, Japan). For MBN analysis, the extracts were digested with potassium persulphate. Briefly, 3.0-ml of potassium persulphate were added to a bottle containing 1-ml of soil extract. Samples were autoclaved for 20 minutes at 121 $^{\circ}$C and analysed in an Autoanalyser (Bran and Luebbe AA 3; Seal Analytical, Southampton, UK) to determine total nitrogen content. Soil MBC and MBN were estimated by the difference in content between fumigated and unfumigated subsamples, without correction for extraction efficiency.

4.2.9. Soil ammonium-N and nitrate-N extraction.

I extracted ammonium-N and nitrate-N from soil samples using a KCl solution. As the conversion of ammonium to nitrate can occur rapidly after sampling, the extraction process started during sampling in the field. From the composite soil sample for each plot (see section 2.3.2. above), 2 g were subsampled and added to each a falcon tube containing 20-ml of 2M KCl solution. All tubes were kept cool and brought back to the lab to be processed within 24 h. Samples were shaken at 200 rpm for 1 h, allowed to settle for 30 minutes and filtered (Whatman 42 filter paper). Samples were then refrigerated until analysis for NH_4^+-N and NO_3^--N on an Autoanalyser (Bran and Luebbe AA 3; Seal Analytical, Southampton, UK).

4.2.10. Statistical analysis

All statistical analyses were performed in R version 3.2.4 (R Core Team, 2016) using the vegan (Oksanen et al., 2017) and lme4 packages (Bates et al., 2015) for multivariate and mixed model analyses, respectively.

I used Principle Components Analysis (PCA; *rda* function) to explore the initial differences in soil properties among the six soil types. I included all soil variables measured (C, N, MBC, MBN, C:N ratio, pH, ammonium-N and nitrate-N) and scaled them for direct comparison. The scores of the first two ordination axes (PC1 and PC2) were included as explanatory variables in linear models (*lm* function), to investigate the effect of initial soil properties on mean pre-treatment soil CO_2 efflux, whereby CO_2 efflux was modelled as a function of PC1 and PC2 as explanatory variables; with block as included as an error term. The full model included the interaction between PC1 and PC2, and the best model was achieved by dropping non-significant terms to identify the most parsimonious model. The final model fit was assessed using diagnostic plots (Crawley, 2007).

I used linear mixed effects models (*lmer function*) to determine the influence of tree species on pre-treatment soil respiration and the effect of litter treatments on soil respiration. For pre-treatment soil respiration models, the full model included tree species as a fixed effect, and block and time as random effects. To explore the influence of litter treatment and tree species on soil respiration, the full model included litter treatment and tree species as explanatory variables and block and time as random effects. Due to the strong influence of temperature on soil respiration, soil temperature was included as a covariate in all models of soil respiration. The significance of individual terms was determined by dropping terms individually using AICs and *p* values to check for model improvement, until the best fit was reached. All final models were tested against the appropriate null model and the model fit was assessed using diagnostic plots.

To determine the effects of litter treatment on soil variables, I calculated response ratios using the equation: *RR = ln (Rx / Rc)*; where *'ln'* is natural logarithm; *'Rx'* is the value for treatment;

and *'Rc'* is the value for the control. Then I used linear models to determine the influence of litter treatment on soil properties (C, N, MBC, MBN, pH and C:N), including block as an error term.

Finally, for months in which I measured a disproportionate increase in soil respiration with litter addition, I calculated priming effects for each species from the differences soil respiration among litter treatments as:

$$PE = (SR_{LA} - SR_{CT}) - (SR_{CT} - SR_{LR}),$$

where PE is the priming effect, SR_{LA} is the soil CO_2 efflux in the litter addition treatment, SR_{LR} is the soil CO_2 efflux in litter removal treatments, and SR_{CT} is the soil CO_2 efflux in the controls.

4.3. Results

4.3.1. Litter quality and initial soil properties

Litter properties varied among species (Table 4.1.): pine litter had the highest C content, C:N ratio and the highest ratio of lignin to nitrogen (L:N ratio). Accordingly, mixed litter in which pine was one of the constituent species also had high carbon and lignin content. Alder had the highest N content and the lowest C:N ratio. Using the L:N ratio as a proxy for 'litter quality', alder and alder- oak were the litter types with the 'highest quality', while pine and oak-pine were 'low-quality' litter. Initial soil properties also varied among soil types (Table 4.1.). Single species plots of pine had the highest total C, while alder soils had the highest total N and the lowest MBC, MBN and C:N ratio. At the same time, oak soils had the highest MBC and MBN. In soils from mixed species plots, the alder-pine mixture had the highest C, MBC, N and MBN; and soils in the mixed pine–oak plots had the lowest N content and C:N ratio (Table 4.1.). Accordingly, ordination showed clear separation of species and species mixtures linked to initial soil properties (Figure 4.1.). Total C, total N

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and ammonium-N concentrations were closely aligned with the first ordination axis, which explained the variation between alder-pine and alder-oak soils. On the other hand, soil pH, soil nitrate-N concentrations and C:N ratios were aligned with the second ordination axis, which explained the clear separation of alder soils from soils under oak-pine and oak. When the scores from the first two ordination axes were included as explanatory variables in linear models of soil respiration, the second ordination axis (PC2) explained a significant proportion of the variation in pre-treatment respiration rates (R^2 = 0.25, p = 0.01), where alder-oak plots had the highest soil CO_2 efflux and pine plots had lowest (Figure 4.3.a).

Table 4.1. Leaf litter properties of different tree species and soil properties from plots planted with a single species or a two-species mixture at Gisburn Forest, UK. The table shows carbon (C), nitrogen (N), lignin (L) and cellulose content, and C:N and L:N ratios for freshly fallen leaf litter. And of alder (A), oak (O), pine (P), and two-species litter mixtures (AO = alder and oak, AP = alder and pine and OP= oak and pine) containing an equal mass of both species.

		Total C (%)	Total N (%)	C:N ratio	Lignin (%)	Cellulose (%)	L:N ratio		
	alder	49.00	3.60	13.60	5.55	37.73	1.54		
	oak	47.01	1.19	39.64	8.20	10.79	6.89		
	pine	63.55	0.93	59.10	16.37	12.60	17.6		
Litter	alder-oak	48.01	2.40	26.62	6.88	24.26	4.22		
	alder-pine	56.27	2.27	36.35	10.96	25.16	9.57		
	oak-pine	55.28	1.06	49.37	12.29	11.70	12.25		
		Total C (%)	Total N (%)	MBC	MBN	C:N ratio	pH	NH_4-N	NO_3-N
	alder	9.24	0.5	260.25	55.00	18.76	4.03	68.31	20.11
	oak	7.97	0.37	362.10	83.00	21.57	4.59	57.72	2.43
	pine	12.94	0.67	353.19	69.53	20.75	4.35	80.12	3.21
Soil	alder-oak	12.22	0.61	449.99	82.79	20.33	4.12	62.79	15.38
	alder-pine	16.47	0.79	461.32	92.31	20.63	4.11	68.53	6.19
	oak-pine	9.33	0.43	365.25	76.09	21.74	4.62	64.34	3.19

4.3.2. Soil pre-treatment respiration and litter decomposition

Soil pre-treatment respiration varied among soil types (Figure 4.3.). Mean respiration from soils, in which alder was present (alder, alder-oak and alder-pine), had the highest CO_2 efflux; whereas pine, oak and pine-oak plots had the lowest. Accordingly, litter decomposition also varied significantly among species ($R^2 = 0.94$, $p = 0.001$; Figure 4.3.b), with alder and alder-oak litter ('high quality') decomposing significantly faster than all other litter types, whereas pine ('low quality') had the slowest decomposition rate.
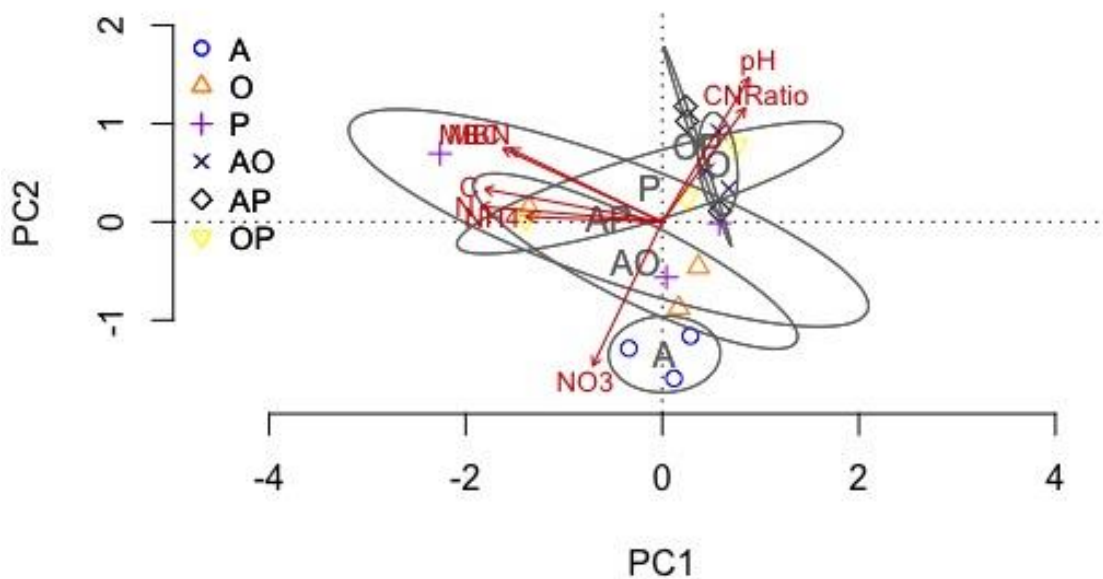
Figure 4.2. PCA ordination showing the initial differences in soil properties in field plots planted with different tree species and two-species mixtures at Gisburn Forest, UK; where A, O and P are monoculture plots of alder, oak and pine respectively; AO is a 50:50 mix of alder and oak, AP is 50:50 mix of alder and pine, and OP is 50:50 mix of oak and pine; vectors show the relative influence of each soil property on the distribution of samples in ordination space, where C is

carbon, N is nitrogen, MBC is microbial biomass carbon, MBN is microbial biomass nitrogen, pH is soil pH, NH4 is ammonium-N and NO3 is nitrate-N.

4.3.3. The effect of litter manipulation on soil CO_2 efflux.

Soil CO_2 efflux in all species plots showed strong seasonal variation, where colder months caused a pronounced drop in soil CO_2 efflux in alder and oak plots, but there was a weaker decrease in pine and mixed-species plots. Soil CO_2 efflux in all plots responded to the interactive effect of litter treatment and tree species, with soil CO_2 efflux differing among species and a significant decrease in soil CO_2 efflux in LR treatments ($x^2 = 59.194$, $p = 0.001$; Figure 4.4.). soil CO_2 efflux was lower in LR mesocosms compared to controls in single-species plots of alder and oak, ($x^2 = 114.24$, $p = 0.001$; Figure 4.4.), but not in pine plots. There was also no significant effect of litter treatment on soil CO_2 efflux in mixed-species plots, but soil CO_2 efflux varied with species mixture ($x^2 = 57.448$, $p = 0.001$; Figure 4.5.), whereby alder-oak plots had the highest respiration rates and oak-pine plots the lowest.
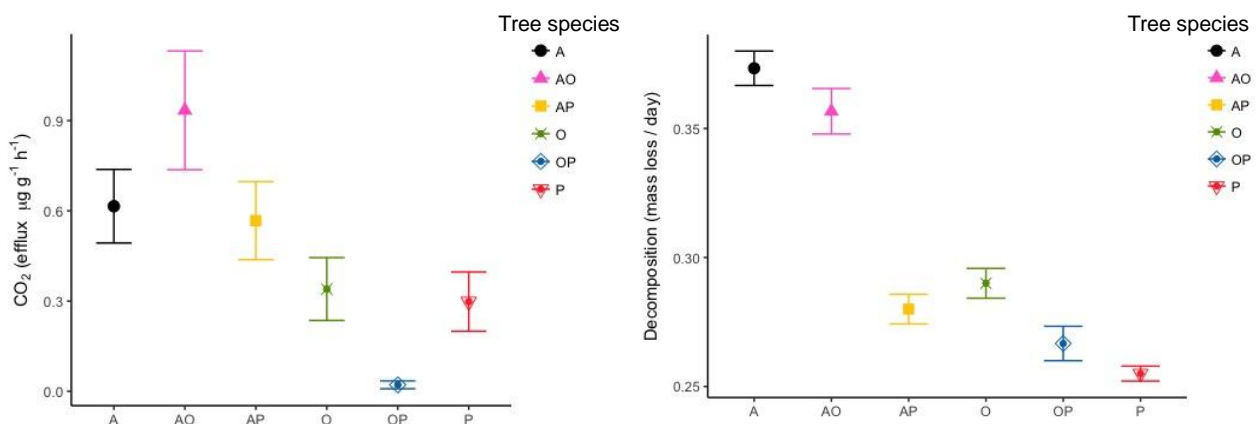
Figure 4.3. Pre-treatment mean soil CO_2 efflux and litter decomposition rates from field plots ($n = 3$ plots) planted with different species and two-species mixtures at Gisburn Forest, UK. a) Mean soil CO_2 efflux during three months before the start of litter treatments. b) Mean mass loss of litter from

different tree species during 6 months of decomposition, measured using litterbags. Bags in each plot contained leaf litter of the corresponding species and were collected at two time points over six months to calculate mass loss. Means and standard error bars are given for $n = 3$ per tree species and mixture.

Surprisingly, I observed no clear or consistent increase in soil respiration in LA mesocosms and consequently, priming effects occurred too infrequently for formal analysis. However, there was a disproportionate increase in soil respiration in LA mesocosms in all alder plots in May and June 2016 (Figure 4.6.), which lends partial support to my third hypothesis
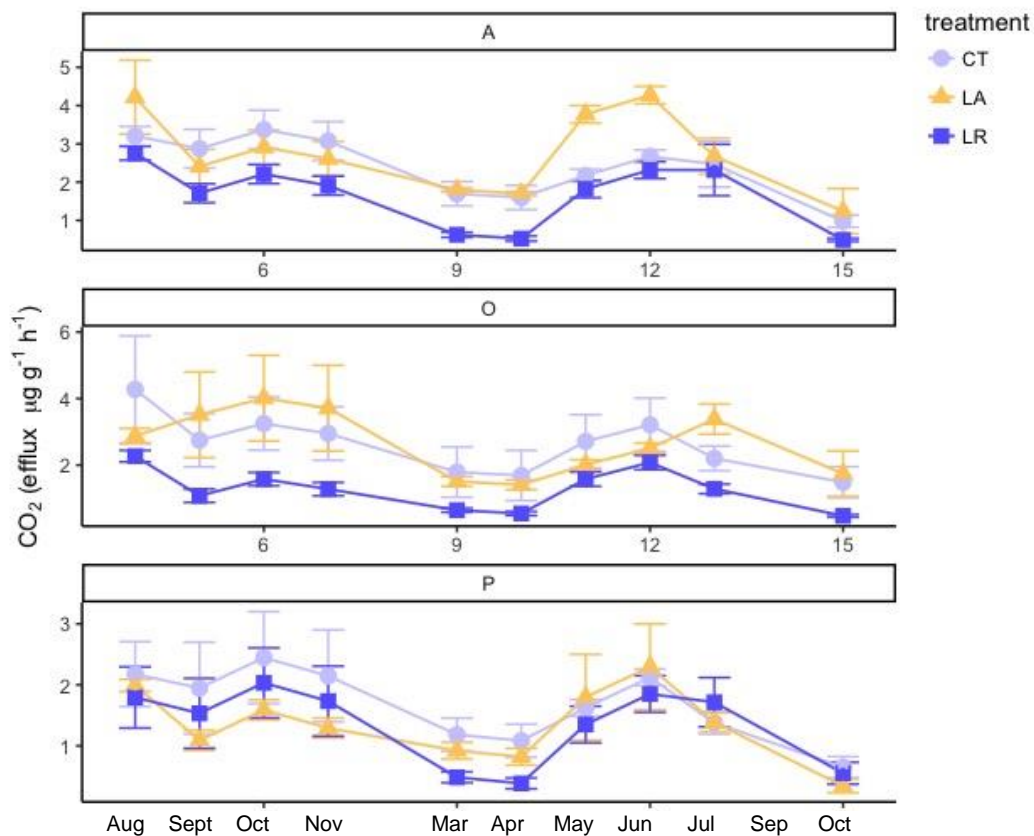
Figure 4.4. Soil CO_2 efflux from mesocosms with different litter quantity in plots planted with single tree species at Gisburn Forest, UK. The lines show the monthly mean soil CO_2 efflux from each species plot (n = 3 plots per species) in response to litter treatment during 15 months of observations; where CT is control, LA is litter addition and LR is litter removal; and A, O and P are plots of alder, oak and pine respectively.
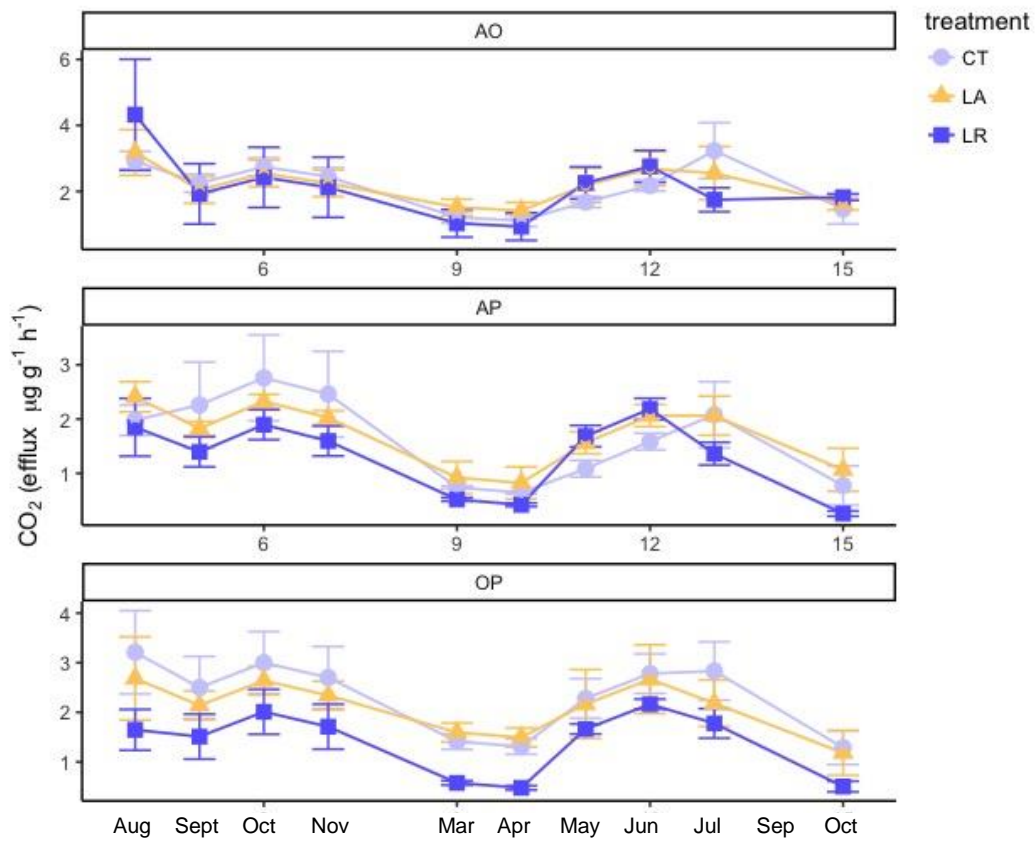
Figure 4.5. Soil CO_2 efflux from mesocosms with different litter quantity in plots planted with two-species mixtures at Gisburn Forest, UK. The lines show the monthly mean soil CO_2 efflux from each mixed species plot (n = 3 plots per mixture) in response to litter treatment during 15 months of observations; where CT is control, LA is litter addition and LR is litter removal; and AO is a 50:50 mix plot of alder and oak, AP is 50:50 mix plot of alder and pine, and OP is 50:50 mix plot of oak and pine
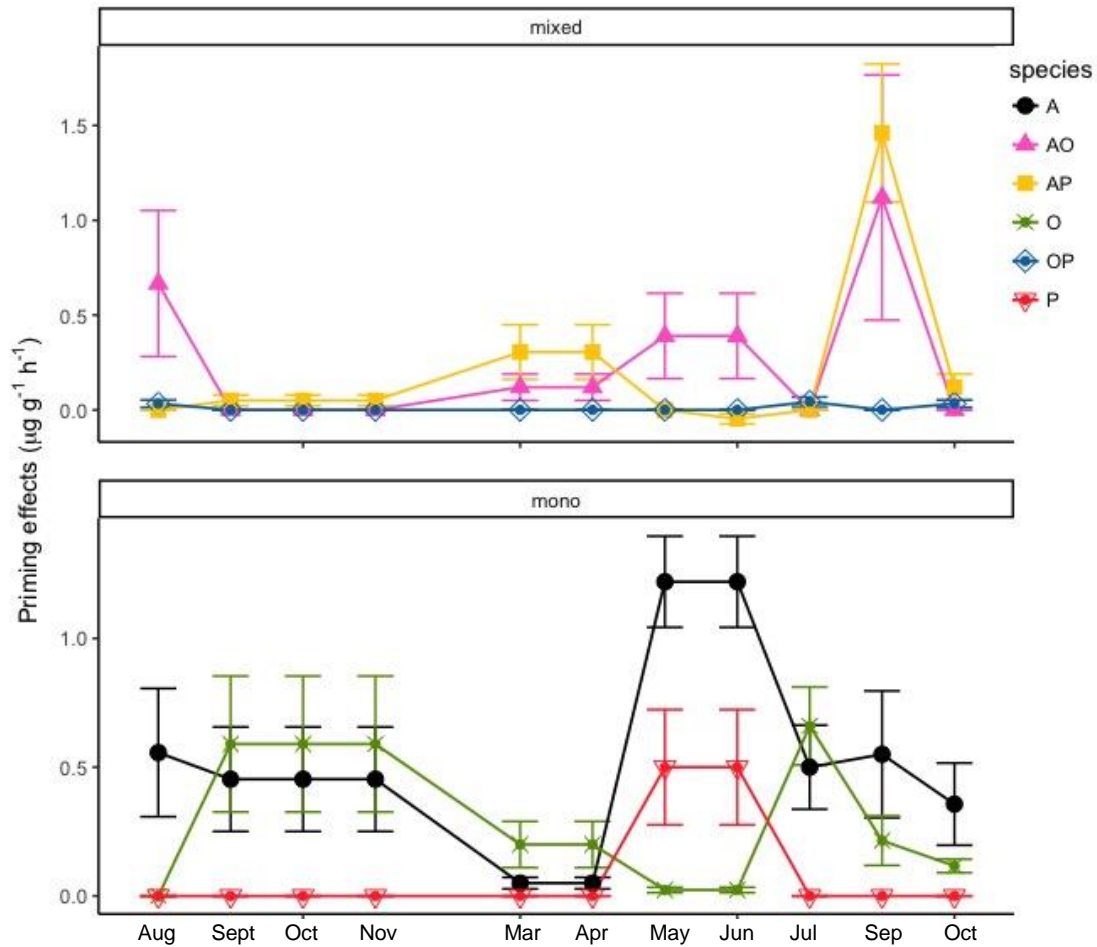
Figure 4.6. Priming effects from different tree species plots planted with single tree species and two-species mixtures at Gisburn Forest, UK; Monthly means are shown during 15 months of observations (n = 3 plots per species), where A, O and P are single species plots of plots of alder, oak and pine respectively; and AO is a 50:50 mix plot of alder and oak, AP is 50:50 mix plot of alder and pine, and OP is 50:50 mix plot of oak and pine.

4.3.6. The effect of litter manipulation on soil properties

The effect of litter manipulation in soil properties at the end of the experiment varied among tree species (Figure 4.7.) and mixtures (Figure 4.8.). Soil C and N declined in oak plots during the study regardless of litter treatment, but there were no significant changes

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in alder and only an increase in soil N with litter addition in pine plots. By contrast soil MBC, and MBN was lower in both LR and LA treatments relative to controls within pine and alder, but not in oak plots.
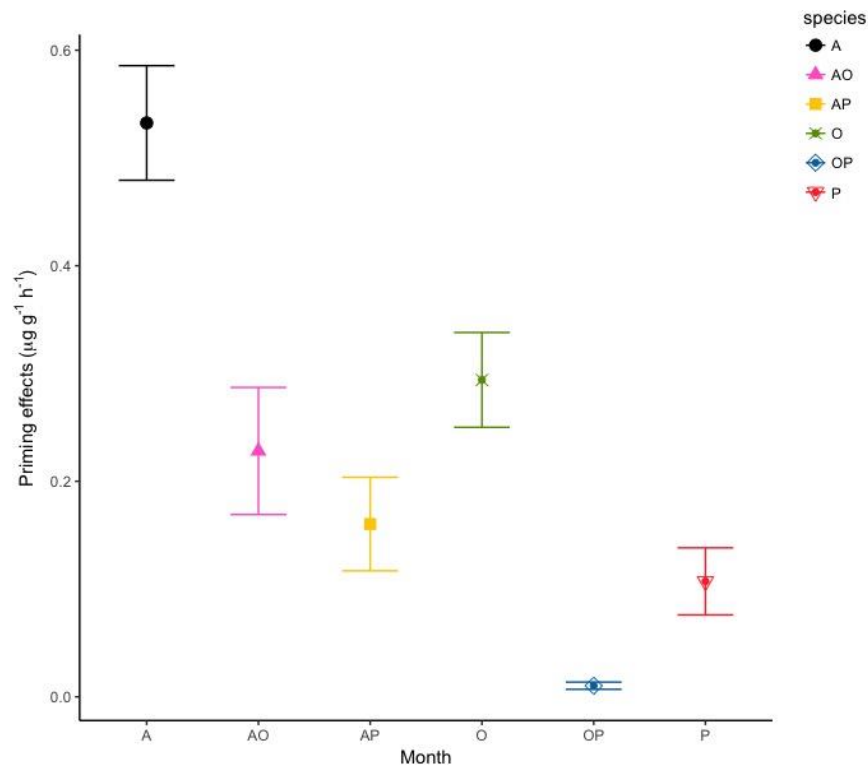
Figure 4.7. Mean priming effects from different tree species plots planted with single tree species and two-species mixtures at Gisburn Forest, UK; Dots represent means that were calculated from 15 months of observations (n = 3 plots per species), where A, O and P are single species plots of plots of alder, oak and pine respectively; and AO is a 50:50 mix plot of alder and oak, AP is 50:50 mix plot of alder and pine, and OP is 50:50 mix plot of oak and pine.

4.4. Discussion

My study assessed how different tree species affect C dynamics in temperate forest soils. Specifically, it provided an insight into how differences in litter quality and quantity interacted to affect soil respiration, soil properties, and the occurrence of priming effects.

4.4.1. The link between soil properties, litter decomposition and soil respiration.

Soil pH and C:N ratios affected pre-treatment soil respiration and explained a large portion of the separation in ordination space between tree species plots. The lower soil pH in plots in which alder was present could be due to the established influence of alder to soil acidification (Rhoades et al, 2001). Additionally, the differences in C:N ratios among plots planted with different species, were mainly driven by higher concentrations of total N in soils where alder was present. This can be explained by the association of alder roots with the N- fixing bacteria, such as *Frankia alni,* which converts atmospheric N into usable compounds like ammonium-N and nitrate-N (Mitchell and Ruess, 2009). Alder not only benefits from the association with N-fixing bacteria, but can also promote higher nutrient content of adjacent tree species when planted in mixtures (Binkley *et al.,* 1992). Hence, the similarity in soil properties and soil CO_2 efflux in plots where alder is present is likely to be largely due to the role of alder in increasing the nutrient content of the soil and neighbouring plants.

The soil C:N ratio has long been used as an indicator for soil quality (Batjes, 1996).This is because greater inputs of available soil nitrogen increases microbial activity, as microbes are less likely to be limited by N, which promotes higher soil CO_2 efflux (Tarrant and Trappe, 1971). By contrast, the higher C:N ratio in oak and oak-pine plots explains low pre-treatment soil respiration rates.

In support of my first hypothesis, differences in soil respiration largely reflected the species-specific rates of litter decomposition in my study (Figure 4.3). Litter decomposition in terrestrial ecosystems directly regulates the input of nutrients and C entering the soil, thus affecting respiration (Veen et al., 2015). At the same time, differences in litter decomposition are regulated by litter quality, with higher quality litter decomposing faster (Wardle, 2002). According to the litter properties I measured in my study, I expected to see the most rapid mass loss in alder litter, followed by oak, and then the alder-oak mixture with corresponding patterns in soil CO_2 efflux. Whereas the decomposition rates conformed to expectations, soil respiration tended to be higher in alder-oak mixed plots than in alder, and in alder-pine mixed plots compared to oak plots. This finding suggests both a key role of N availability from alder litter and supports previous research on the facilitative effect of litter mixtures over decomposition via microbial activity and soil respiration (Wardle et al. 1997).

The effect of litter manipulation on soil CO_2 efflux and the occurrence of priming effects.

The effects of litter removal treatments can be attributed to the differences in decomposition rates of leaf litter present on each plot. Slow decomposition rates can often promote the accumulation of organic matter in the forest-floor. This is particularly important at Gisburn Forest, as the soils have considerably high moisture and high water tables (Mcnamara et al, 2008), which can in turn slow down litter decomposition and promote accumulation of organic matter (Strakova et al, 2012). As I only removed litter that was clearly identifiable as leaves or pine needles, I expected the LR treatment to have a smaller effect in pine and oak-pine plots, as a result of greater availability of organic carbon in the forest floor and low respiration rates from low-quality litter. Accordingly, litter removal had no, or only a minor effect in pine and in mixed-species plots. At the same

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time, the removal of the litter in the LR treatments had a significant effect on respiration rates in single-species alder and oak plots, which had higher decomposition rates (Figure 4.3.). Nonetheless, it is important to note that litter removal had no effect in alder-oak mixtures, despite high rates of litter mass loss.

Contrary to my second hypothesis, litter addition (LA) treatments had no consistent effects. The effects of litter addition were temporary in single-species plots but lasted longer in alder and oak compared to pine, which follows the pattern of litter quality and decomposition of these species. However, doubling the amount of litter inputs not only doubles the quantity of C and nutrients entering the soil, but also increases the amount of compounds that have a negative impact on microbial communities and decomposition (Facelli and Pickett, 1991). For instance, the high content of tannins and phenolic acids in pine litter reduce microbial activity and therefore, affect C dynamics by reducing soil respiration (Nierop et al, 2006). The effect of litter addition in mixed-species plots was unclear and in all plots, the increase in soil respiration with litter addition was mainly limited to warm months when decomposition is expected to be rapid (Figures 4.4. & 4.5.).

Given the limited effects of litter addition, it is unsurprising that I did not observe clear soil CO_2 release by priming effects in my study. However, it is important to note that I measured a disproportionate increase in soil respiration in response to litter addition treatments in single-species alder plots during months when soil respiration was at its highest (May and June 2016). This finding provides partial support to my third hypothesis that high-quality litter is more likely to produce priming effects by stimulating soil microbial activity.

My results provide evidence for the influence of variations in tree species, litter quality and quantity on C dynamics, in managed temperate forest soils. Increased litter inputs did not have the same effect on soil respiration and the occurrence of priming effects across all

species and mixtures, because the effects of the experimental treatments were regulated by litter quality and decomposition rates. Additionally, my results give an insight on how increased litterfall, which is likely to increase under climate change, could have variable effects on soil C dynamics in single-species and mixed-species plantations.

4.5. Conclusion

My results demonstrate the influence of tree species on soil C dynamics in managed temperate forest soils via the quality and quantity of leaf litter. Increased litter inputs had variable effects across species and mixtures, because the effects of the experimental treatments were regulated by decomposition rates. Additionally, my results give an insight into the potential impact of altered litterfall on soil C dynamics in single-species and mixed-species plantations under climate change.

Increased litterfall is expected as rising atmospheric CO_2 levels promote forest productivity (Raich and Schelsinger, 1992). My experiments demonstrate variable responses of soil respiration and soil properties to changes in litter inputs under different tree species. Without appropriate species selection, plant-soil interactions could either increase or reduce the release of CO_2 from temperate forests, highlighting the importance of improving our understanding of tree species responses to environmental changes. My study demonstrates that mixed-species plantations have lower soil respiration rates overall and also seem to be less sensitive to increased litter inputs, suggesting that the right mixture of species might be key to increasing soil carbon sequestration in future.

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Chapter 5. Tree species identity and litter quality regulate soil carbon dynamics in response to inputs on 'foreign' litter in a managed temperate woodland.

Abstract

An informed selection of tree species for rotations in managed forests could reduce forest CO_2 emissions in the UK. However, it is still unclear how the rotation of tree species can improve the stocks of carbon (C) in forest soils and mitigate the rise of atmospheric CO_2 efflux. The 'home field advantage' (HFA) of litter decomposition is likely to be particularly important in this context, as planting one tree species in soil conditioned by another could also modify the input of C and nutrients into the soil during the first years of a new rotation period. Here, I conducted a field experiment in Gisburn forest, UK, to explore the effects of 'foreign' litter additions in soil C dynamics. I measured key litter properties of alder, oak and pine, and measured soil properties in single-species plots of the same species. I used a reciprocal transplant experiment in mesocosms within the plots to assess how the HFA influences litter decomposition and soil CO_2 efflux (soil respiration) in 'home' or 'away' soils during 15 months. Additionally, I measured the changes in soil properties after 'foreign' litter inputs. The occurrence of HFA varied among species, with alder soils favouring the decomposition of 'home' litter when compared to 'foreign' oak or pine litter. In contrast, decomposition in pine plots seemed to be driven by litter quality, rather than the HFA. The HFA was also apparent in soil CO_2 efflux, with the highest respiration rates over 'home' litter in alder and oak, but not in pine plots. Soil properties varied with 'foreign' litter addition, and increased total soil C content of pine soils with alder litter suggested that the addition of 'high-quality' litter could promote C storage. By contrast, the results suggest that the addition of 'foreign' litter might reduce C stocks in oak soils, irrespectively of litter quality.

My results demonstrate how an informed selection of species for forestry rotations could affect soil C dynamics and provide an insight into the possible role of the HFA for increasing soil C stocks during forestry rotations.

5.1 Introduction

Afforestation and land management practices aimed to reduce CO_2 emissions while ensuring sustainable timber production, is influencing land-use policy in the UK (Jenkins et al., 2011). Practices such as afforestation are designed to contribute to mitigating the rise in atmospheric CO_2, helping nations worldwide meet their Kyoto targets (van Kooten & Johnston, 2016). Increased afforestation across the UK is a current policy target, which is most likely to use low-quality agricultural land (McNamara, 2008). Indeed, the afforestation of grassland or moorland has become common practice, as it has great impact on reducing inputs of CO_2 to the atmosphere (Calder, 1990; Fowler, 1989; Soulsby and Reynolds, 1994). At the same time, afforestation can also alter nutrient cycling and soil processes as consequence of forest growth (Robertson, 2008). However, these effects can vary with tree species, climate and soil type (Ulrich, 1983; Cape et al., 1991; Brown and Iles, 1991; Parker, 1983; Miller et al., 1990). Forests are important in global C cycling and sequestration as they contain *c.* 80% and 40% of all global terrestrial aboveground and belowground C stocks respectively (Kirschbaum et al., 1996; Schimel et al., 2001). However, many C offset calculations are based primarily on aboveground biomass in forests because we still have a limited understanding of belowground C storage and its response to changes in climate (Six et al., 2002). Additionally, despite the importance of a careful selection of tree species used in afforestation of land for environmental reasons, the main criteria for tree species selection are usually based on the economic value of the timber (Jenkins et al., 2011) and the effect of different species on soil C dynamics remains

unclear (Vesterdal et al., 2012), which can potentially reduce the effectiveness of afforestation as a tool to reduce CO2 emissions.

In many temperate forests, the soil contains more than twice as much C as the aboveground biomass (Eswaran et al., 1993; Goodale et al., 2002). In these ecosystems, the effects of tree species on the storage of C should be given special attention because they help regulate forest functioning (Grigal and Ohmann, 1992; Yang et al., 2005). Different tree species, and particularly differences in trees functional traits, can have a large impact on forest processes (Bardgett and Wardle, 2010). Trait differences and their ecosystem implications have been widely discussed in ecology (e.g. Grime 1997; McGill et al., 2006; Wright et al., 2013), with studies demonstrating that conifers, which are usually adapted for low nutrient conditions, characteristically have low tissue production, lower leaf area, and lower leaf nutrient concentration compared to broadleaf trees (Wright et al. 2004; Aerts and Chapin 2000). Importantly, plant traits also define litter quality, whereby plant species adapted to low nutrient conditions generally produce litter with low concentrations of water soluble compounds and high concentrations of lignin and cellulose, compared to species adapted to high nutrient conditions (Bardgett and Wardle, 2010). Consequently, differences in tree species' functional traits will regulate the concentration of C and nutrients input into forest soils via leaf litter decomposition.

During leaf litter decomposition, large quantities of C and nutrients enter the forest soil (McNaughton et al., 1989), which is estimated to contribute about 70% to the annual forest C flux, with leaf litter decomposition as the primary source of soil C and nutrients to the soil (Warembourg and Paul, 1977). During leaf litter decomposition, nutrients from organic materials are mineralised by microorganisms and made available for plant uptake, whereas C is either stabilised and stored in the soil or released back to the atmosphere as CO_2 (Aber and Melillo, 1991; Schlesinger, 1997; Gartner and Cardon, 2004). The turnover, storage and release of C and nutrients during decomposition is the result of many complex

interactions, which are influenced by soil type, decomposer communities, climate conditions and tree species (Vesterdal and Raulund-Rasmussen, 1998; Côté et al., 2000; Callesen et al., 2003; Jandl et al., 2007; Prescott, 2010). At the same time, leaf litter decomposition facilitates the recycling of nutrients and chemical elements, and regulates forest restoration and productivity (Cleveland et al., 2011). The interaction of the physical and chemical environment (e.g., temperature, humidity), litter quality (e.g., C:N, lignin:N), and soil decomposers (e.g., bacteria, fungi, and invertebrates) regulate leaf litter decomposition (Hättenschwiler et al., 2005; Prescott, 2005). It follows that leaf litter from different tree species, which vary in their physical and chemical properties, will show differences in their rate of decomposition and therefore, affect soil processes differently. Additionally, it is still unclear the close interaction that exist between decomposer and substrate, which can often favour decomposition of certain species over other (Ayres et al., 2009).

A range of decomposers present in the soil play a key role in leaf litter decomposition as they may particularly be better at decomposing litter from a specific plant with which they are associated (Veen et al., 2015). Previous research suggests that litter may decompose faster in the habitat from which it was derived than in other habitats. This phenomenon has been called the 'home-field advantage' (HFA) effect (Gholz et al., 2000; Ayres et al., 2009).

The HFA occurs after a competitive adaptation, in which soil decomposers and litter type create coexistence mechanisms, as litter is the main source of nutrients and energy for soil organisms (Lin et al., 2017) In this substrate–microbial interaction, plant species can influence the activity of the soil microorganism community directly through leaching or the release of exudates (Pfeiffer et al., 2013), or indirectly by affecting competitive interactions among soil decomposers (Cesarz et al., 2013; Austin et al., 2014). After a long term

interaction characterised by a continual input of substrate with same characteristics, microbial decomposers develop a preference for decomposing this particular kind of litter (Ayres et al., 2009), generating specificity of decomposers for a particular litter type.

Ayres et al. (2009) calculated the magnitude of HFA in forest ecosystems, showing that positive HFA accelerated litter mass loss by approximately 8% using reciprocal transplant experiments. However, Gießelmann et al. (2011) showed that the microorganisms and mesofauna asociated to decomposition had no significant effects on HFA in an Atlantic rainforest. Similarly, St. John et al. (2011) found no HFA effect in a forest–grassland reciprocal transplant experiment and attributed the result to an adaptation of soil microbial communities to different litter resources. These studies suggest that the magnitude and direction of the HFA effect can vary and attribute a great part of this variation to litter quality (Ayres et al., 2009; Veen et al., 2015). Also, it has been shown that litter translocation will affect species decomposition rates. For instance, low-quality litter that contains recalcitrant or toxic secondary compounds may generate a large HFA because fewer soil communities can decompose these compounds (Austin et al., 2014; Chomel et al., 2015). By contrast, high-quality litter, which contains easily degradable compounds, could be expected to have a lower HFA because most soil decomposers can decompose them (Ayres et al., 2009; Austin et al., 2014; Veen et al., 2015b). Accordingly, it is expected that decomposition will vary according to litter quality of tree species, however it is unclear how decomposition will be altered following a transplantation of litter.

Building on the work described in Chapter 3, the experiment I present here aimed to assess the whether the home-field advantage affects litter decomposition and soil respiration *in situ*, and to determine the impact of 'home' *vs.* 'foreign' litter on soil properties. I used a litter transplantation experiment in monoculture stands of three different tree species to test the following alternative hypotheses:

H1) The effect of decomposing litter on soil C dynamics will depend on litter quality, with high quality litter promoting higher respiration, regardless of litter origin.

H2) The home-field advantage of litter decomposition will be reflected in soil respiration rates, whereby soil respiration will be higher for litter decomposing at "home" compared to litter decomposing "away".

H3) The effects of "foreign litter" on soil properties will depend on litter quality, with high quality litter increasing MBC in response to fast litter decomposition.

My results demonstrate how an informed selection of species for forestry rotations could affect soil C dynamics and provide an insight into the possible role of the HFA for increasing soil C stocks during forestry rotations.

5.2. Methods

5.2.1 Field site

I conducted my study within a long-term tree growth trial established by Forest Research in 1955, which is located in Gisburn Forest, northwest England (henceforth "Gisburn"; Figure 1). The site is *c.* 35 km inland from the coast (54° 1′ N; 2° 22′ W), sloping slightly to the south-west with an elevation ranging from 260 to 290 m. The harvest of the first rotation was done after a heavy storm knocked down most of the adult trees in 1990. For the harvesting process, tree trunks were flown out by helicopter to reduce the impact cause by heavy machinery in the soil. Consequently, the soil in each plot has been under the influence of the same tree species or mixture of species for over 60 years and has remained undisturbed. The trial is currently in its second rotation (planted in April 1991), in which all plots were planted with the same tree species as the first rotation. For the present study, I selected single-species plots (44.72-m x 44.72-m) of black alder (*Alnus glutinosa L.;* henceforth 'alder'), sessile oak (*Quercus petraea L.*; henceforth 'oak') and

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Scots pine (*Pinus sylvestris L.*; henceforth 'pine') replicated in three blocks for a total of nine plots.

I collected freshly fallen litter of all species from two litter traps placed in each plot during October and November 2015. Litter traps were constructed using polypropylene tubes and mesh (1 m above the soil surface, 1-m^2 area). Leaf litter samples were brought back to the lab and oven-dried to constant weight at 60 ºC. Litter used in field experiments was left intact, and litter used for decomposition bags was manually chopped and sieved to 1 cm. The remaining litter was ground using a ball mill (Retsch MM400, Hann, Germany) for chemical analyses.

5.2.2. Treatment addition

To determine how 'home' vs. 'foreign' litter affects soil C dynamics, I used a reciprocal litter transplantation experiment. Mesocosms were used to contain treatment effects within a discrete area and avoid disrupting long-term measurements at the sites. In each plot, I installed three mesocosms, consisting of polypropylene tubes (20-cm inner diameter and 12-cm height) that were sunk into the soil to 3-cm depth and at least 1-m from the nearest tree trunk. All

Figure 5.1. Aerial photographs of Gisburn Forest, UK.: a) Gisburn Forest, Lancashire, UK. b) Diagram representing the distribution of plots and experimental set up at Gisburn Forest. (Images were obtained from: Wytham Woods-Imagery ©2017 Infoterra Ltd &

Bluesky, Digital Globe, Map Data ©2017 Google; and Gisburn Forest-Imagery ©2017 Getmapping plc, Map Data ©2017 Google).

mesocosms were installed in May 2014 and the vegetation and litter within each mesocosm was carefully removed. All mesocosms were left undisturbed for one month before the start of measurements and checked monthly to remove growing plants.

Two mesocosms per plot received equal quantities of 'foreign' litter inputs from each of the other two species, and the third mesocosm received litter (18 g) from the 'home' species. Litter addition followed a factorial design with all litter types decomposing within mesocosms on each soil type. I conducted a single litter addition in July 2015 and the experiment terminated in October 2016 for a total of 15 months of observations. To avoid naturally falling litter entering the mesocosms, wire mesh 'hats' were placed on top of each mesocosm.

5.2.3. Soil respiration measurements

To determine the effects of different litter types on soil respiration, I took monthly soil CO_2 efflux measurements over each mesocosm from using a soil survey system comprising an infrared gas analysed attached to a soil survey chamber (LI-8100A, LiCor BioSciences, Lincoln, Nebraska, USA). To eliminate the effects of turbulence from chamber closure, a 15-sec post-purge period and a 15-sec dead band period were set for each measurement. To determine differences in initial soil respiration, I measured soil respiration monthly for three months before starting treatments (May-July 2015). I then made monthly measurements over the decomposing litter from August 2015 to October 2016, when the experiment ended. Soil respiration in December 2015 and January 2016 was not measured because the soil was frozen.

5.2.4. Litter processing

To assess litter quality, I analysed leaf litter from composite samples of each study species (three analytical replicates per species, 9 samples in total). I determined the total C and N content and conducted fibre analysis of the litter samples following the Van Soest method (Van Soest 1963). I measured the C and N content of the leaf litter on oven-dried (60 ºC) ground subsamples using a Vario ELIII Element Analyser (Elementar, Hessia, Germany). Acid detergent lignin (ADL) and cellulose were determined on 1 g oven-dried (60 ºC) knife-milled (1 mm) litter samples. I performed the extractions using a Fibertec[TM] 1020 hot extraction unit (Foss, Hilleroed, Denmark). Briefly, each sample was placed in a glass crucible and 1-g of celite was added as a filtration aid. Total acid detergent fibre (ADF) was obtained after washing the samples with boiling acid detergent solution (0.5 M H_2SO_4 + CTAB) for 1 h, followed by a 5-min acetone soak; the resulting ADF samples were drained and oven-dried at 105 ºC for 5 h. Cellulose was then solubilized by soaking the ADF samples in H_2SO_4 for 3 h, followed by washing with hot deionised H_2O (until acid-free). The remaining sample was dried for 2 h at 130 ºC and then ashed in a furnace for 3 h at 525 ºC. Cellulose content was calculated from the mass loss after solubilization and lignin content was calculated from the mass of the residue.

5.2.5. Litter decomposition experiment.

I used litterbags to measure the rate of decomposition of leaf litter from my study species. Litterbags (10-cm x 10-cm) were made using nylon mesh (aperture 1-mm), filled with 3 g of single-species litter and placed in the field in July 2015. Vegetation and litter was removed from the soil and the litterbags were pinned in place to ensure good contact with the soil. I placed four litterbags per species in each plot within 1 m of the mesocosms. To determine mass loss over a six-month period, I collected two litterbags per species and

plot in September 2015 and two in January 2016. After collection, litter was extracted from bags, oven dried (60 ºC) and weight to calculate mass loss over time.

5.2.6. Soil collection and analysis

To measure soil properties in the study plots, I collected six soil samples at 0-10 cm depth using a 2.5-cm diameter soil corer and mixed them to create one composite sample per replicate plot. Following the same procedure, I collected three soil cores from within each mesocosm at the end of experiment to determine the effect of litter type on soil properties. All samples were sealed in plastic bags and brought back to the lab to be processed within 24 h of collection or oven-dried for further analysis.

5.2.7. Soil water content and pH.

I determined gravimetric soil water content using 20-g subsamples of fresh soil. I measured the fresh weight of the soils within 24 h of collection and then dried the samples at 105 ºC for 48 h to calculate soil water content. I measured soil pH on a slurry of soil in deionised water (1:3 ratio) using a S220 Seven Compact pH meter (Mettler Toledo, Columbus OH, USA).

5.2.8. Soil total C, total N and microbial biomass

I measured the total C and N content of the soil on ground subsamples of oven-dried soil using a Vario ELIII Element Analyser (Elementar, Hessia, Germany). To determine soil microbial biomass carbon (MBC), I performed the chloroform fumigation extraction method on paired subsamples (8-g dry weight equivalent) of fresh soil following Vance et al. (1987) with modifications (Jones & Willet, 2006). Briefly, one subsample was fumigated with ethanol-free chloroform for 24 h and both subsamples were extracted in 40 ml 0.5M

K$_2$SO$_4$, shaken at 200 rpm for 1 h, centrifuged at 3000 rpm, and filtered. The extracts were refrigerated for *c.* 10 days until analysis for total C on a TOC-L combustion analyser coupled with a TNM-L unit (Shimadzu Corp, Kyoto, Japan). Soil MBC was estimated by the difference in C content between fumigated and unfumigated subsamples, without correction for extraction efficiency.

5.2.9. Statistical analysis

All statistical analyses were performed in R version 3.2.4 (R Core Team, 2016). I used Principle Components Analysis (PCA; *rda* function in the vegan package; Oksanen et al., 2017) to explore the initial differences in soil properties among the three soil types. I included all soil variables measured (C, N, MBC, MBN, C:N ratio, pH, ammonium-N and nitrate-N) and scaled them for direct comparison. The scores of the first two ordination axes (PC1 and PC2) were included as explanatory variables in linear mixed effects models (*lmer function*), to investigate the effect of initial soil properties on pre-treatment soil CO$_2$ efflux, whereby mean CO$_2$ efflux was modelled as a function of PC1, PC2 and their interaction as explanatory variables and with block as a random effect. The models were assessed by comparing them using likelihood ratio tests, dropping terms until the best model was determined, using AICs and *p* values for comparison and model improvement. The best model was then compared to the corresponding null model and the model fit was then assessed using diagnostic plots (Pinheiro and Bates, 2000).

I used linear mixed effects models to determine the influence of soil type and litter type on soil respiration from field mesocosms. In these models, I included soil type and litter type as fixed effects, and block and time as random effects. I also included soil temperature in all models as a covariate and selected the best model following the same steps as described above.

To determine changes in soil properties within the mesocosms, I calculated the response ratio for each mesocosm using the equation: *RR = ln(Rx / Rc)*; where '*Rx*' is the value for each soil property at the end of the experiment; and '*Rc*' is the value at the beginning of the

experiment, which were used as a control (REFERENCE). I then used linear models *(lm function)* to determine the influence of soil type and litter type on the response ratios of soil properties (C, N, MBC, MBN, pH, NO_3, NH_4, and C:N) using block as an error term. I also used linear models to determine the influence of soil type and litter type on decomposition rates, using block as an error term. In both cases, the full model included the interaction between soil type and litter type, and non-significant terms were dropped until the best-fit model was obtained (Crawley, 2007).

5.3. Results

5.3.1. Litter quality

Litter properties varied among species (Table 5.1.): alder had the highest N content, but the lowest C:N and lignin to nitrogen (L:N) ratio. By contrast, pine had the highest C concentration, C:N ratio and L:N ratios. For the purpose of classifying litter in this study, I used the L:N ratios as an indicator of litter quality, whereby low values indicate high quality. Therefore alder is considered as high quality litter, oak as medium quality, and pine as low quality.

5.3.2. Initial soil differences and respiration

Ordination of initial soil properties revealed clear separation of plots planted with different tree species (Figure 5.2.). Plots planted with alder or oak were tightly grouped, whereas pine soils varied widely along the first ordination axis (PC1). Alder soils were separated from oak and pine along the second ordination axis (PC2), corresponding to differences in nitrate-N, pH and C:N ratios. Soils under alder and pine differed in their C and N content, with pine having the highest C and N content, whereas soils under alder had the highest NO_3 concentrations (Table 5.1.).

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Table 5.1. Leaf litter and soil properties of different tree species used in a litter translocation experiment at Gisburn Forest, UK. Analysis on freshly fallen litter from alder, oak and pine trees; showing carbon (C), nitrogen (N), lignin (L) cellulose content, C:N ratios and L:N ratios for n = 3 analytical replicates. Soil properties were analysed from a composite sample per plot (n = 3 plots per species) collected from single-species plots of alder, oak and pine. (n = 3 replicates per species).

		Total C (%)	Total N (%)	C:N ratio	Lignin (%)	Cellulose (%)	L:N ratio		
Litter	Alder	49.00	3.60	13.60	5.55	37.73	1.54		
	Oak	47.01	1.19	39.64	8.20	10.79	6.89		
	Pine	63.55	0.93	59.10	16.37	12.60	17.60		
		Total C (%)	Total N (%)	MBC	MBN	C:N ratio	pH	NH_4-N	NO_3-N
Soil	Alder	9.24	0.5	280.25	55.06	18.76	4.03	68.31	20.11
	Oak	7.97	0.37	352.16	63.69	21.57	4.59	57.72	2.43
	Pine	12.94	0.67	383.19	69.83	20.78	4.35	80.12	3.12

Soils under oak trees had the highest C:N ratio. Accordingly, second ordination axis also explained the variation in mean pre-treatment soil respiration values, ($x^2 = 4.455, p = 0.034$; Figure 5.4.), where soils under the influence of alder showed a higher pre-treatment mean CO_2 efflux (1.61 µg C g h^{-1}), compared to oak (1.34 µg C g h^{-1}) and pine soils (0.97 µg C g h^{-1}).
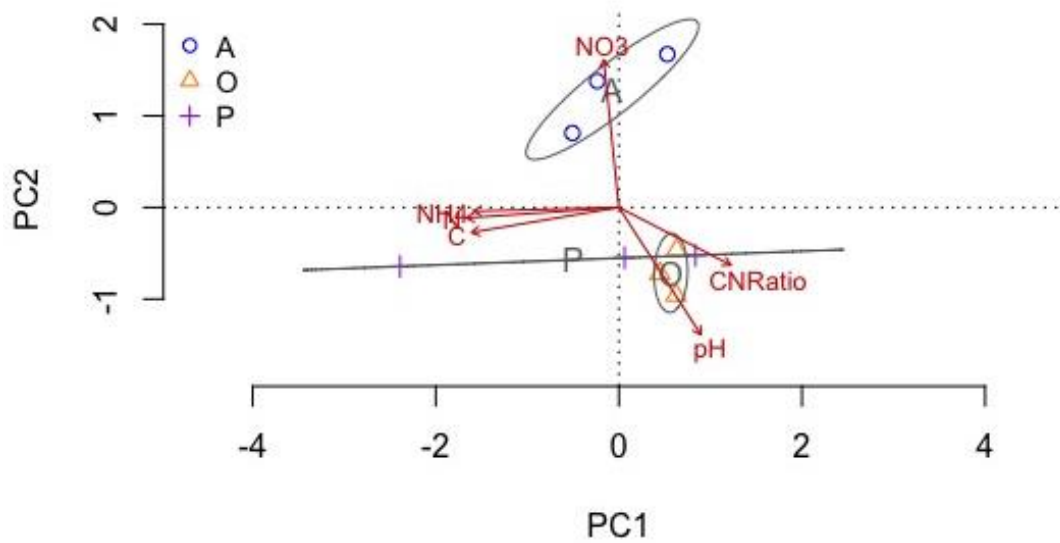
Figure 5.2. PCA ordination plot of initial differences in soil properties for plots planted with oak (O), alder (A) or pine (P) trees at Gisburn Forest, UK. Vectors show the relative influence of each soil property on the distribution of samples in ordination space, where C is carbon, N is nitrogen , pH is soil pH, NH_4 is ammonium-N and NO_3 is nitrate-N.

5.3.3. The home-field advantage of litter decomposition

Decomposition rates varied significantly as a result of the interaction between litter type and soil type (R^2 = 0.96, p = 0.001). In alder soils, 'home' litter decomposed much faster than both types of 'foreign' litter, whereas in oak soils, the decomposition of 'home' and 'foreign' litter was very similar. Finally, in the pine soils, the 'foreign' alder litter decomposed faster than either oak or pine litter.
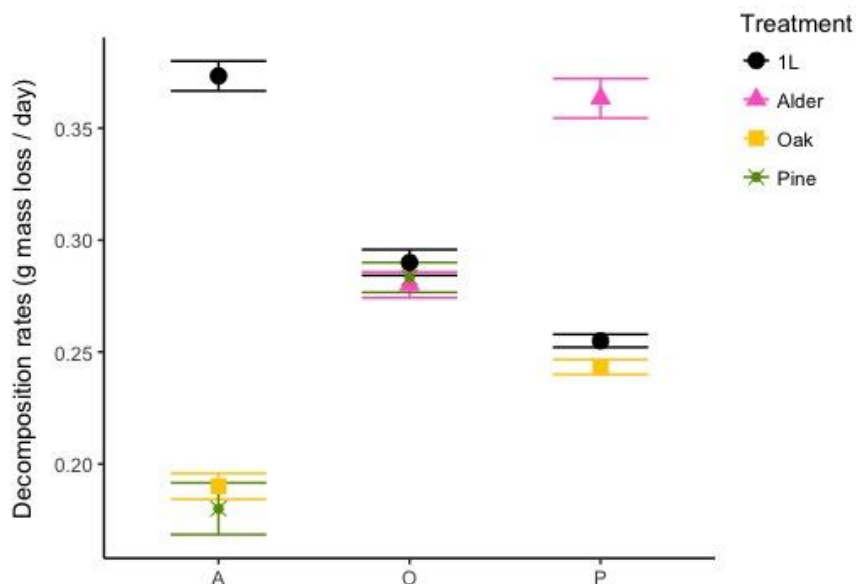
Figure 5.3. Mass loss from decomposing alder, oak and pine litter during an *in situ* reciprocal transplant experiment using litterbags at Gisburn Forest. Dots represent mean values for decomposition rates (% mass loss per day) and error bars show standard errors for $n = 3$ per litter type, where A, O and P are soils within monoculture plots of alder, oak and pine, respectively; black dots indicate 'home litter' (CT) for each soil, and 'foreign' litter is shown as pink triangles for alder litter, yellow squares for oak litter and green crosses for pine litter.

5.3.4. 'Home' and 'foreign' litter addition and their effect on soil CO_2 efflux

Decomposition rates varied significantly as a result of the interaction between litter type and soil type ($R^2 = 0.96$, $p = 0.001$; Figure 5.3.). In alder soils, 'home' litter decomposed much faster than both types of 'foreign' litter, whereas in oak soils, the decomposition of 'home' and 'foreign' litter was very similar. Finally, in the pine soils, the 'foreign' alder litter decomposed faster than either oak or pine litter.
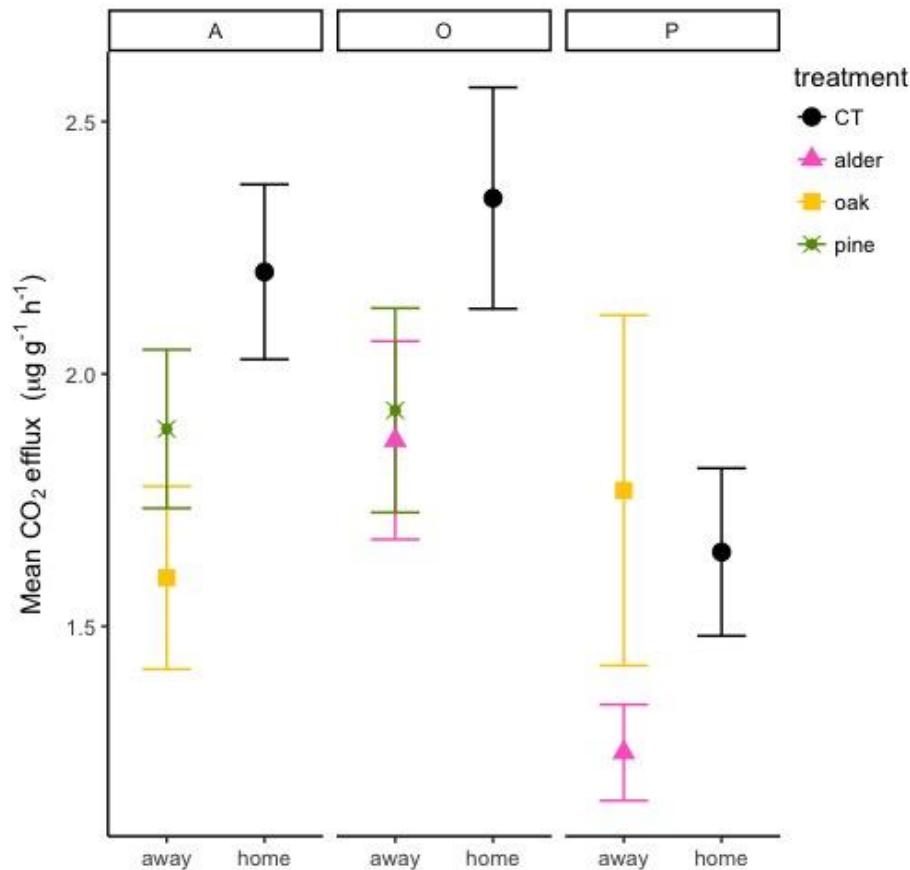
Figure 5.4. Mean soil CO_2 efflux in single species plots of alder (A), oak (O) and pine (P) as influenced by litter of the same species in a reciprocal transplant experiment at Gisburn Forest UK. Black dots indicate 'home litter' (CT) for each soil, and 'foreign' litter is shown as pink triangles for alder litter, yellow squares for oak litter and green crosses for pine litter. Mean monthly soil CO_2 efflux were calculated from 15 months of observations and error bars show standard errors for $n = 3$ per litter and soil type.

Similarly, the interaction between soil type and litter type significantly affected soil CO_2 efflux ($\chi^2 = 37.72$, $p = 0.001$). There was a strong seasonal pattern in soil CO_2 efflux across all treatments (Figure 5.5), with higher respiration rates during autumn and summer. Overall, pine soils had lower rates of CO_2 efflux than alder and oak soils. Evidence for the home-field advantage was observed in alder and oak plots, where mesocosms containing 'home'

litter had higher respiration rates than foreign litter (Figures 5.3. and 5.4). By contrast, in pine plots the highest respiration rates were measured over oak litter.
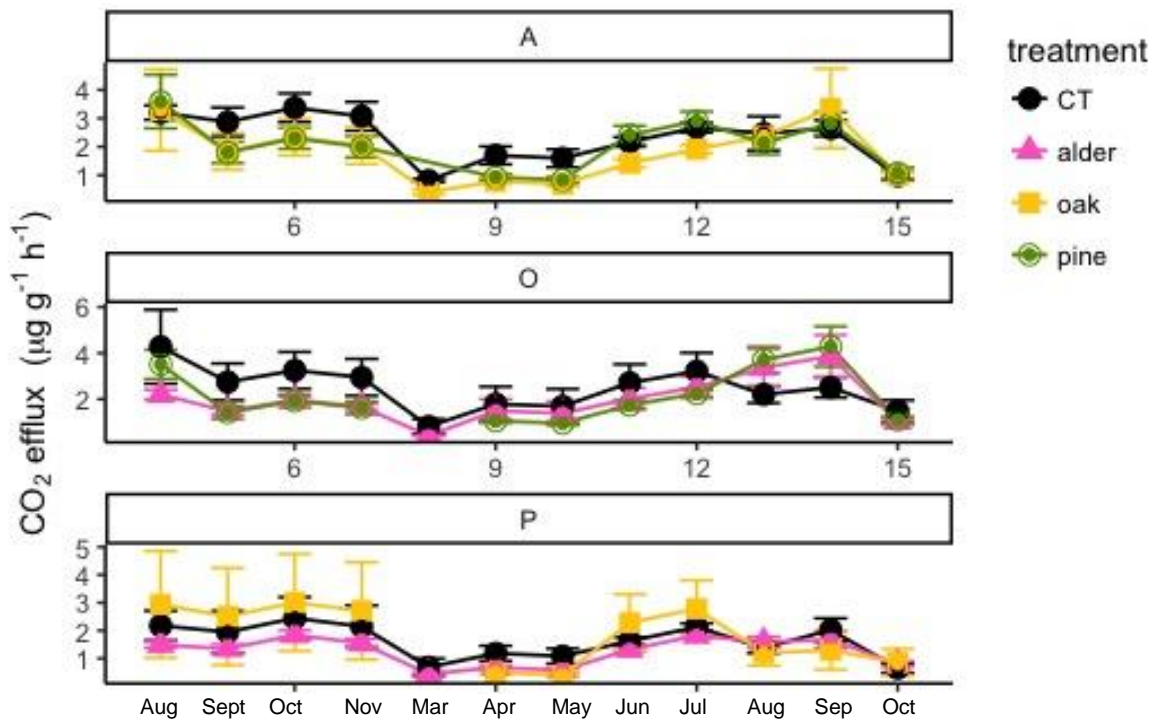
Figure 5.5. Soil CO_2 efflux during 15 months in single species plots of alder (A), oak (O) and pine (P), as influenced by litter of the same species in a reciprocal transplant experiment, where black dots indicate 'home litter' for each soil, and 'foreign' litter is shown as pink triangles for alder litter, yellow squares for oak litter and green crosses for pine litter. Symbols indicate mean monthly soil CO_2 efflux and error bars show standard errors for $n = 3$ per litter and soil type.
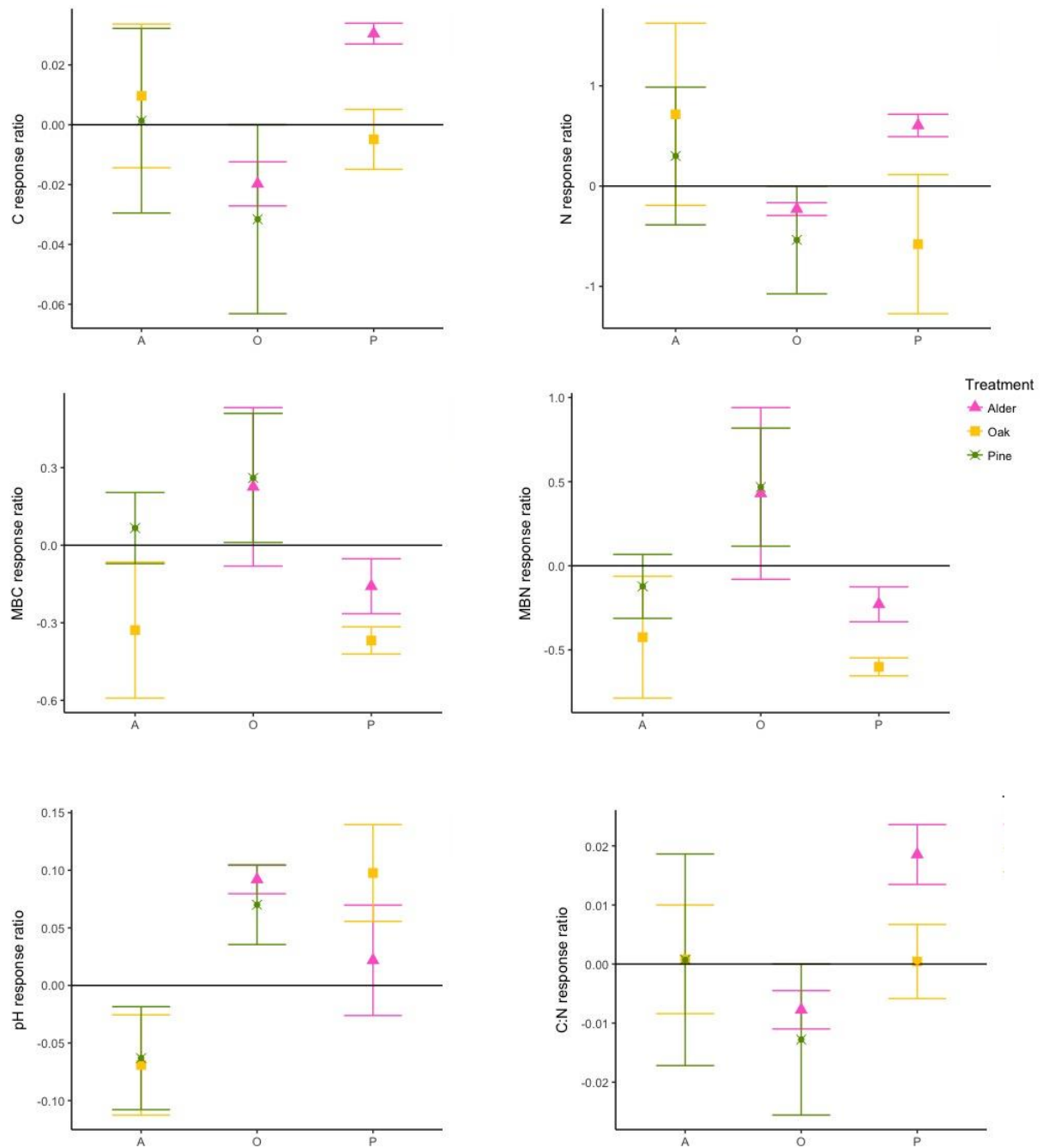
Figure 5.6. Response ratios showing changes in soil properties in single-species plots of alder (A), oak (O) and pine (P) at the end of a reciprocal litter transplant experiment in field mesocosms at Gisburn Forest, UK. Dots and error bars represent means and standard errors (n = 3 plots per species) for response ratios of total carbon (C), total nitrogen (N), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), C:N ratio and pH after the addition of 'foreign' litter, where pink triangles for alder litter, yellow squares for oak litter and green crosses for pine litter

5.3.5. The effect of foreign litter on soil properties

Different 'foreign' litters varied in their effect on soil properties (Figure 5.6). There was a significant increase in C, MBN and C:N response ratios with alder litter in pine plots (χ^2 = 7.97, p = 0.004; χ^2 = 7.99, p = 0.004; χ^2 = 8.10, p = 0.004; Figure 6) . In contrast, there were no significant changes in soil variables in alder plots except pH, which decreased with the addition of foreign litter. However, in oak plots MBC, MBN and pH increased and total C and total N decreased after the addition of both species of 'foreign litter'.

5.4. Discussion

My experiments assessed how different litter from 'foreign' tree species affects C dynamics in temperate forest soils. Specifically, the study provides an insight into how species-specific litter quality affects soil respiration and soil properties and demonstrates links between the 'home-field-advantage' of litter decomposition and soil respiration.

Linking litter quality, soil properties and soil respiration.

The ratio of lignin to nitrogen is a well-known predictor for litter quality, as it regulates litter decomposition and soil microbial (Taylor et al., 2014). Litter properties can also shape soil properties, which is evident from previous work characterising soils in single and mixed-species plots (see Chapter 4, section 4.3.1.). In my study, this is particularly noticeable in soils under alder because alder roots association with the nitrogen fixing bacterium *Frankia alni,* helps converting atmospheric nitrogen into ammonium-N and nitrate-N (Tarrant and Trappe, 1971, Yiqi and Zhou 2010). Therefore, it is not surprising that the high concentrations of total N observed in alder litter are reflected in the nitrate-N content of alders' soils. Additionally, high concentrations of N in the soil promote the activity and growth of the microbial communities (Taylor et al., 2014) and explain the higher soil

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respiration observed in alder plots compared to oak and pine. At the same time, the 'low quality' of oak and pine litter, which can be mainly attributed to high concentrations of lignin, help explain the low pre-treatment rates of soil respiration in plots dominated by those species. Lignin is a recalcitrant structural component of leaf litter, which slows litter decomposition because only certain taxa can synthesise the enzymes needed to break down lignin into more labile C compounds (Sariyildiz et al., 2003). Hence, the litter properties of different species shape soil properties and 'condition' soil microbial communities. The interaction between litter quality, soil properties and soil microbial communities produces the 'home-field advantage' (HFA) of litter decomposition, whereby litter is expected to decompose fastest in its location of origin (Gholz et al., 2000).

Home-field advantage of litter decomposition

The HFA of litter decomposition was not consistent among species (Figure 5.5.). Alder soils favoured the decomposition of 'home' litter, but the differences in the quality of 'foreign' litter did not affect their rate of decomposition in alder plots. Many decomposer organisms may be adapted to break down particular litter types (Ayres et al. 2009), and there is growing evidence for species-specific decomposer communities on litter, suggesting that plant–decomposer interactions can favour the decomposition of 'home' litter that the soil communities are adapted to (Veen et al., 2014). Accordingly, it is likely that the HFA in alder plots was due to the preferential decomposition of 'home' litter via decomposers adapted to litter with high N and low lignin content. By contrast, litter decomposition in pine plots seemed to be mainly driven by litter quality and there was no evidence of HFA, suggesting that the microbial community under pine is not highly specialised or that less recalcitrant foreign litter represents an attractive alternative resource to microbes. Surprisingly, 'home' and 'foreign' litter also had similar decomposition rates in oak, providing no evidence of HFA or preference of decomposers

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for 'high-quality' litter. This contrasts with evidence that decomposition of litter from broadleaf trees is faster than decomposition of conifer litter (Prescott et al., 2000) and might be related to microclimate differences that influence decomposition (Perez-Suarez et al., 2011).

'Home' and 'foreign' litter addition and their effect on soil CO_2 efflux and properties.

In support of my second hypothesis, the HFA of decomposition in alder plots was reflected in patterns of soil CO_2 efflux, where alder soils favoured the decomposition of 'home' litter, and consequently promoted higher respiration rates than 'foreign' litter. However, the quality of 'foreign' litter did not influence soil respiration or soil properties of alder soils, contrary to my expectations. This might be explained by the very similar low rate of decomposition of oak and pine litter in alder soil. As litter decomposition is the main factor regulating the rate of carbon and nutrient inputs into the soil, slowly decomposing litter would result in lower inputs of C and nutrients into the soil during the study period, with only a minor effect on soil properties.

The pattern in oak soils was less clear, as there was no HFA of decomposition for oak litter and yet soil respiration was higher for a large part of the experiment (Figure 5.4.). Finally, the largest increase in respiration rates in pine plots was measured over oak litter, which was not explained by the HFA or litter quality.

I also observed links between changes in soil CO_2 efflux and changes in soil properties with additions of 'foreign' litter. Interestingly, the increase in soil C in pine plots in response to the addition of alder litter (Figure 5.6.), goes in hand with the high decomposition rates of alder litter and low soil CO_2 efflux from alder litter in pine plots. The increase in soil C can be attributed to a high input of C into the soil via decomposition, that is being incorporated to the soil rather than respired, suggesting that additions of 'high-quality' litter in pine soils could promote C storage. Finally, the increase in microbial biomass in oak

plots with the addition of pine or alder litter (Figure 5.6.) suggests that much of the C and N from the litter is being incorporated into the microbial biomass, rather than being respired. My results show that the HFA and the effects of 'foreign' litter addition vary among different combinations of soil and litter. Instead of general patterns of the HFA, I found that interactions between litter quality, soil type, and the balance between microbial activity vs. growth determine litter decomposition and soil C dynamics in different soil-litter species combinations. My results also highlight the importance of expanding our knowledge about the effect of species rotation on C stocks in managed temperate forest soils.

Conclusions

My experiments demonstrate differences in the way that litter decomposition and soil respiration respond to the addition of litter from 'foreign' tree species. The selection of tree species for rotation in forestry will affect forests emissions of CO_2 and the interactions between 'home' soils and 'foreign' litter inputs present a potential option for rotating species in tree plantations to maximise C sequestration. Replanting a 'foreign' species in plots formerly dominated by alder or oak could reduce soil respiration rates and increase soil C sequestration. My results also suggest that a rotation of a 'high litter quality' species following a pine plantation might have similar effects in reducing soil respiration and increasing C sequestration. This intriguing possibility merits further attention in future research.

General Discussion

Tree species identity plays a key role in the C dynamics of temperate forests (Aponte et al., 2013; Ahmed et al., 2016), whereby the quantity and quality of litter inputs into the soil directly influence rates of decomposition, soil respiration and soil properties (Aerts, 1997). Such plant-soil interactions regulate the belowground carbon (C) balance of forest ecosystems (Wardle et al., 2009), but we know little about how species-specific responses to global change will influence plant-soil interactions and soil C dynamics in future (Facelli and Steward, 2008). The work presented in this thesis aimed to investigate how species-specific differences in litter quality shape soil properties and soil C dynamics, and how altered litter inputs could influence these processes.

First, I established a laboratory microcosm experiment to study the interactive effects of litter quality and soil properties from stands of ash, oak, sycamore, and mixed stands of all species (Chapter 3). I found evidence of strong interactions between litter type and soil properties, which influenced both soil respiration (CO_2 efflux) and the incorporation of C into the soil microbial biomass. I observed a direct relationship between litter quality and soil respiration, whereby peak soil respiration was greatest for sycamore with "high-quality" litter characterised by low lignin and high nitrogen (N) content, and lowest for oak, with had "low-quality" litter with high lignin and low N content. However, the magnitude of this response was modified by soil type. In particular, soil type largely explained changes in soil microbial biomass carbon (MBC) and pH after litter addition, regardless of litter type. As fast-growing tree species tend to have high-quality litter, these findings suggest that there could be a trade-off between above- and belowground C storage. In this trade-off, litter quality will influence soil C dynamics over the short-term via C and nutrient inputs, but tree species can also influence soil C storage over the long-term by modifying key soil properties, which modulate the response of the soils to different types of litter inputs.

Based on the key results of my initial laboratory study, I established an *in situ* mesocosm experiment to study the effects of altered litter inputs in single and mixed-species plots in a managed temperate forest in Gisburn, UK (Chapter 4). Litter inputs are likely to increase as rising levels of atmospheric CO_2 enhance forest productivity (Prevost-Boure et al., 2010), which could also alter litterfall patterns (Sayer et al., 2006). However, given the strong influence of species identity and litter quality on decomposition processes, it is unclear how altered litter inputs will affect soil C dynamics under different tree species. I explored this in a 61-year old forestry trial comprising single-species plots of alder, oak and pine; and mixed-species plots of all pairwise combinations of the same species. My results showed that litter properties varied among tree species, and consequently there was a clear separation in soil properties among plots planted with different species. The separation of soil types was mainly driven by differences in soil pH, carbon to nitrogen ratios and concentrations of available nitrate-N in the soil, which were linked to the presence of alder, a nitrogen fixing tree species. I then explored the interaction between soil properties and litter quality and their effect on litter decomposition and soil CO_2 efflux, and determined that the rapid decomposition of "high-quality" litter promotes higher rates of soil respiration. The response of soil C dynamics to altered litter inputs also varied strongly by species, whereby soil CO_2 efflux declined with litter removal treatments in all plots except those in which pine was a constituent species, which has low-quality litter that decomposes slowly. At the same time, the effects of litter addition were inconsistent but the increase in soil CO_2 efflux generally lasted longer in alder and oak compared to pine plots. Importantly, neither litter addition nor litter removal had a substantial effect on soil respiration in mixed species plots. Taken together, these findings suggest that the effects of changes in litter inputs are linked to litter quality and decomposition, and that the ecosystem response to altered patterns of litterfall under climate change will depend on

the dominant species. Interestingly, my results suggest that soil C dynamics in single-species plots are more sensitive to increased litterfall than those planted with a mixture of species. It is possible that functional complementarity during decomposition processes may buffer changes in soil C dynamics in response to minor perturbations, and this intriguing possibility merits further study in future.

Finally, I explored the interactions between litter and soil type in more detail using a reciprocal litter translocation experiment in mesocosms within the Gisburn experimental plots to study soil C dynamics *in situ* (Chapter 5). Understanding changes in soil C dynamics with different combinations of plant inputs and soil properties are particularly relevant for planning forestry rotations, as an informed selection of species could have an impact upon forest CO_2 emissions and soil C stocks. My reciprocal transplant experiment compared the decomposition of 'home' vs. 'foreign' litter and then explored whether the 'home-field advantage' or litter quality explained changes soil CO_2 efflux and soil properties. Although there was great variation in litter decomposition among tree species, the variation was regulated by two key factors: i) the ratio of lignin to nitrogen in leaf litter, representing litter quality, and ii) the occurrence of the 'home field advantage' (HFA). There was a clear HFA in alder plots, whereas litter decomposition in pine plots seems to be driven by litter quality, favouring the decomposition of "high-quality" litter with no evidence of HFA. These patterns were largely mirrored in my measurements of soil respiration, where I observed a HFA in alder and oak but not in pine. Importantly, I measured an increase in soil C with the addition of alder litter in pine plots and greater microbial biomass in oak plots with additions of foreign litter. These results suggest that it might be possible to develop a planting scheme, using different species in rotation, to increase soil C stocks in plantations.

In conclusion, the body of work presented in this thesis demonstrates the influence of tree species on soil C dynamics. The links between litter quality, soil respiration and soil properties were similar in laboratory incubations, using soils and litter collected from natural woodland, and in field experiments within managed forestry plantations.

Litter quality has a key role in the C cycle and a direct influence on decomposition rates, but the extent of the influence of litter quality varied across species and mixtures, and was further modified by soil properties. However, in most cases litter decomposition was a good predictor of soil respiration rates and litter quality appeared to regulate the response of soil C dynamics to altered litter inputs.

My study also demonstrates that mixed-species plantations have lower soil respiration rates overall and also seem to be less sensitive to increased litter inputs, suggesting that the right mixture of species might be key to increasing soil C sequestration in future.

Additionally, my results showed that an informed species selection in forestry rotation is crucial, as replanting a 'foreign' species in plots formerly dominated by alder or oak could reduce soil respiration rates and increase soil C sequestration. At the same time, this study suggests that a rotation of a 'high litter quality' species following a pine plantation might have similar effects in reducing soil respiration and increasing C sequestration.

Although my experiments focussed on manipulating the quality and quantity of litter inputs to different soils, my results raise a number of new questions about the potential contribution of different tree species to reduce CO_2 emissions and increase soil C stocks. In particular, the decomposition of root litter does not mirror leaf litter decomposition within temperate tree species (Hobbie et al., 2010), and additional work is needed to establish the influence of species-specific root traits on soil processes. Growth trials with saplings could be used to corroborate the patterns I have revealed with leaf litter treatments, and test further interactions between species and soils with whole plants.

Overall, the work presented in my thesis provides important knowledge to develop research into the role of tree species identity in forest functioning under climate change, which could inform forestry practices in future.

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List of amendments by Eduardo Medina Barcenas to PhD thesis after corrections from examiners.

General

- The title of the thesis was changed to better fit the aims and results of the experiments.
- A list of figures and tables was added.
- The list of references was re-done.
- The list of minor correction was amended.

Chapter 1

- The introduction was expanded, including a section on roots contribution on soil C dynamics. It also presents more examples (based on the literature) of the state of the art of how different tree species can affect soil C dynamics. Also, it includes and expanded section on the role of soil biota in litter decomposition. The discussion was amended to fit accordingly.

Chapter 2

- Methodology on soil moisture and soil temperature field measurements was added.

Chapter 3

- The introduction was restructured and visits literature on similar studies that focus on short-term responses. The discussion is limited to frame results in a short term context and gives more clarity when long term extrapolation are used.

Chapter 4

- More clarity of key message in the introduction and hypothesis were re-structured.

Chapter 5

- The introduction was re-written with more focus on HFA and also highlighting the importance of litter quality for decomposition processes. The discussion was amended to fit accordingly.