Webs of influence: Investigating the effects of the forest mycorrhizosphere on soil carbon storage in a changing world

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Statement of originality

I hereby confirm that this PhD thesis is an original piece of work conducted independently by the undersigned and all work contained herein has not been submitted for another degree.

All research material has been duly acknowledged and cited.

Signature of candidate:

Nina Lindstrøm Friggens

Date:

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General Abstract

Anthropogenic climate change is broadly accepted to be the biggest threat to ecosystems in the 21st century, with the most rapid change occurring in Arctic regions. It is necessary to understand the consequences of on-going warming, such as changing vegetation and northward advance of Arctic treelines, as well as examining the robustness of proposed mitigation strategies, such as intensified tree planting. Using field based approaches in soil carbon rich sub-Arctic and high latitude boreal regions, I found that *Betula pubescens* roots and associated mycorrhizal fungi extend 3-4.5 m away from trees, thereby covering open forest gaps, possibly creating a 'wood-wide-web'. However, I found no evidence of common mycelial networks between trees or the understorey in these forests. My findings indicate consistent high production of roots and mycorrhizas throughout the forest floor, coupled with declining soil organic carbon (SOC) stocks with increasing distance from trees. In the Scottish uplands, with comparable tree and understorey species, I found that planting *B. pubescens* onto heather moorland leads to a 58 and 50% loss of SOC stocks 12 and 39 years after planting, resulting in no net gain in ecosystem C. Long term tree planting experiments provide empirical evidence for the consequences of tree planting schemes as a climate change mitigation strategy and the potential effects of warming-driven encroachment of Arctic treeline forests onto globally important ericaceous soil carbon stores. Combined, my results show how *B. pubescens* mycorrhizospheres - their roots and associated mycorrhizas - effectively explore throughout the forest floor and shape the spatial dynamics and depletion of soil carbon stocks in Arctic and boreal regions most vulnerable to climate change. Furthermore, this work suggests that, although urgent action on climate change is

needed, awareness of the ecological context is crucial if planting trees is to be a robust strategy for climate change mitigation.

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General Introduction

1.1 Plant-soil interactions

Climate change is broadly accepted to be the biggest threat to ecosystems, and all that depends on them, in the 21st century (IPCC, 2018). In order to achieve successful mitigation of climate change, it is critical to understand how ecosystems are affected by and respond to climate driven changes. Although climate change has adverse effects in a wide range of ecosystems, this work focuses on high latitude terrestrial ecosystems. In most terrestrial ecosystems plant-soil interactions are a key component of ecosystem function and create dependent links between above-ground plant communities and below-ground microbial communities (Wardle *et al.*, 2004). As a result of this relationship between the above- and below-ground communities, the functioning of one cannot be sustained without the contribution of the other (Barrios, 2007). Based on this co-dependent relationship it has been postulated that functional or species composition changes above-ground may affect function or species composition below-ground, and vice versa (Mitchell *et al.*, 2010b, 2012a).

It is increasingly recognised that plant-soil interactions play an important role in structuring above-ground plant communities and ecosystem responses to environmental change (Ehrenfeld *et al.*, 2005; Bardgett & Wardle, 2010; van der Putten *et al.*, 2016). One such response to environmental change is species range expansion and shifts in ecosystem boundaries and productivity as observed at the sub-Arctic forest-tundra ecotone (Tømmervik *et al.*, 2009; Hagedorn *et al.*, 2014; Reichle *et al.*, 2018). These ecosystem shifts in both range and productivity affects

plant-soil interactions in these regions which in turn shape plant community dynamics (Bennett *et al.*, 2017) and exert strong controls on ecosystem carbon (C) and nutrient cycling (Guo & Gifford, 2002; Ehrenfeld *et al.*, 2005; Lee *et al.*, 2012). Therefore, when investigating the role of soils and primary producers on ecosystem services, both above- and below-ground communities and processes must be considered simultaneously to understand how global changes will affect one or, more likely, both of these ecosystem sub-systems.

1.2 Treeline advance in the sub-Arctic

One of the many regions in the world where anthropogenic climate change is predicted to cause major shifts in above-ground plant communities with likely cascading effects on below-ground soil communities, C turnover and storage, is in northern circumpolar boreal and Arctic ecosystems. In the Arctic, climate warming has resulted in large areas of tundra becoming more productive, with some landscapes showing increases in above-ground biomass of 10 g m^{-2} yr^{-1} from 1982-2010 (Epstein *et al.*, 2012a; Figure 1.1). In many of these areas, shrubs and trees have been observed to increase in cover and height (Myers-Smith *et al.*, 2011; Elmendorf *et al.*, 2012; Bjorkman *et al.*, 2018) and are generally thought to contribute to an increase in Arctic "greenness" (Epstein *et al.*, 2012b).

Model analyses predict (Pearson *et al.*, 2013) that substantial regions of the ~1.63 million km^2 of circum-polar Arctic vegetation communities in Circumpolar Arctic Vegetation Map (CAVM; Walker *et al.*, 2018) categories G4 ('Tussock-sedge, dwarf-shrub, moss tundra'), S1 ('Erect dwarf-shrub, moss tundra') and S2 ('Low-shrub, moss tundra') have the potential to shift to forest classes (Bastin *et al.*, 2019; Raynolds *et al.*, 2019). This amounts to a predicted 52% increase in woody cover

across the Arctic by 2050 (Pearson *et al.*, 2013). The predicted shift from tundra to forest is in line with the climate driven encroachment of trees and tall shrubs into tundra areas previously predicted and observed in these high latitude regions (Callaghan *et al.*, 2002; Skre *et al.*, 2002; Rundqvist *et al.*, 2011).

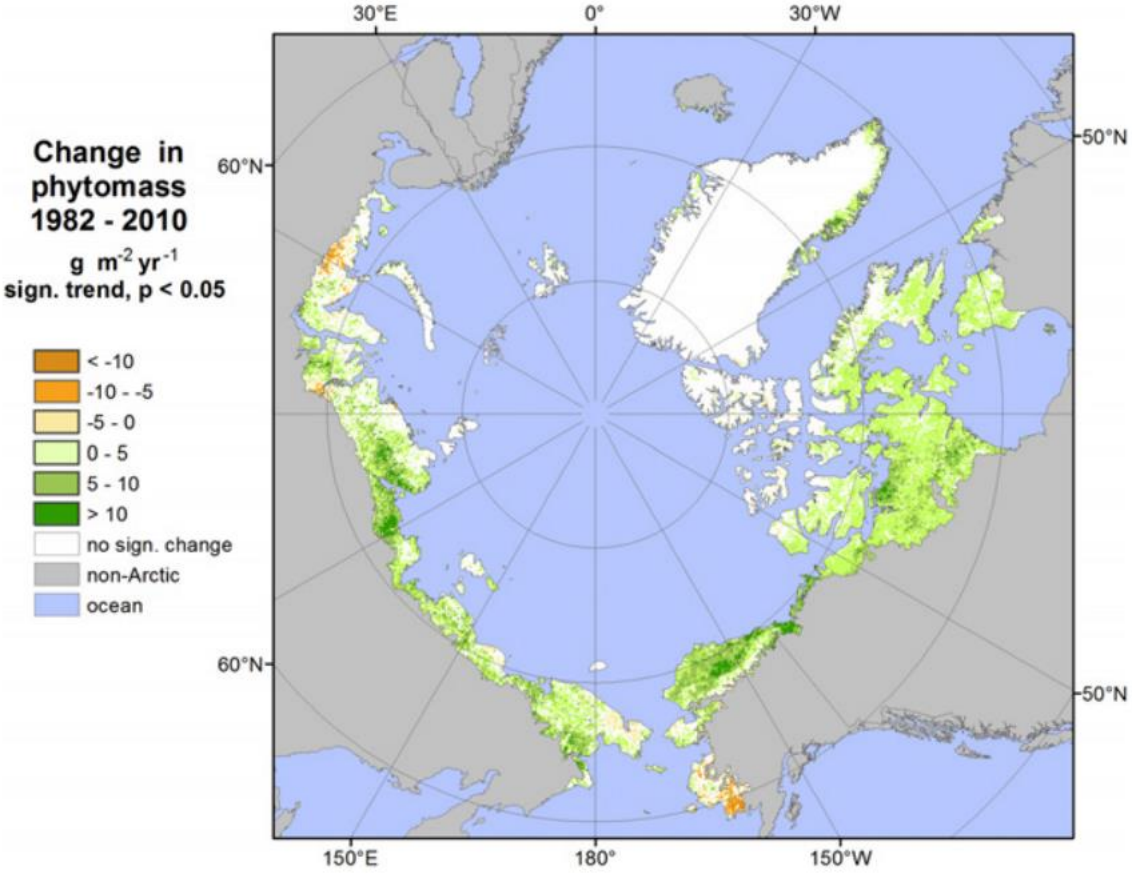

Figure 1.1. Significant changes ($p < 0.05$) in above-ground tundra phytomass from 1982 to 2010. From Epstein *et al.* (2012a).

The predicted vegetation change will most likely have significant consequences for the C cycle and storage in high latitude regions. Existing patterns of above- and below-ground biomass and C stocks along spatial vegetation transitions or ecotones may hold clues regarding the possible consequences of temporal shifts in vegetation communities in the future. In a study using such a ‘space-for-time substitution’ approach across multiple sites in the Scandes mountains in Sweden and Norway,

comparing treeline forests with adjacent tundra heath, Sjogersten and Wookey (2009) found higher rates of leaf litter decomposition and soil respiration as well as a shallower depth of the soil organic horizon in treeline forests compared to adjacent tundra heaths. This finding was corroborated by Hartley *et al.* (2012) who found that whole ecosystem C storage (the combination of above- and below-ground C) is greater in tundra heaths than in mountain birch forests in the Swedish sub-Arctic and predict that increased plant growth may lead to loss of soil C through decomposition. Similar patterns have been found in Alaskan boreal ecosystems where increases in soil temperature corresponded with increases in above-ground C pools and decreases in below-ground C pools (Kane & Vogel, 2009). Focussing on shrub as well as tree expansion in the Arctic, Parker *et al.* (2015) used replicated vegetation gradients in the Swedish sub-Arctic to show that a small C pool with rapid C turnover is present under mountain birch forest and shrub vegetation, whereas a much larger C pool with slower C turnover is present under tundra-heath vegetation. This evidence indicates that colonisation of tundra heath by mountain birch forests will lead to increases in decomposition rates and CO_2 release from potentially large pools of recalcitrant C stored in tundra soils (Sjögersten & Wookey, 2009; Hartley *et al.*, 2012). Contradictory evidence was observed in the Alaskan tundra where a 20 year warming experiment resulted in increases in woody plant species and changes to the soil community composition with no changes to soil C or nitrogen (N) stocks (Sistla *et al.*, 2013). Sistla *et al.* (2013) therefore infer that 20 years of warming increased total ecosystem C rather than causing significant C release as found in the Scandinavian sub-Arctic. However, given the relatively short time scale over which this vegetation shift was observed it is likely that a lag in soil C stock change (Sistla & Schimel, 2013) may mask the true ecosystem C stocks at equilibrium.

Rapid soil C cycling and loss of stored soil C in more productive ecosystems is particularly important given the predicted climate driven changes in plant productivity (Epstein *et al.*, 2012a) and the observed high soil C densities at high latitudes (Hugelius *et al.*, 2013; Köchy *et al.*, 2015); this soil C is potentially vulnerable to be lost through both the direct (Karhu *et al.*, 2014) and indirect effects of warming. It is therefore vital, now more than ever, to understand the mechanisms and plant-soil interactions that mediate these climate driven changes in ecosystem C cycling and storage.

1.3 The importance of soil

It has been estimated that more C is stored in soil globally than in vegetation and the atmosphere combined (Tarnocai *et al.*, 2009) and a large portion of this is stored in high latitude regions (Figure 1.2) due to low temperatures and relatively poor plant litter quality (Wookey *et al.*, 2009; Köchy *et al.*, 2015; Crowther *et al.*, 2019). A recent synthesis also found, not only that a large amount of soil C is stored at high latitudes, but that these large stocks of soil C are associated with low stocks of above-ground plant C (Crowther *et al.*, 2019).

Several teams have attempted to estimate just how much soil C is stored at high latitudes. Jobbágy and Jackson (2000) estimated that global soil organic C was 2344 Pg within the top 3 m of soil, whilst Ping *et al.*, (2008) estimated that North American Arctic soils alone contain 98.2 Pg of C and argue that previous estimates of global soil C stocks are likely to be an underestimation. Similarly Tarnocai *et al.*(2009) estimate that northern circumpolar permafrost soils contain 1672 Pg organic C which is approximately 50% of global reported below-ground C pools, whereas Hugelius *et*

al.(2014) report a revised estimate of ca. 1300-1370 Pg soil organic C in circumpolar permafrost regions.

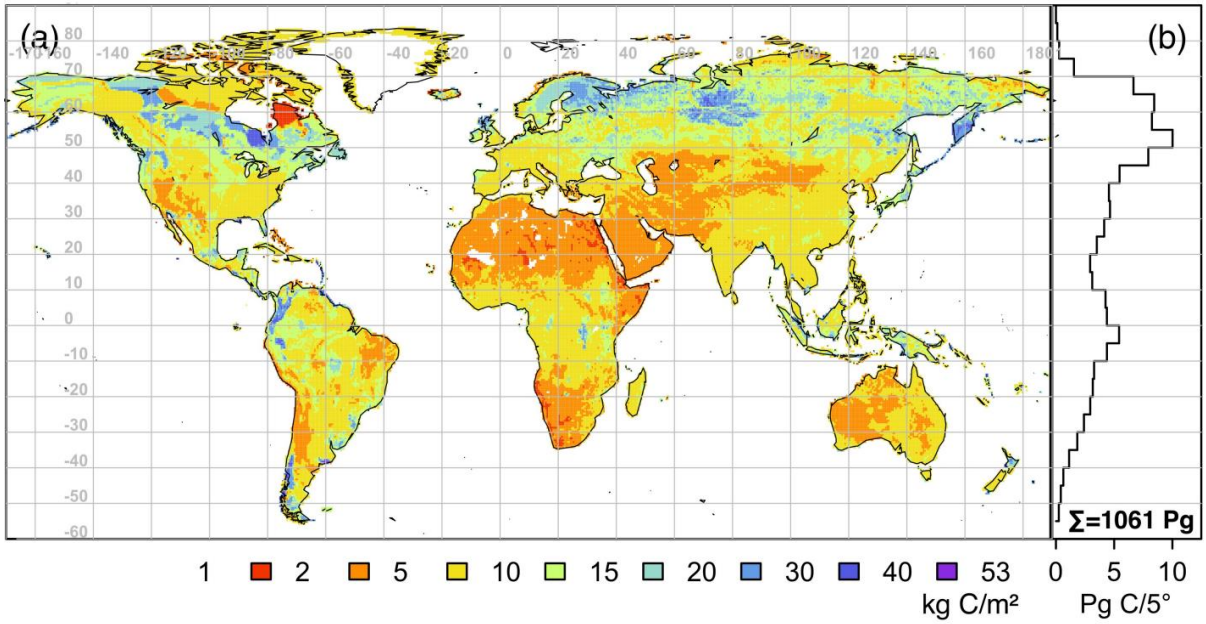

Figure 1.2. Global stock (a) and mass (b, per 5°latitude) of organic carbon in the top 1 m of the terrestrial soil calculated from HWSD v.1.1-adjusted. From Köchy *et al.* (2015).

Climate change and global warming has been predicted to stimulate the decomposition of this stored organic C causing the release of 68-508 Pg C into the atmosphere with potential positive feedback effects on climate change (MacDougall *et al.*, 2012). Other studies on the vulnerability of permafrost soil C estimate that 24.4-34.5 Pg C will be lost by 2050 given different climate change scenarios (Schädel *et al.*, 2014). It is clear that, despite the challenge of accurate quantification and the discrepancies among estimates, there is a substantial pool of C stored in Arctic and circumpolar soils, which are vulnerable to being lost to the atmosphere (Karhu *et al.*, 2014) as the climate warms.

C storage or sequestration in soils occurs when the C inputs in the form of plant litter, root exudates and microbial biomass exceed the C lost through decomposition, leaching and respiration (Wardle *et al.*, 2004). These key soil processes which, in the

right combination, result in C sequestration, are mediated by the above-ground plant community that influence litter composition (Dorrepaal *et al.*, 2005; Brovkin *et al.*, 2012; Parker *et al.*, 2018) and root exudates (Smith, 1976; Melvin *et al.*, 2015). A strong control is also exerted by the below-ground microbial community which facilitates decomposition of organic C (Gregory, 2006; Jastrow *et al.*, 2007). Soils globally, and particularly high latitude soils, can act as C sinks. However changing climates and precipitation may cause soils to become net sources of C (Epstein *et al.*, 2004; De Deyn *et al.*, 2008; MacDougall *et al.*, 2012; Gutinas *et al.*, 2013; Obu *et al.*, 2015). Furthermore, changes in soil microbial communities and associations with symbiotic mycorrhizal fungi may alter the source-sink relationship of soil C (Read & Perez-Moreno, 2003). The source-sink relationship of soil depends, in part, on the response of the soil microbial community to increased temperatures and climate change (Xue *et al.*, 2016). Another element of soil source-sink function is soil quality (specifically C:N ratios; Read & Perez-Moreno, 2003; Schädel *et al.*, 2014). Incubation studies of soils from across the Arctic and permafrost regions show that the quality of stored organic C in soils may have a stronger influence on C release to the atmosphere than temperature and could be used to predict pan-Arctic C release in various climate change scenarios (Schädel *et al.*, 2014). The vital role of both above- and below-ground organisms in combination with other biotic and abiotic factors in the C cycle and sequestration processes further underpins the importance of understanding the combined responses of these communities to climate change.

1.4 The importance of mycorrhizas and the mycorrhizosphere

The focal link between plants and soil is the rhizosphere and the maintenance of symbiotic relationships with mycorrhizal fungi which explore the soil for water and

nutrients and, in turn, receive plant photosynthates (Anderson & Cairney, 2007; Smith & Read, 2010). This root-fungus-soil interface is also referred to as the mycorrhizosphere (Sommer *et al.*, 2017). The importance of these symbiotic mycorrhizosphere relationships for plant health is well established, with >90% of all terrestrial plants forming mycorrhizal associations (Cairney, 2000). However, the significant role played by mycorrhizal associations in shaping ecosystem properties and geographic patterns proposed 35 years ago (Read, 1984) is still under debate today (Read & Perez-Moreno, 2003; Wurzburger *et al.*, 2017). Mycorrhizal fungi are increasingly recognised as key players in shaping ecosystem function (Hazard and Johnson 2018), forest population dynamics (Bennett *et al.*, 2017) and nutrient cycling (Zhang *et al.*2019). Mycorrhizal fungi fall into several functional types based on the structures that create the physical interactions with their host, the family of host plants with which they associate, and their ability to mobilise soil nutrients (Read, 1991; Read & Perez-Moreno, 2003). The most common functional types of mycorrhizas are; ectomycorrhizas (ECM) which form external sheaths over plant roots, arbuscular mycorrhizas (AM) which form arbuscule structures within plant root cells, ericoid mycorrhizas (ERM) which associate with ericoid plants, and orchid mycorrhizas (ORF) which associate with orchids (Read, 1999).

The effect of microorganisms and mycorrhizal fungi on soil C-cycling and C stored as microbial biomass depends on the functional type and growth form of individuals in a microbial community (Cornelissen *et al.*, 2001; Clemmensen *et al.*, 2015). The functional type and growth forms of mycorrhizal mycelia can vary significantly depending on the soil environment and above-ground plant communities (Grayston & Prescott, 2005; Hazard & Johnson, 2018). Within functional types of mycorrhizal

fungi, there are different exploration types defined by the structure and distances over which the mycelial and hyphal networks explore the soil (Agerer, 2001).

Within ECMs, some mycorrhizal mycelia have short exploration distances or can be contact-dependent and some mycorrhizal mycelia have long exploration distances, known as cord-forming extraradical mycelia (Agerer, 2001). Significantly more C can be stored in cord-forming or long distance extraradical mycelia due to having more biomass than shorter exploration types with lower biomass. However, due to the rapid growth and exploratory role of long distance extraradical mycelia they have more labile and readily decomposable necromass and have higher rates of hyphal turnover (Agerer, 2001; Ekblad *et al.*, 2013; Koide *et al.*, 2014; Clemmensen *et al.*, 2015). Contrastingly, ERMs produce melanised hyphae and mycelia which resist necromass decomposition and contribute to the long term build-up of soil organic matter (Clemmensen *et al.*, 2015). Mycorrhizas also contribute to organic matter decomposition by breaking down complex organic material to scavenge for N which they supply to host plants (Read & Perez-Moreno, 2003; Read *et al.*, 2004; Talbot *et al.*, 2008; Lindahl & Tunlid, 2015). Despite early recognition that mycorrhizas are active in litter turnover to facilitate nutrient supply to hosts, ectomycorrhizas have primarily been considered biotrophs which receive C from the host plant(s) (Shah *et al.*, 2016). Due to the photosynthetic C received by mycorrhizal fungi from host plants, mycorrhizas have not traditionally been considered to contribute to the decomposition of soil organic matter and are therefore often not included in C cycle models or predictions of climate change effects on soil C release (Read & Perez-Moreno, 2003; Talbot *et al.*, 2008). However, the ability of mycorrhizal fungi to synthesise and utilise extracellular enzymes has long been recognised (Read & Perez-Moreno, 2003; Read *et al.*, 2004) but primarily linked to their mineral nutrient

scavenging properties (Read *et al.*, 2004; Averill *et al.*, 2014). Mineral nutrient scavenging properties varies with mycorrhizal type which varies according to biome and latitude (Figure 1.3).

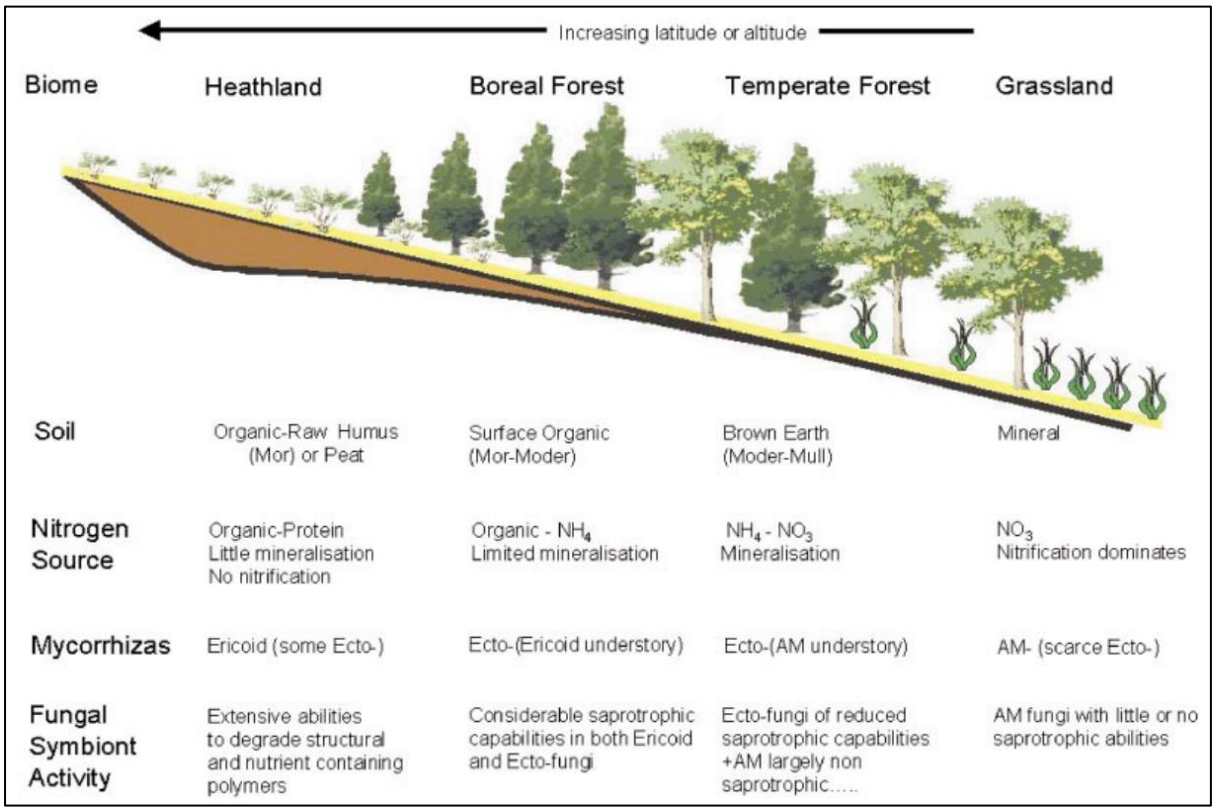

Figure 1.3. The proposed relationships, on a northern hemisphere based global scale, between the distribution of biomes along environmental gradients and the roles of the prevailing mycorrhizal association in facilitation of N and P capture by the characteristic functional groups of plant. From Read & Perez-Moreno (2003).

Talbot *et al.* (2008) proposed three hypotheses to explain how mycorrhizal fungi may directly decompose soil organic matter: (i) the *alternative C source* hypothesis, where mycorrhizas decompose soil organic matter when photosynthates from the host are low; (ii) the *coincidental decomposer* hypothesis, where soil organic matter is decomposed as a bi-product of the biochemical reactions used by mycorrhizas to scavenge for mineral nutrients; and (iii) the *priming effect* hypothesis, where the

supply of labile C from the host allows mycorrhizas to decompose organic matter sources inaccessible to other saprotrophs (Talbot *et al.*, 2008). Talbot *et al.*(2008) argue that, given the apparent role of mycorrhizas in soil organic matter decomposition, this should be taken into consideration in C cycle and global change models as changes in the abundance, activity and functional type of mycorrhizal fungi are likely to impact soil C dynamics.

Mycorrhizal fungi have been identified as key players in positive soil priming. Positive soil priming involves enhanced decomposition of older soil organic matter (SOM) pools mediated by the supply of energy by new labile C inputs from plants (Fontaine *et al.*, 2007; Hartley *et al.*, 2012). This phenomenon has been detected from tropical forests (Nottingham *et al.*, 2012) to temperate forests (Prévost-Bouré *et al.*, 2010) and peatlands (Walker *et al.*, 2016) to Arctic permafrost soils (Wild *et al.*, 2016) and may become a more prevalent mechanism, causing increased CO_2 release and soil C loss in regions with large soil C pools affected by climate driven changes in plant communities, such as the circum-polar north (Wookey *et al.*, 2009; Wild *et al.*, 2016).

Given the complex plant-mycorrhiza-soil interactions presented, it appears that despite the traditional view of mycorrhizas not contributing significantly to decomposition activity, soil organic matter degradation by mycorrhizas does have an important role to play in organic matter stabilisation and turnover in soils (Lindahl & Tunlid, 2015; Shah *et al.*, 2016).

1.5 Spatial patterns of soil C cycling within a sub-Arctic treeline forest

The mountain birch (*Betula pubescens* Ehrh. ssp *czerepanovii* (Orlova) Hämet Ahti) forest – tundra treeline ecotone at Nissunsnuohkki, South of Abisko, Sweden, is a

valuable model system for studying the effects of above-ground vegetation on fundamental soil processes and C cycling in a region vulnerable to the effects of climate change. Previous work in the treeline forests near Abisko has focussed on differences in vegetation types and soil C storage and turnover at the landscape scale, e.g. comparing forests or shrublands with tundra (Sjögersten & Wookey, 2002; Hartley *et al.*, 2012; Parker *et al.*, 2015). However, it remains unclear what the influence of different plant species is on soil processes such as C storage and turnover at the level of individuals within these treeline forests. Parker *et al.* (2016) observed that respiration was higher at the base of mountain birch trees compared to 150 cm away, indicating 'hot-spots' of soil activity close to trees. However, their work lacks further information on possible variation in key soil processes, such as respiration and mycorrhizosphere productivity at finer scales, as well as at greater distances from tree bases. As contrasting vegetation types, and individual birch trees, affect key soil processes (Hartley *et al.*, 2012; Parker *et al.*, 2016), I investigate (in Chapter 2) how far roots and associated ectomycorrhizal networks extend away from individual trees, and to what extent this influences surrounding soil C cycling and storage. Understanding fine-scale variation in soil processes is particularly relevant in ecotone mountain birch forests, as many of these are open in structure with large spacing between trees as is common for many Arctic treelines across North America and Eurasia (Payette & Lavoie, 1994; Kullman & Öberg, 2009).

One could hypothesize that a single plant (e.g. a mountain birch tree) with its associated leaf litter, microbial community and mycorrhizas, can strongly influence the soil C storage and turnover in its immediate surroundings and might be considered an 'ecosystem engineer' (Mitchell *et al.*, 2010a). From that follows the

question of how far the influence of one such ecosystem engineer extends within the open forest and what that means for mapping soil processes in the sub-Arctic and circumpolar north? What is the extent of the spatial/lateral influence of a single tree with roots and associated mycorrhizas on soil processes in the sub-Arctic mountain birch forest?

There appear to be two, mutually exclusive, possibilities; either the birch trees strongly influence soil processes in their nearest vicinity and less so further away, creating 'hot-spots' of activity within the forest as observed in a recent study by Parker *et al.*(2016). Or the spheres of influence of each individual are contiguous or overlapping causing there to be similar rates of soil processes throughout the forest, essentially forming a mycorrhizal network throughout the forest floor, or a so-called *wood-wide-web* (Peter, 2006; Beiler *et al.*, 2010).

The influence of trees and associated mycorrhizas on key ecological processes is highly relevant for global change modelling to understand fundamental ecosystem functioning in high latitude regions vulnerable to the effects of climate change. Therefore, characterising the spatial complexity of soil processes (or lack thereof) in these treeline forests is critical to understanding how soil C cycling may change with potential climate driven forest expansion.

1.6 The spatial 'reach' of the mycorrhizosphere in a treeline forest

ECMs that associate with mountain birch trees have a wide range of exploration types including long distance extraradical mycelia which can extend substantial distances (up to several decimetres) from the host root (Agerer, 2001). These extraradical mycelia are thought to be the main mediators of soil nutrient uptake by

ECMs, ultimately leading to the tree root and shoot (Read, 1992; Wallenda & Kottke, 1998). Nitrogen, essential for plant growth and photosynthesis, is generally considered the most limiting nutrient in high-latitude sub-Arctic ecosystems (Sjögersten & Wookey, 2005). Although N is a limiting nutrient in Arctic and boreal ecosystems it must be acknowledged that other key nutrients such as phosphorus (P) and potassium (K) can be limiting and that fundamental nutrient limitation assumptions are based on early NPK fertilisation experiments (Shaver & Chapin, 1995). Furthermore, the interaction and combination of multiple mineral nutrients such as N and P can alter or alleviate plant nutrient limitation in tundra ecosystems (Street *et al.*, 2018a). Plants can use N in both inorganic and organic forms particularly in systems where N is limited (Schimel & Bennett, 2004). The uptake of organic N is facilitated by mycorrhizal fungi deploying hyphal exploration to scavenge for N using extracellular enzymes to degrade organic material (Schimel & Bennett, 2004) as seen by ECMs associated with mountain birch trees (Read & Perez-Moreno, 2003). ECMs compete for soil N with free living saprotrophic fungi which have a much larger arsenal of extracellular enzymes for the decomposition of soil organic matter (Bending & Read, 1995). However, due to the supply of photosynthates that ECMs receive from their host trees, they are able to grow through areas of low N to find patches of high N concentration in the soil, which they then mine extensively (Bending & Read, 1995).

Given that N is limited in these forests and ECMs are capable of long distance growth through nutrient depleted soil, it raises the question of the extent to which mountain birch tree roots and ECMs explore and mine the forest soil for mineral nutrients, including N, and from how far away a tree can obtain its N, facilitated by ECM mining.

N stable isotope (^{15}N) labelling techniques can be used to measure available N and N flow through soil and plant pools in the ecosystem (Schimel & Bennett, 2004). These techniques have been used extensively to investigate transfer of N from source to plant tissues mediated by mycorrhizas in controlled environments (Finlay *et al.*, 1988; Johansen *et al.*, 1992; Hogberg *et al.*, 1999; Taylor *et al.*, 2004) and in field experiments (Clemmensen *et al.*, 2008; Högberg *et al.*, 2008; Jones *et al.*, 2009; Remy *et al.*, 2016). Göttlicher *et al.*(2008) were able to trace a ^{15}N label from soil to coniferous trees 9.5 m away from the source in a boreal forest.

Given that ECM scavenging for soil N can cause decomposition of soil organic matter (Talbot *et al.*, 2008; Lindahl & Tunlid, 2015) and that ECM extraradical mycelia have the potential to extend over large distances (Agerer, 2001), I sought to investigate (in Chapter 3) how far away from its base a mountain birch tree can access soil N, facilitated by ECMs, in the sub-Arctic treeline forest. Such a ^{15}N tracer experiment has not, to my knowledge, been used to investigate N uptake over long distances within sub-Arctic treeline forest ecosystems. If the ECM networks in these sparse density forests extend far into open forest gaps they may be contiguous or even overlapping with ECM networks of other trees and potentially form a so called *wood-wide-web* (Wiemken & Boller, 2002; Simard & Durall, 2004; Beiler *et al.*, 2010) whereby the entire forest floor is explored by hyphal and mycelial networks. Much knowledge is still to be gained from studying the presence and strength of a wood-wide-web in sub-Arctic mountain birch forests and the consequences of this for decomposition and nutrient cycling within these important ecosystems.

1.7 Common mycelial networks

As well as increasing the surface area for nutrient and water uptake of individual plant rhizospheres, ECMs can associate with multiple hosts at once and thereby form common mycelial networks (CMNs) which occur in all major terrestrial ecosystems (Simard *et al.*, 2012; Hazard & Johnson, 2018). CMNs are possible due to the often non-specific nature of plant-fungi associations (Selosse *et al.*, 2006), where the same plant can associate with multiple mycorrhizal species as well as the same mycorrhiza associating with multiple plant hosts (Smith & Read, 2008; Van Der Heijden *et al.*, 2015, Figure 1.4). The ability of plants to associate simultaneously with multiple mycorrhizal fungi of multiple genotypes increases the fungal diversity available to the host plant and may promote resilience to an ecosystem facing environmental change (Hazard & Johnson, 2018).

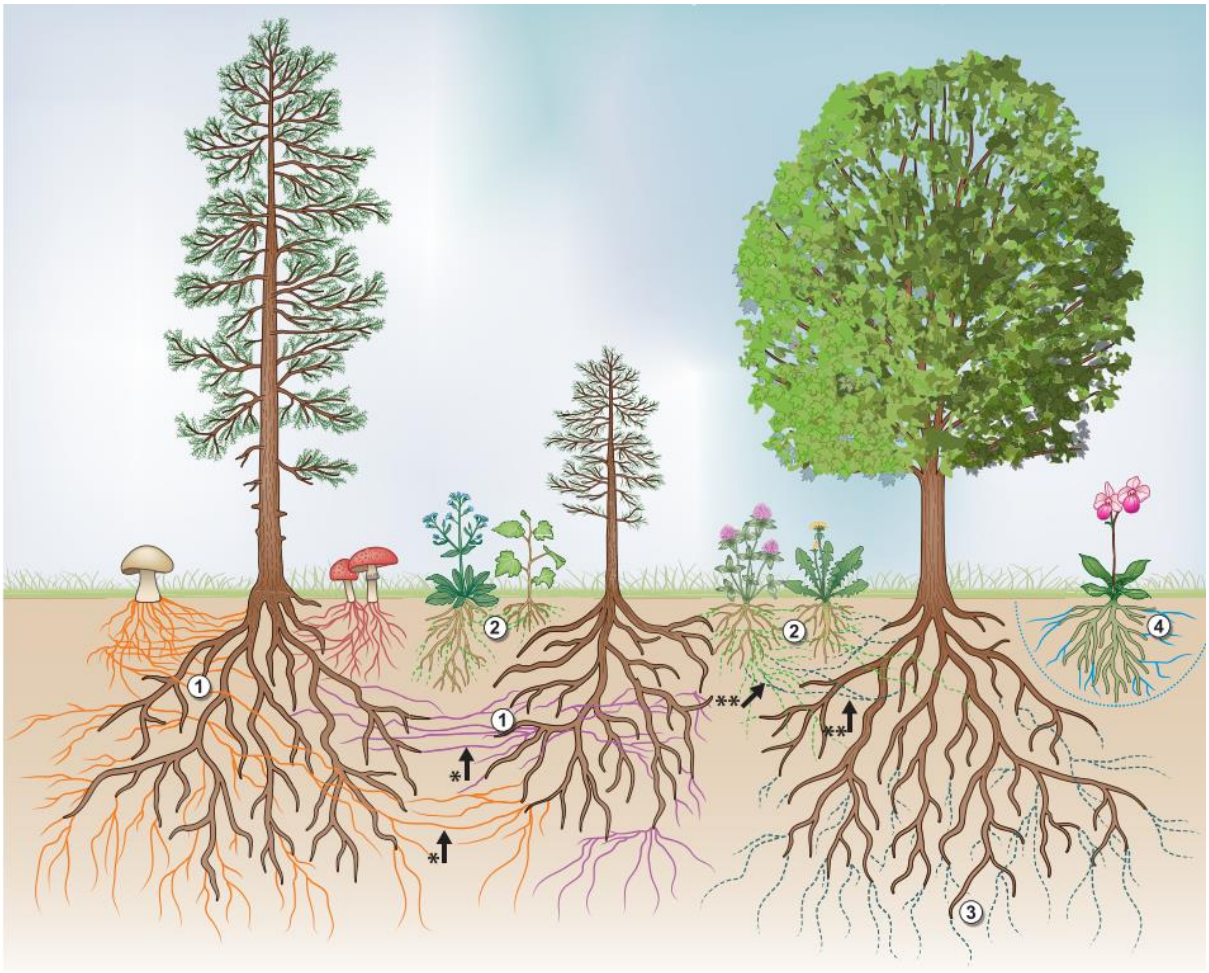

Figure 1.4. Drawing of a hypothetical plant community consisting of plant species that associate with different types of mycorrhizal fungi and which form three separate underground networks.(1) Trees forming networks with ectomycorrhizal (EM)fungi (solid thin lines) are interconnected (seearrow*); (2) various plant species and a tree (3) form arbuscular mycorrhizal (AM) networks and are also interconnected (see dashed lines, arrow**), and (4) an orchid forms a third underground network. The different colors represent different mycorrhizal fungal species for EM fungi (solid thin lines) and AM fungi (dashed thin lines). Note that other combinations are possible (e.g. temperate forests with EM trees often harbor an understory of shrubs (e.g. Vaccinium) that form ericoid mycorrhizal associations). In these forests some fungi form both EM and ericoid mycorrhizal associations, meaning that there might be interlinkages between the two networks (composite by Ursus Kaufmann, Agroscope). From Van Der Heijden *et al.* (2015).

CMNs form a cytoplasmic link between individual plants facilitated by the mycorrhizal fungus which can act as below-ground avenues for the transfer of C and other nutrients among plants within a community (Simard *et al.*, 2012). The transfer of C (Simard *et al.*, 1997a; Selosse *et al.*, 2006; Teste *et al.*, 2010; Deslippe & Simard,

2011; Babikova *et al.*, 2013b; Pickles *et al.*, 2017) and N (Ek *et al.*, 1997; He *et al.*, 2005) between plants has been demonstrated in the lab and in the field (Simard *et al.*, 2012).

In the Arctic the transfer of C through CMNs has been found in the dwarf birch, *Betula nana*, using a ^{13}C pulse-chase labelling experiment (Deslippe & Simard, 2011). Deslippe and Simard (2011) found that 10.7 ± 2.4 % of photosynthetic C was transferred between conspecific pairs of *B. nana* and argue that the magnitude of this C transfer may alter competitive plant interactions in the Arctic tundra, potentially affecting birch tree and shrub community structures. They also found the C transfer to be linked to ambient temperatures and suggest that this may lead to positive feedback, increasing *B. nana* monodominance with increasing temperatures within Arctic ecosystems affected by climate warming. These findings are important in light of climate driven shrub and tree expansion in the Arctic and circum-polar north (Myers-Smith *et al.*, 2011; Hofgaard *et al.*, 2013; Bjorkman *et al.*, 2018; Raynolds *et al.*, 2019).

Several recent studies have found evidence of transfer of C between conspecifics via CMNs (Deslippe & Simard, 2011; Pickles *et al.*, 2017), however much less is known about heterospecific C transfer via CMNs (Figure 1.4). Although mycorrhizas have traditionally been divided into functional groups based on their host plants, new evidence is emerging that the same genet of mycorrhizal fungi can associate with different plant species commonly thought to only host specific mycorrhizal functional types (Grelet *et al.*, 2009, 2010; Leopold, 2016). Grelet *et al.* (2010) found that the mycorrhizal fungi *Meliniomyces variabilis* can simultaneously colonise Scots pine (*Pinus sylvestris*), an ECM tree species, and *Vaccinium vitis-idaea,* an ERM

understorey species, but they found no evidence of the formation of large CMNs between the two.

This work in both Arctic and boreal forest ecosystems prompts the following questions: (1) Do common mycelial networks (CMNs) exist in mountain birch forests, and can the transfer of photosynthate derivatives from one individual to another be traced? (2) If mycorrhizas can simultaneously colonise canopy-forming (tree) and understorey species, can C be transferred from the canopy to the understorey species via heterospecific CMNs?

Based on the evidence of the spatial reach of mountain birch mycorrhizospheres from Chapters 2 & 3, suggesting the potential for overlapping mycorrhizal networks, I sought to investigate the presence and strength of both con- and hetero-specific CMNs in these sub-Arctic treeline forests (Chapter 4). Understanding CMNs in the sub-Arctic is increasingly important as they may confer resilience to ecosystems (Hazard & Johnson, 2018), provide competitive advantages (Deslippe & Simard, 2011) and allow plants to adjust plastically to environmental challenges (Simard, 2018).

1.8 Planting trees on deep organic soils

The research described above has focussed on ecosystem processes and function in the terrestrial sub-Arctic, which is a region of particular interest due to the large amounts of stored soil C (Ping *et al.*, 2008; Hugelius *et al.*, 2014) and the increasing vulnerability of this stored C to be released into the atmosphere as a consequence of climate change (Luo, 2007; Karhu *et al.*, 2014). These findings are also relevant in Scotland where, in common with high latitude regions, large amounts of C are stored in the soil (Bradley *et al.*, 2005; Köchy *et al.*, 2015) particularly in upland heath

areas, which are dominated by ericoid plant communities similar to those found in Abisko. Within the UK, Scottish upland areas have the highest density of C stored in the top 1 m of soil (Bradley *et al.*, 2005), the majority of which is subject to a range of land uses and affected by global changes (Ostle *et al.*, 2009). Ostle *et al.*(2009) call for a recognition of the potential losses of soil C occurring with land use changes in the UK and to balance these with C sequestration elsewhere in order to manage UK land for net C sequestration.

In February 2018 the Scottish Government published its Climate Change Plan (Scottish Government, 2018) outlining proposals and policies to mitigate climate change and its impacts between 2018-2032. In the section on land use change and forestry, they propose to increase forest cover in Scotland from 18% in 2018 to 21% by 2032 by increasing woodland creation targets from 10,000 ha $year^{-1}$ in 2018 to 15,000 ha $year^{-1}$ by 2024 (Scottish Government, 2018). These proposed climate change mitigation steps focus and rely on the sequestration of carbon dioxide (CO_2) by the generation of tree biomass, but do not consider storage of C in soils. In a paper commissioned to inform the Scottish Government's Woodland Expansion Advisory Group on which types of land in Scotland are best for planting, it was reported that 34% of Scotland's land area may have potential for woodland expansion (Sing *et al.*, 2013). Areas were deemed unsuitable for planting if they had peats deeper than 0.5 m (8% land area), but peats and peaty soils with <0.5 m depth, which may still have large C stores in the top 0.5 m, will have been considered potentially appropriate for planting. The afforestation included in the Scottish Government's Climate Change Plan (Scottish Government, 2018) is in accordance with recent promotion of global forest restoration (Bastin *et al.*, 2019). Here, the authors claim that forest restoration is "our most effective climate solution"

and will sequester 205 Gt of C (Bastin *et al.*, 2019). However, they do not consider how this would affect soil C dynamics. The implementation of this would mean large-scale changes to above-ground vegetation in areas of high soil C storage, which, due to the aforementioned links between above-ground vegetation and below-ground microbial communities, is likely to have profound effects below-ground on mycorrhizosphere communities and C dynamics. Both the direction and magnitude of these changes, however, remain unknown.

To understand some of the consequences of increased woodland cover in the Scottish uplands the Moorland Colonisation Project (MOORCO) was set up by researchers at the James Hutton Institute (formerly the Macaulay Land Use Research Institute; MLURI). The MOORCO project aims to study how expansion of native woodland species, Scots Pine and both Silver and Downy Birch, onto heather moorland affects biodiversity and ecosystem services (MOORCO, 2018). Using a unique experimental platform of established chronosequences and both planting and felling plots, the researchers have found that planting native trees onto heather moorland changes the soil microbial community from fungal-dominated to bacterial dominated (Mitchell *et al.*, 2012b), increases rates of plant litter decomposition and decreases total soil C storage and organic matter depth (Mitchell *et al.*, 2007, 2010a). These effects of afforestation, and the contrast between ecosystem processes in forests and ericaceous moorlands, are consistent with the differences seen between the mountain birch forest and the tundra heath in sub-Arctic Abisko by Hartley *et al.*(2012) and Parker *et al.*(2015). There are strong similarities in plant functional types and controls on soil organic matter dynamics between the MOORCO experimental platform and substantial regions of the ~1.63 million km^2 of circum-polar north predicted to have the potential to support forest due to climate

warming (Pearson *et al.*, 2013; Raynolds *et al.*, 2019). Given the functional relevance of Scottish ericaceous heathlands to wider global climate change scenarios, I ask: What is the effect of planting selected native tree species in the Scottish uplands (with species compositions comparable to sub-Arctic environments) on soil C dynamics and storage?

The experimental platform set up and maintained by the MOORCO project across the Scottish uplands is, to the best of my knowledge, currently the largest experimental afforestation, both spatially and temporally, and can provide unique empirical insights into the effects of planting trees onto soil C rich ericaceous heathlands. Using the MOORCO plots, I sought to investigate (Chapter 5) how planting trees onto Scottish heathlands affects soil C turnover and whole ecosystem C storage. This work is both timely and highly relevant to policy makers given the recent promotion of afforestation as a climate change mitigation strategy (Bastin *et al.*, 2019).

1.9 The effects of the forest mycorrhizosphere in a changing world

The overall aim of this research is to understand the links between the above- and below-ground communities within sub-Arctic and high latitude boreal ecosystems vulnerable to climate warming. By focussing on specific plant-soil interactions at fine spatial scales, as well as at landscape scales in both the Swedish sub-Arctic and the Scottish uplands, I aim to draw important links between the C cycles and the organisms which facilitate them in two important C-rich ecosystems relevant for global change modelling and land use policy.

Chapter 2:
Spatial patterns in soil organic matter dynamics are shaped by mycorrhizosphere interactions in a treeline forest

Note:
This chapter has been published in *Plant and Soil* and can be found at:
Friggens, N.L., Aspray, T.J., Parker, T.C. et al. Plant Soil (2019).
https://doi.org/10.1007/s11104-019-04398-y

2.1 Abstract

In the Swedish sub-Arctic, mountain birch (*Betula pubescens* ssp *czerepanovii*) forests mediate rapid soil C cycling relative to adjacent tundra heaths, but little is known about the role of individual trees within forests. Here I investigate the spatial extent over which trees influence soil processes. I measured respiration, soil C stocks, root and mycorrhizal productivity and fungi:bacteria ratios at fine spatial scales along 3-m transects extending radially from mountain birch trees in a sub-Arctic ecotone forest. Root and mycorrhizal productivity was quantified using in-growth techniques and fungi:bacteria ratios were determined by qPCR. Neither respiration, nor root and mycorrhizal production, varied along transects. Fungi:bacteria ratios, soil organic C stocks and standing litter declined with increasing distance from trees. As 3 m is half the average size of forest gaps, these findings suggest that forest soil environments are efficiently explored by roots and associated mycorrhizal networks of *B. pubescens*. Individual trees exert influence substantially away from their base, creating more uniform distributions of root,

mycorrhizal and bacterial activity than expected. However, overall rates of soil C accumulation do vary with distance from trees, with potential implications for spatio-temporal soil organic matter dynamics and net ecosystem C sequestration.

2.2 Introduction

Above-ground plant communities and below-ground microbial communities, comprised of saprotrophic and mycorrhizal fungi, bacteria and archaea, form intimate and interdependent relationships (Wardle *et al.*, 2004). Ultimately, any changes to above-ground vegetation communities could have cascading below-ground effects on microbial communities, carbon (C) sequestration and turnover (Wookey *et al.*, 2009).

The mountain birch (*Betula pubescens* Ehrh. ssp *czerepanovii* (Orlova) Hämet Ahti) forest – tundra treeline ecotone south of Abisko, Sweden, provides a suitable natural system for studying the effect of above-ground vegetation on fundamental soil processes and below-ground C cycling. The mosaic of forest and tundra heath across the landscape offers several vegetation transitions, in permafrost-free areas, reducing potential confounding hydrological effects. Previous research at the treeline near Abisko has established that shrub and tree cover have expanded between 1976 and 2009 (Rundqvist *et al.*, 2011), and that there are clear differences in organic C storage between forest and tundra heath soils (Sjögersten & Wookey, 2002). Extensive transect work has further shown that the relatively productive mountain birch forest and deciduous shrubs, with higher above-ground C stocks, have significantly less C and nitrogen (N) stored below-ground compared to the relatively less productive adjacent tundra heath (Hartley *et al.*, 2012; Parker *et al.*, 2015). These differences in below-ground C stocks and rates of soil C cycling have been

linked to differences in associated below-ground mycorrhizal communities between forest and tundra (Parker *et al.*, 2015).

Previous work has focussed on differences in vegetation at the landscape scale, but little is known about the zone of influence of individual trees within treeline forests. Characterising the spatial complexity of soil processes (or lack thereof) in these forests is critical to understanding how soil C cycling may change with potential forest expansion. In a recent study, Parker et al. (2016) observed that respiration was higher at the base of mountain birch trees compared to 150 cm away, indicating 'hot-spots' of soil activity close to trees. However, their work lacks further information on possible variation in key soil processes, such as respiration and rhizosphere productivity at finer scales, as well as at greater distances from tree bases. As contrasting vegetation types, and individual birch trees, affect key soil processes (Hartley *et al.*, 2012; Parker *et al.*, 2016), we sought to investigate to what extent individual trees, and their associated root and ectomycorrhizal networks, influence surrounding soil C cycling. We also sought to determine how far these networks extend and, in addition, we deployed indirect techniques to identify any spatial shifts in the balance between autotrophic and heterotrophic soil CO_2 efflux (Subke *et al.*, 2006; Subke & Bahn, 2010). Quantifying and understanding fine-scale variation in soil processes is particularly relevant in the mesic ecotone mountain birch forests, as many of these are open in structure with large spacing between trees. This sparse density forest is also common for many Arctic treelines across North America and Eurasia (Kullman and Öberg 2009; Payette and Lavoie 1994).

Given this ecological context there are two, potentially mutually exclusive, possibilities regarding spatial variation in the mycorrhizosphere within the forest. (i) if the influence of trees is limited in range, then there may be pockets within forests

that function more similarly to tundra heath in terms of their soil processes, with lower respiration, high soil C stocks and ericoid fungal dominance (Cornelissen *et al.*, 2001; Read & Perez-Moreno, 2003). Alternatively, (ii) if the influence of trees and their associated mycorrhizosphere extends beyond the mean maximum distance between trees, there may be contiguous, or overlapping, networks of ectomycorrhizas exploring and exploiting resources throughout the forest floor. This could indicate the presence of a so called *wood-wide-web* (Beiler et al. 2010; Simard and Durall 2004; Wiemken and Boller 2002) and the potential for common mycelial networks (CMNs) where mycorrhizas associate with multiple plants, and vice versa (Hazard and Johnson 2018; Simard et al. 2012).

Understanding the coupling between plant and microbial communities in the Arctic, and the consequences for fundamental soil processes, is particularly important due to the large amounts of C stored in high latitude soils (Kuhry et al. 2013; Ping et al. 2008), as well as the substantial allocation of plant biomass below-ground (Iversen *et al.*, 2015). Much of this C is potentially vulnerable to be released to the atmosphere, if temperatures rise, through its metabolism by soil organisms, constituting a potential positive feedback to climate forcing and the acceleration of climate change (Karhu et al. 2014; Luo 2007). The direct interface between plants and soil is the rhizosphere and the maintenance of symbiotic relationships with mycorrhizal fungi which explore the soil for water and nutrients and receive plant photosynthates (Anderson & Cairney, 2007; Smith & Read, 2010). In the sub-Arctic, the majority of mycorrhizal associations are those of ericaceous sub-shrubs with ericoid mycorrhizas (ERM), and betulaceae (both tree and dwarf birch) with ectomycorrhizas (ECM) (Cornelissen *et al.*, 2001; Read & Perez-Moreno, 2003).

The effects of microorganisms and mycorrhizal fungi on ecosystem function and soil C-cycling depend on the growth form and morphotype of individuals in a microbial community (Clemmensen et al., 2015; Cornelissen et al., 2001). These can vary significantly depending on the soil environment and the above-ground plant communities (Grayston & Prescott, 2005; Hazard & Johnson, 2018). One example is of ericaceous ERM dominated systems capable of significant C sequestration in melanised hyphal biomass vs boreal ECM dominated systems with more rapid biomass turnover and low C sequestration (Clemmensen *et al.*, 2013, 2015). Another example is where soils below ericaceous dwarf shrubs are dominated by fungi, while those below herbaceous vegetation or temperate woodlands are dominated by bacteria (Högberg *et al.*, 2007; Mitchell *et al.*, 2010b). This is thought to be related to differences in the quantity and quality of above- and below-ground litter and root exudates, C:N ratios in the soil (Fierer *et al.*, 2009) and soil pH (Mitchell *et al.*, 2010b). As fungi have higher C assimilation efficiency (Zhang *et al.*, 2005), and higher recalcitrance of necromass, than bacteria (Six *et al.*, 2006; Strickland & Rousk, 2010), fungal dominated soils sequester more C and have lower respiration rates than bacterial dominated soil (Averill and Hawkes 2016; Clemmensen et al. 2013, 2015). Understanding the link between vegetation, soils, and the ecological processes they mediate is key to understanding soil C sequestration and turnover at local and landscape scales.

Given the strong influence of mountain birch trees on soil processes around them, we hypothesise the following:

1) Production of fine roots and ectomycorrhizal hyphae declines significantly at increasing distances from tree bases, reflected in declining contributions of autotrophic inputs towards total respiration.

2) Respiration rates show corresponding patterns of decline with increasing distance from tree bases.
3) The ratio of soil fungi:bacteria increases with distance away from tree bases, creating conditions similar to more open heath.
4) The patterns of soil organic carbon stocks will reflect the relative consequences of (a) declining litter deposition, and (b) declining mycorrhizosphere activity, with increasing distances from trees. All other factors remaining equal, the former would tend to contribute to higher C stocks closer to trees (denoted Hypothesis 4a), while the latter would tend to result in slower turnover of soil C, and higher C stocks, further away from trees (Hypothesis 4b).

2.3 Materials and Methods

2.3.1 Site descriptions

All studied trees were selected within a permafrost-free area (approx. 1 km^2) in the sub-Arctic treeline ecotone at Nissunsnuohkki, south of Abisko, Sweden (ca. 68°18'56.2"N 18°49'18.2"E), ~600 m asl. The treeline forest comprises mountain birch (*Betula pubescens* ssp *czerepanovii*) and has an open canopy structure with an ericaceous understorey consisting of *Vaccinium vitis-idaea, V. myrtillus* and *Empetrum nigrum* ssp *hermaphroditum*. In this area the *Betula pubescens* above-ground biomass was estimated to be 0.066±0.036 kg m^{-2} (mean±SD) and LAI estimated to be 0.17±0.11 m^2 m^{-2} (Dahlberg *et al.*, 2004).

Forest soils are ‘microspodosols’ with a thin O horizon (< 5 cm) underlain by glacial till on a bedrock typically of hard-shale (Sjögersten & Wookey, 2002); Soil pH in the organic horizon is 4.3 ± 0.1 (Parker *et al.*, 2015).

2.3.2 Tree selection and transect set-up

Three blocks of 3 individual trees (n = 9) were selected for study. The blocks were within a 500 m radius area and at least 150 m apart. Mountain birch trees of varying size and number of polycormic stems were selected, all located in open forest areas with ≥6 m to any neighbouring trees in the direction of the transect. Care was taken to select various topographical aspects in order to account for variation of influencing factors such as water or snow accumulation. Crown width of all trees was measured in the N-S and E-W orientation and averaged (Appendices Table 7.1).

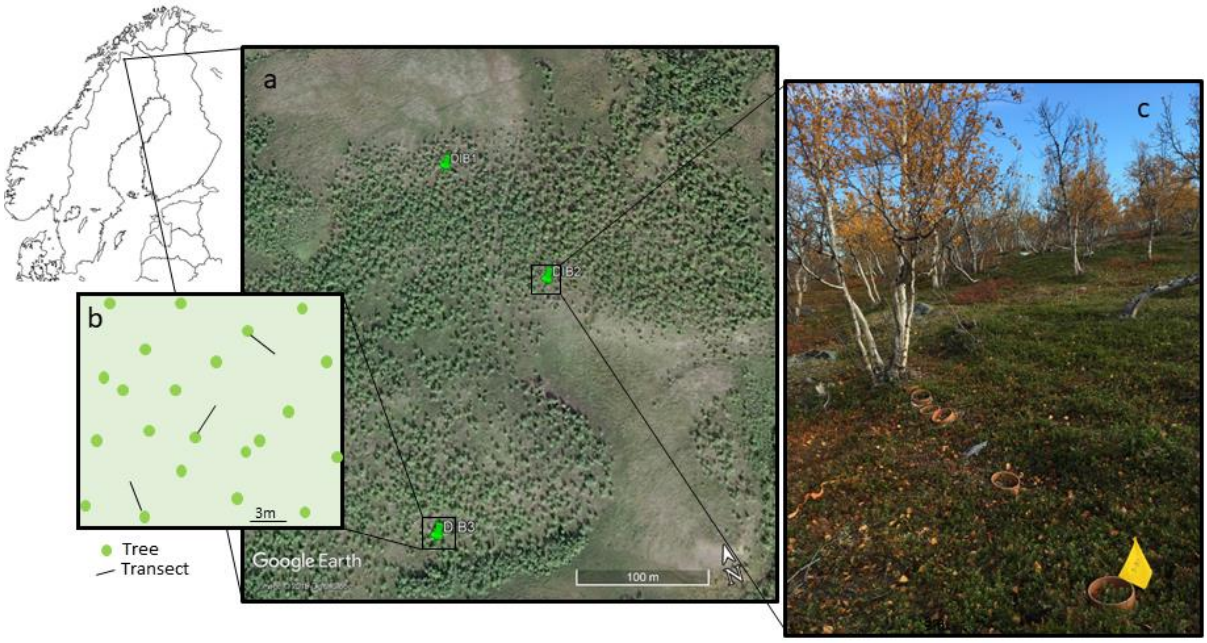

Figure 2.1 Site map and transect set ups. a) Site map of blocks in the open mountain birch forest south of Abisko. b) Representative schematic of transects extending 3 m into forest gaps. c) Photo of transect at block 2.

Transects were set up in a straight line from the base of each selected tree with measurement locations at 25, 50, 75, 100, 200 and 300 cm from the tree base. Directions of transects were intentionally varied such that each block had transects of differing compass direction to account for shading, prevailing winds or snow drifting, and to allow transects to radiate into open forest gaps.

Digital photographs of each respiration collar were taken from directly above. Image analysis software ImageJ2 (Rueden *et al.*, 2017) was used to determine the percentage vegetation cover of *Vaccinium vitis-idaea, Empetrum nigrum* and cryptogams in collars.

2.3.3 Soil and understorey C fluxes

At each point along the transects, PVC collars of 15 cm diameter and 6 cm height were secured to the soil surface with the centre of the PVC ring corresponding to a given distance. Non-setting putty (Plumber's Mait®, Bostik Ltd, Stafford, UK) was used to secure and seal the PVC collars to the soil in order to minimise disturbance of the soil and prevent severing of any roots or fungal hyphae. The forest floor vegetation was parted to allow placement of each collar resulting in vegetation inside and outside the collar (Table 2.1).

An EGM-4 infrared gas analyser with a darkened CPY-3 chamber (PP Systems International, Amesbury, MA, USA) was used to measure respiration. Respiration in this study is defined as the sum of microbial, root and shoot (including cryptogam) respiration within the chamber, and therefore represents forest soil and understorey respiration. Plants were not initially removed from collars in order to minimise disturbance to the system. Respiration rates were calculated as the slope of a linear function of CO_2 concentration increase within the closed system over a period of 96 seconds. Respiration measurements of all 3 blocks were always completed within 3 h and the order in which the blocks and transects within each block were measured was randomised each time. Measurements were conducted regularly through the full growing season in 2017 from bud swelling (08/06/17) to full leaf-out (23/06/17) and peak growing season (23-26/07/17) through to leaf senescence (22/09/17). A total of

11 repeated measurements were conducted at each collar. Additionally, on June 11th (12:00-16:40 hrs) CO_2 flux measurements were taken with a clear chamber allowing photosynthesis as an estimate of understorey gross primary production (GPP). As the vegetation canopy in the forest is higher than the measurement chamber, respiration measured here is the sum of root, soil heterotroph and limited understorey leaf respiration (65.2 ± 31.3 % cover), referred to as *respiration*. Soil temperature and moisture were measured every hour at 5 cm depth using ONSET (Bourne, MA, USA) 12-Bit Temperature Smart Sensor and EC5 Soil Moisture Smart Sensor, respectively, logged on a HOBO microstation. Temperature response curves were generated for the entire growing season for all plots (seasonal Q_{10} values); however, data from trees not in full leaf and data from extremely dry days were excluded from the analysis due to the known break-down of the temperature response at the extremes of low moisture (Sjögersten & Wookey, 2002). We note that these fluxes incorporate both autotrophic and heterotrophic flux contributions, which vary throughout the growing season. Rather than being inherent temperature sensitivities of soil decomposition, these “virtual” Q_{10} values (*sensu* Subke and Bahn 2010), integrate total below-ground metabolic activity, with higher values associated with areas of high autotrophic contributions during peak season.

2.3.4 Hyphal in-growth

Hyphal in-growth bags made of 6 x 6 cm 37-µm nylon mesh (allowing hyphal in-growth while excluding roots), and sealed with a heat sealer, were filled with 18 g of sand from the shore of lake Torneträsk (68°21’N,18°49’E) that had been washed, sieved and autoclaved twice before being oven dried. Individual hyphal in-growth bags were deployed on June 12th 2017, 15 cm to the left (when facing the tree) of each PVC collar along each transect, creating a parallel transect. Each hyphal in-

growth bag was flattened to ensure an even width of approximately 0.5 cm in each bag. This geometry was chosen to allow hyphae from various mycorrhizal species to grow into and explore through the sand depleted of organic carbon and into the organic carbon rich soil on the other side, thereby avoiding biasing for any particular species according to their characteristic ability to grow in low nutrient environments. Each bag was deployed in the organic soil horizon (just below the litter layer) at a 45° angle from the vertical (both to provide good contact with the soil, but also to avoid any biases associated with lateral or geotropic hyphal growth), thus covering a vertical depth of approximately 4 cm.

All hyphal in-growth bags were harvested on September 17th 2017, resulting in a total of 97 days in the organic soil layer. Bags were transported to the research station within 4 hours of retrieval, and all content transferred to sterile plastic bags. Samples were stored at -80°C for three days, then freeze-dried for 72 hours in a ModulyoD freeze drier (ThermoFisher Scientific, Waltham, MA, USA). The majority of in-grown hyphae are assumed to originate from ECMs, as found by Wallander et al. (2001).

Hyphae were extracted by suspending 1.5 g of sand in 25 ml deionised water and sonicating the solution for 10 minutes. The separated hyphae and 10-15 ml water solution were aliquoted into a 15-ml falcon tube to allow mixing and further separation of hyphae from sand. This was then passed through a Büchner funnel with 25-mm glass microfiber filters (Whatman ^{TM}). The hyphae-filter matrix was then analysed for carbon content using a FLASH SMART elemental analyser (ThermoFisher Scientific, Waltham, MA, USA) after drying for 72 hours at 50 °C. This process was repeated for 8 laboratory blank samples that had not been incubated in

the field and the percentage carbon content (0.34 ± 0.05 %C) was subtracted from all samples before further data processing.

2.3.5 Root in-growth

Root in-growth bags of 2 mm plastic mesh were constructed from 8 x 9 cm squares of mesh resulting in a final height of ~5 cm and a cross-sectional area of ~3 cm^2 after being filled and sealed using a heat sealer. The bags were loosely filled with organic soil collected from the tundra adjacent to the studied forest plots to mimic the loose structure of the organic soils within the forest. The collected soil was first oven dried at 85 °C for 48 h and sieved to 4 mm with most roots removed, but some small root fragments remaining. The bags were filled with 3.78 ± 0.34 g (mean ± standard deviation) of soil (average amount based on 10 randomly selected filled bags). Once filled, the bags were submerged overnight in deionised water to re-wet the dried soil and minimise loss of soil during transportation and deployment. All root in-growth bags were deployed on June 15th 2017. One root in-growth bag was deployed approximately 15 cm to the right (when facing the tree) of each PVC collar along the transect creating a parallel transect. A 2 cm diameter soil corer was used to extract a soil core to 5 cm depth and the root in-growth bag was inserted into the resulting hole. All root in-growth bags were harvested on September 15th 2017, giving a total of 92 days in the organic layer, incorporating the majority of the root growing season in subalpine birch forests in the Scandinavian sub-Arctic (Blume-Werry *et al.*, 2016). The bags were harvested using surgical grade scalpels to carefully cut any roots growing through the mesh to ensure all new root mass was maintained within the bag. In the lab, any roots protruding out of the mesh were cut off and discarded before the bags were opened; all new roots were collected and washed by finely examining the soil within each bag individually. Roots were not differentiated as

originating from birch trees or ericoid shrubs. The new roots were oven dried at 60°C for 5 days and weighed.

2.3.6 Soil core sampling

Soil cores were taken during root in-growth bag deployment. The full organic horizon depth was measured and the top 5 cm of the organic soil horizon was retained and stored at -18°C for further analysis. Subsequently, samples were oven dried at 75°C for 72 hours and soil organic matter (SOM) content for each sample determined by loss on ignition in a furnace at 550 °C for 4 h (Ball 1964). Organic soil bulk density for forested areas in the Abisko forests is 0.115 ± 0.043 g cm^{-3} (mean ± SD, n=24) and SOM was converted to soil organic carbon (SOC) content using the formula: SOC = SOM*0.5248, parameterized based on extensive data on the relationship between SOM and soil C content around this area (Parker et al. 2015).

2.3.7 Standing litter

Standing litter along transects was collected from a 25 x 25 cm area next to each collar on June 11th 2018. Collected litter was sorted into intact birch leaves and fragmented litter (including some litter from understorey species). The litter was dried at 60°C for 24 hours and weighed.

2.3.8 DNA extraction and qPCR

DNA was extracted from homogenous, representative soil samples using a DNeasy PowerSoil kit (Quiagen, Hilden, Germany) following the manufacturer's instructions. Extracted DNA was diluted 1:10 for qPCR reactions. Several different sets of primers were tested before the eventual selection of primers below. This selection was based on suitable amplicon length for the standards used. qPCR was run on a Step-One™

RealTime PCR system (Applied Biosystems) using the thermocycle programme described in Fierer and Jackson (2005) for Eub338/Eub518 primers, and May et al. (2001) for NS1/GCfung primers. Gradient PCR was performed on a Veriti 96-Well thermocycler (Applied Biosystems) prior to qPCR to validate the annealing temperatures. Primers used for 'All fungi' were NS1 (5'-GTA GTC ATA TGC TTG TCT -3') (White *et al.*, 1990) and GCFung (with GC clamp removed) (5'-CAT TCC CCG TTA CCC GTT -3') (May *et al.*, 2001). Primers used for 'All bacteria' were Eub338 (5'-ACT CCT ACG GGA GGC AGC AG-3') and Eub518 (5'-ATT ACC GCG GCT GCT GG-3') (Fierer & Jackson, 2005). Each 20µl reaction contained 10µl (2x) PerfecTA SYBR Green Fastmix with ROX (QuantaBio, Maine, USA), 1.25µl of each forward and reverse primer, 1.25µl (20 mg ml^{-1}) BSA, 2µl DNA template and 4.25µl nuclease free water. Two technical repeats were run per sample. Standards were created using *Pseudomonas putida* and *Saccharomyces cerevisiae* DNA with bacterial and fungal primers respectively. The resulting PCR product was cleaned using Wizard® SV Gel and PCR clean-up system (Promega, UK) and quantified by UV spectrophotometer (NanoDrop 2000, Thermo Scientific). Standard DNA was serially diluted by 1:10 and 5 point repeated standard curves were generated for bacteria (10^9-10^4, excluding 10^7) and fungi (10^7-10^3). All standard curves had R^2 >0.99. Melt curve analysis was performed for all samples and standards to confirm a single product.

2.3.9 Statistical analysis

Variation in respiration, root production and hyphal production along transects was investigated using a linear mixed effects model (Pinheiro *et al.*, 2012) with block, tree and collar assigned as random effects, accounting for variation between sampling dates. In the respiration model soil temperature and moisture were included as fixed

effects along with distance along the transect. Parametric bootstrapping (x1000) was used to predict 95% confidence intervals of the model. Tree basal area and vegetation cover were removed as covariates from the respiration model as they did not significantly improve the model fit as measured by Akaike Information Criterion (AIC) values. In the root and hyphal production models distance along the transect was included as a fixed effect in the respective models. SOC stocks and vegetation cover were analysed using a similar method with block and tree as random effects and distance along the transect as a fixed effect in their respective models. Seasonal Q_{10} values were modelled for each collar individually using the non-linear model fit *nls.lm* in R package *minpack.lm* (Elzhov *et al.*, 2010) following the exponential regression: $SR = Rate0\ Q_{10}^{(T/10)}$, where SR is fitted soil CO_2 efflux, Rate0 is the basic respiration rate at 0 °C (y-axis intercept), Q_{10} is the fitted temperature sensitivity, and T is soil temperature.

All analyses was carried out using R Version 3.4.0.

2.4 Results

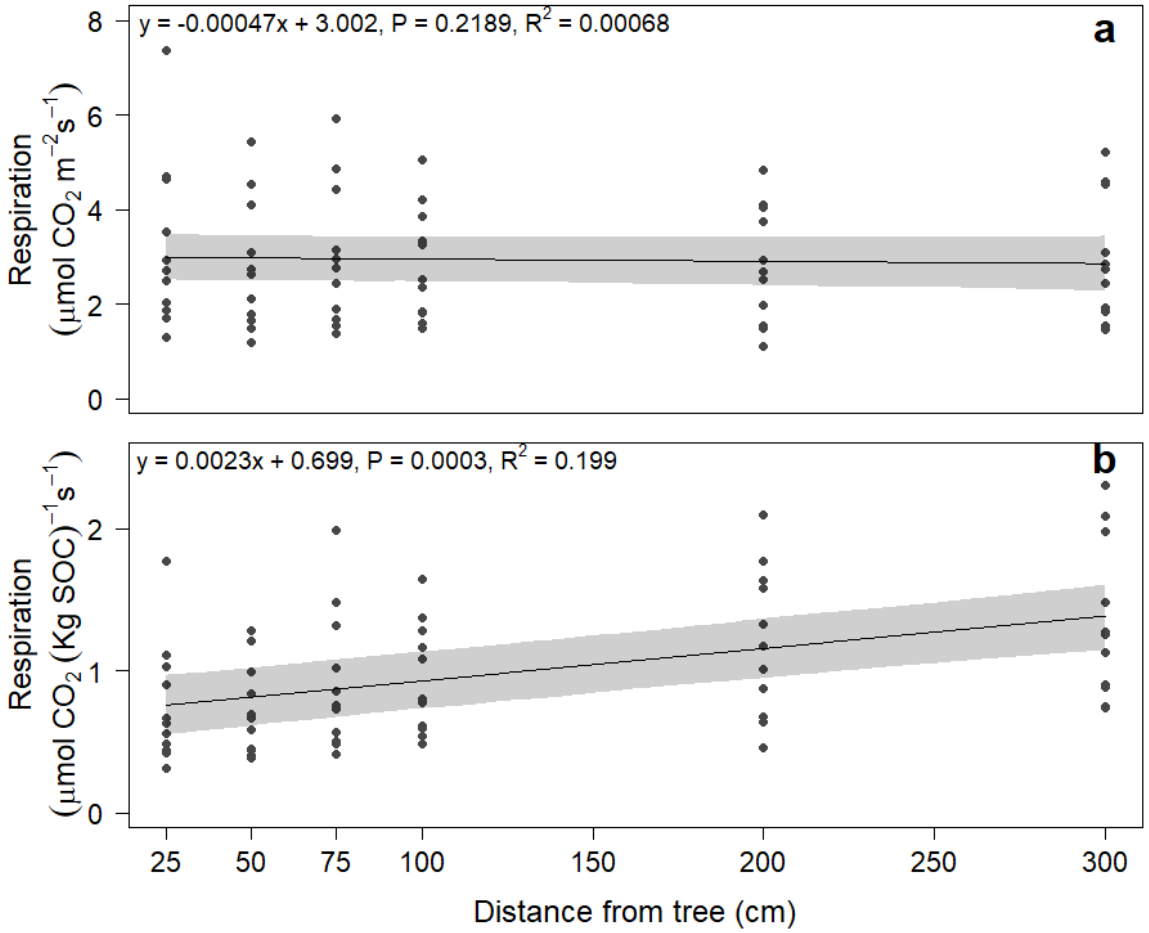

Figure 2.2 Respiration along transects extending 3 m from birch trees. a) Respiration per unit soil area and b) respiration per unit soil organic carbon. Solid line is predicted respiration based on a linear mixed effects model. Points are means at the given distance for each of the 11 measurement days. Grey polygon indicates 95% confidence bands of the predicted line.

Respiration showed no significant differences (*P* = 0.22) between each point measured along transects extending 3 m radially from the base of mountain birch trees. A linear mixed effects model (Figure 2.2a) predicts no difference in respiration ≤3m away from the nearest tree. By contrast, at all points along transects, we found variation by a factor of 2-4 between replicate trees measured.

However, when respiration along transects is presented per unit SOC (Figure 2.2b), rather than per unit area, there is a significant (P = 0.0003) increase in respiration per unit SOC with increasing distance from the tree.

Table 2.1 Soil organic carbon (SOC) content, understorey gross primary productivity (uGPP) and collar vegetation (±Standard Error) along transects extending 3 m from individual trees. P-values refer to the significance of the effect of distance from trees.

Distance from tree (cm)	**Soil Organic Carbon Stocks (kg m^{-2} ±SE)**	**Understorey uGPP (µmol C $m^{-2}s^{-1}$ ±SE)**	**Collar vegetation cover (%±SE)**			
			Vaccinium vitis-idaea	Crypto-gams	*Empetrum nigrum*	Total vegetation
25	4.70 ± 0.48	-2.33 ± 0.54	37.8 ± 9.4	2.2±0.9	21.4±10.6	63.3 ±10.8
50	4.94±0.97	-2.08±0.41	25.4±6.6	13.0±6.3	18.0±10.1	56.4±11.6
75	3.91±0.72	-1.95±0.50	22.1±4.1	11.2±5.3	18.8±8.4	55.8±12.4
100	3.41±0.66	-3.08±0.64	23.8±4.6	20.0±8.9	36.1±13.3	83.2±8.8
200	2.57±0.36	-2.46±0.63	37.7±10.5	6.6±2.6	14.4±4.4	61.6±11.0
300	2.55±0.33	-2.12±0.79	23.6±4.9	10.2±4.3	32.2±8.4	71.0±7.0
P-value	**0.02**	0.94	0.79	0.99	0.50	0.48

The SOC stocks (P=0.02; Table 2.1) and total standing litter (P=0.0009; Supp. Figure 2.2) are significantly negatively related to distance from the tree. By contrast, understorey gross primary production (uGPP), measured on 11 June 2017 as the difference in CO_2 production in dark versus full sunlight conditions, did not vary significantly along the 3 m transects (P=0.94; Table 2.1).

The composition and total coverage of vegetation within the collars used for respiration measurements along transects did not vary significantly with distance (*P*=0.31; Table 2.1).

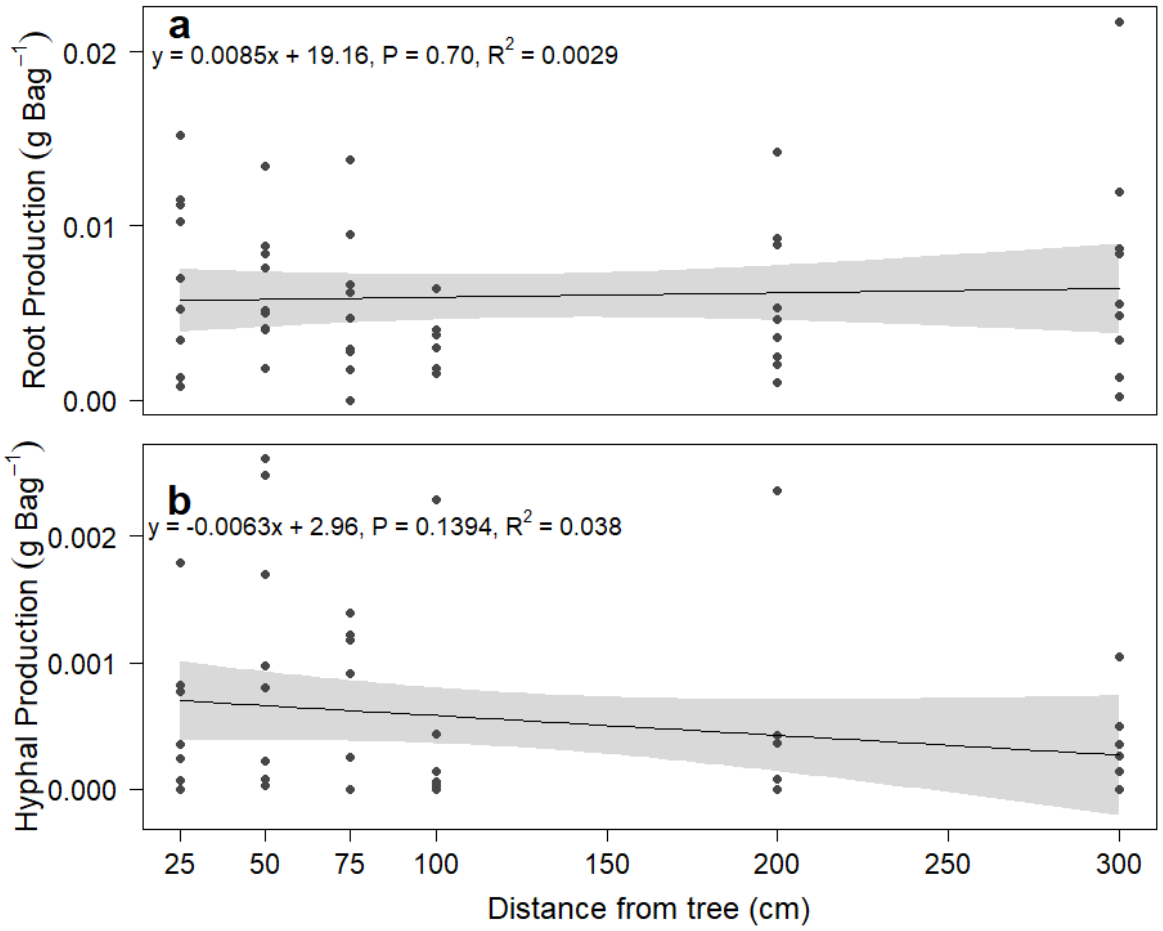

Figure 2.3 Root (a) and hyphal (b) production for 92 and 97 days respectively during the growing season 2017 in in-growth bags along transects extending 3 m from individual trees. Solid line is predicted root production based on a linear mixed effects model. Grey polygon indicates 95% confidence bands of the predicted line.

There was no significant difference in either total root (*P*= 0.69) or hyphal (*P*=0.14) production along transects over growing season (Figure 2.2), with high levels of variation in production between replicate trees observed in both root and hyphal production. There was no significant difference (*P*=0.31) with distance from tree

when roots were normalised by % total vegetation cover. Corresponding to patterns observed in root and hyphal production, the seasonal Q_{10} (Appendices Figure 7.2), does not vary significantly (P=0.064) along transects extending from tree bases (Figure 2.4).

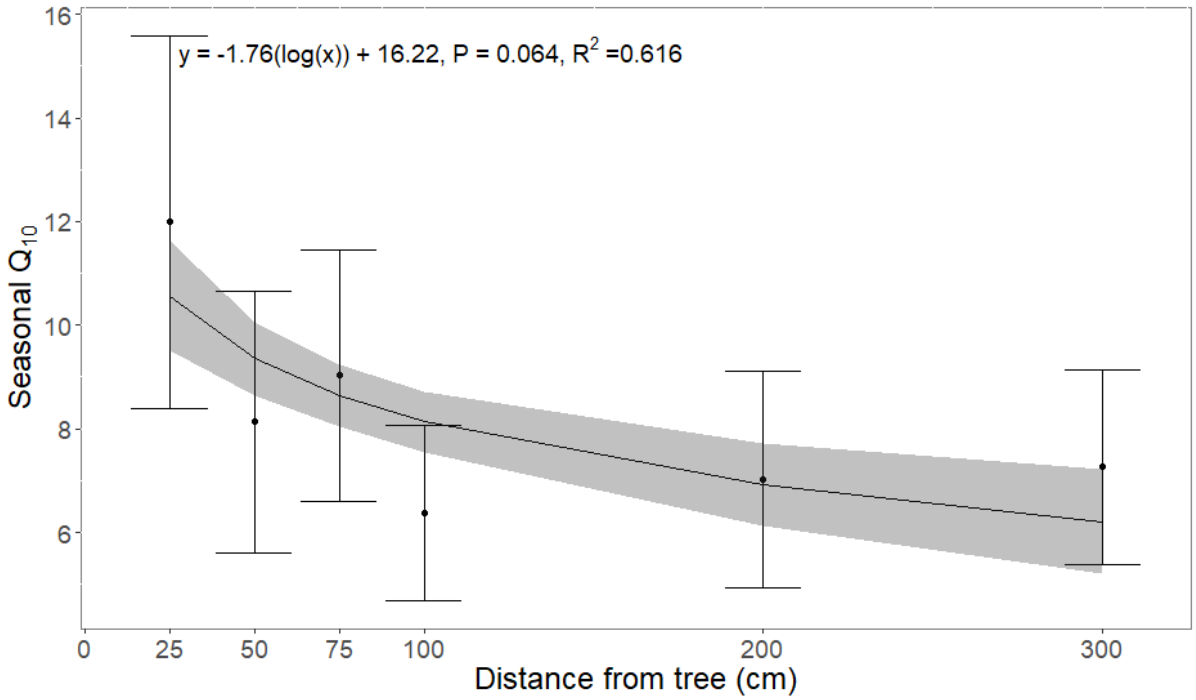

Figure 2.4 Change in seasonal Q_{10} with increasing distance from trees. Seasonal Q_{10} values generated from temperature response curves in Supplemental Figure 5. Grey polygon indicates 97.5% confidence band of the predicted line. Error bars represent standard error of replicates at each distance from trees.

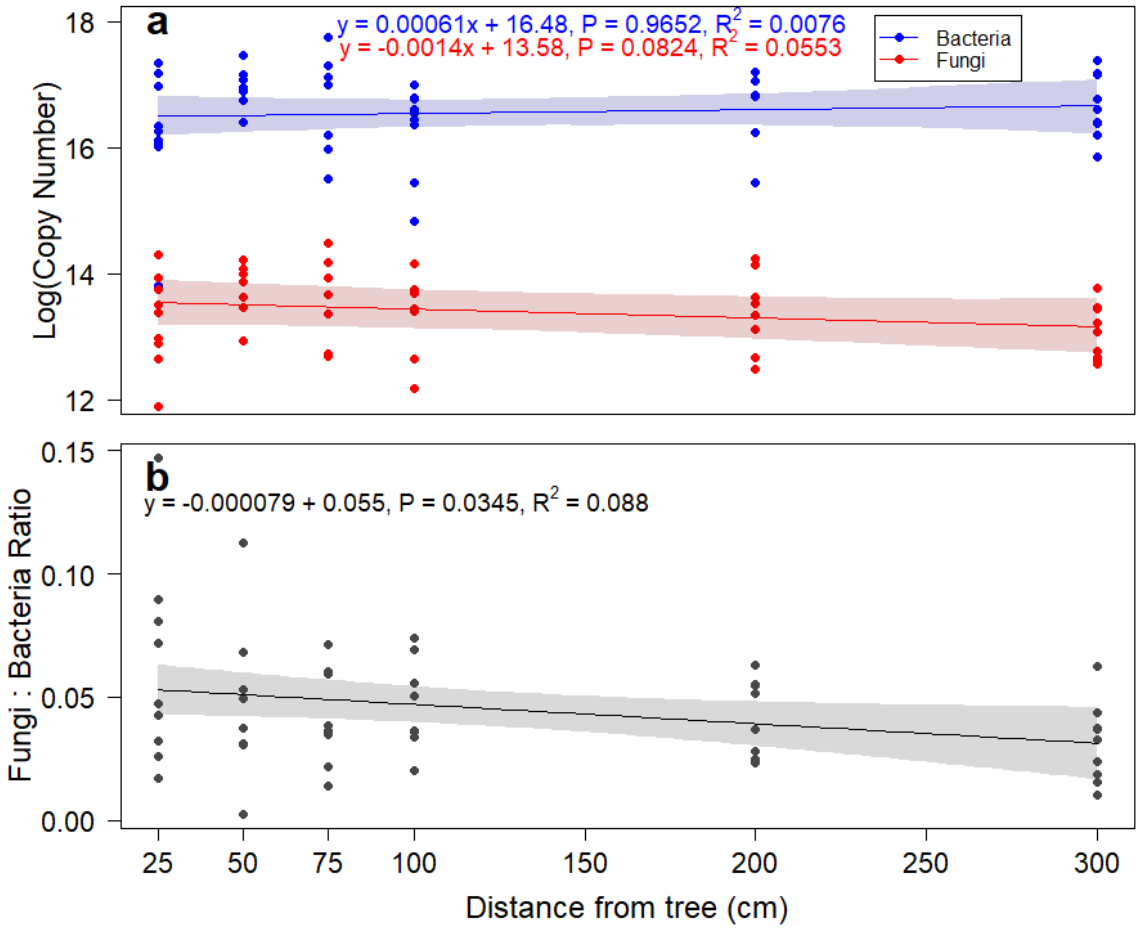

Figure 2.5 Abundance (a) and ratio (b) of fungi and bacteria along 3 m transects extending away from trees. Solid lines are predicted based on a linear mixed effects model. Opaque polygons indicate 97.5% confidence bands of the predicted lines.

The quantity of neither total fungi (*P*=0.082) nor total bacteria (*P*=0.97) varied significantly with distance from trees (Figure 2.5a). These data were used to generate a fungi:bacteria ratio along the transect and here, by contrast, there is a significantly decreasing relationship, declining by 0.077 ± 0.035 per m (*P* = 0.035) with increasing distances from trees (Figure 2.5b).

2.5 Discussion

Through systematic measurements of key soil processes at varying distances from trees, we found no significant trends in respiration and mycorrhizosphere productivity within mesic mountain birch forests (Hypotheses 1 and 2 are therefore not supported by the data). We did, however, detect a significant increase in the ratio of fungi:bacteria with increasing distance from trees (Figure 2.5b), providing support for Hypothesis 3. By contrast, we noted that litter fall and SOC declined significantly with increasing distance from trees (Figures 2.2, 2.3, Table 2.1 & Appendices Figure 7.1), supporting Hypothesis 4a, but not 4b. These findings are summarised graphically in Figure 2.6.

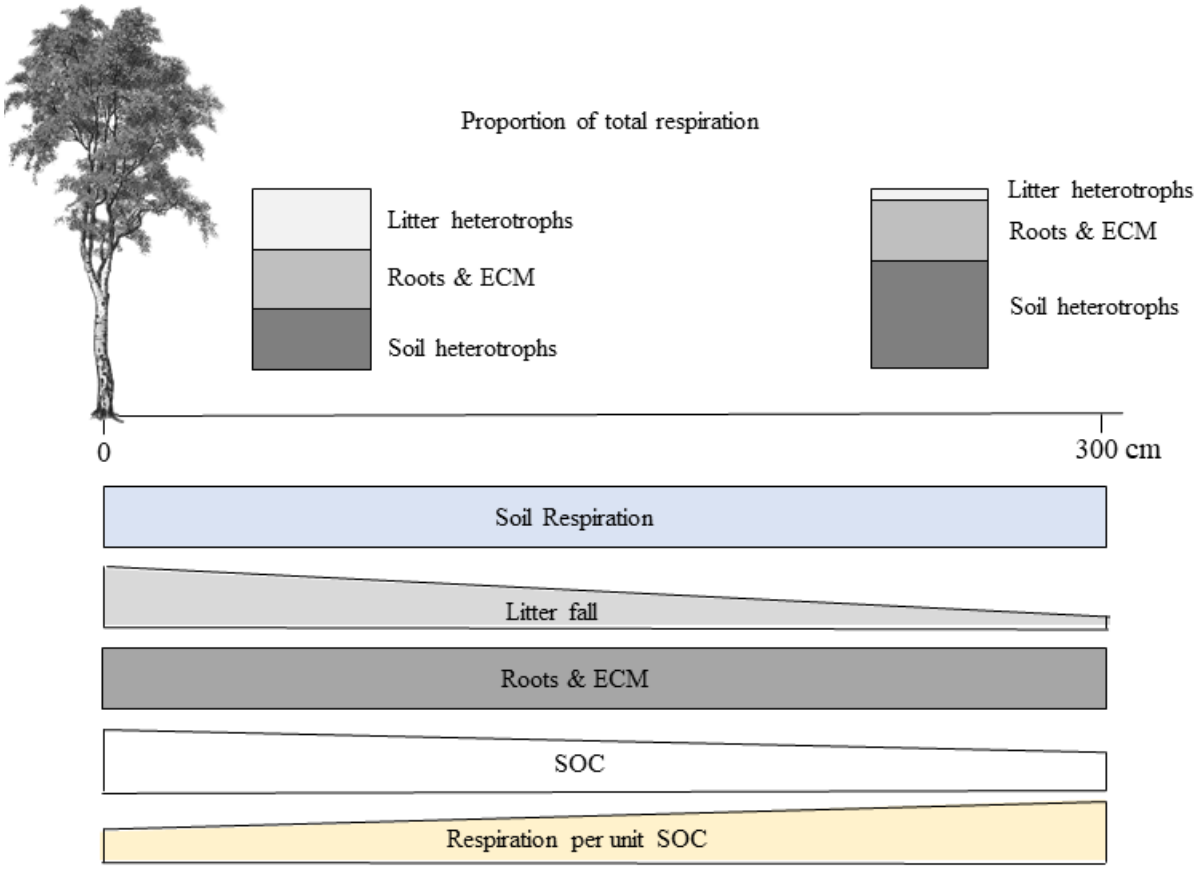

Figure 2.6 Schematic diagram summarising soil processes measured in this study at varying distances away from individual trees. The partitioning of respiration explained by tree-mediated inputs is based on the data presented here, but requires further investigation. SOC: Soil Organic Carbon.

The lack of significant trends in mycorrhizosphere productivity does not signify homogeneity in these soils, as high levels of variation between studied trees were apparent (Figures 2.1 & 2.2). This lack of a clear spatial trend in mycorrhizosphere productivity, however, is particularly relevant in the context of the open structure of treeline ecotone forests. With 2-7 m between trees (Table 7.1), a 3 m transect represents the likely maximum distance obtainable from the nearest tree base without being closer to a neighbouring tree. Thus, if root and hyphal production show no significant trends on a scale of up to 3 m with respect to the nearest tree, then it can be hypothesized that this *lack* of pattern reflects efficient exploitation of the soil resource by root and ECM networks extending throughout these forests (given similar environmental and edaphic contexts). The influence of trees on soil processes such as respiration and mycorrhizosphere productivity up to (and potentially beyond) 3 m away from trees is further supported by similar mean seasonal Q_{10} values across all measured distances along the transect (Figures 2.4 & Appendices Figure 7.2).

The absence of a trend in respiration and mycorrhizosphere productivity with increasing distance from trees within the forest does not support the original Hypotheses 1 and 2. These findings therefore also contrast with those of Parker et al. (2016), where systematic declines in respiration were noted 150 cm away from tree bases compared with 50 cm. It should be noted, however, that Parker et al. (2016) reported fewer fine scale spatial measurements and lower levels of replication than the current work. Our results are also in accordance with 46% of reviewed datasets in a recent meta-analysis across plant communities, which found no significant horizontal spatial variation in ectomycorrhizal communities (Bahram *et al.*,

2015). The lack of spatial variation with respect to respiration and mycorrhizosphere productivity found here may be linked to widespread activity of birch tree roots, ectomycorrhizas and bacteria, directly or indirectly contributing to organic matter and soil carbon decomposition and nutrient cycling (Bödeker et al. 2016; Lindahl and Tunlid 2015; Talbot et al. 2008).

Despite the lack of trends mediated by trees on respiration and mycorrhizosphere production, we identified a significant decline in SOC stocks with increasing distance from trees (Table 2.1), from which we can infer a difference in C accumulation rates and/or turnover with distance from trees (Hypotheses 4a and 4b). A decline in SOC with increasing distance from the tree, or build-up of SOC closer to the tree, is likely a result of higher litter inputs (Appendices Figure 7.1), or higher fungi:bacteria (Figure 2.5b) ratio (and thus slower decomposition; Hypotheses 3 and 4), or a combination thereof. Respiration data (Figure 2.2a) indicate that higher litter inputs close to the tree are not associated with higher rates of decomposition, which implies that, despite the apparent higher availability of C closer to trees, there is not a corresponding increase in microbial activity. This may reflect litter recalcitrance or slowed C turnover in ectomycorrhizal dominated soils potentially hampering early stage decomposition (Sterkenburg *et al.*, 2018). Although we could not detect a trend in respiration per *unit area*, there was a clear increase in respiration per *unit SOC* with increasing distance from trees (Figure 2.2b). This shows greater respiration per unit resource (specifically, here, carbon) available to heterotrophs further away from the tree (thus not supporting Hypothesis 4b). Greater respiration per unit SOC indicates higher potential for SOC loss if the carbon use efficiency (CUE) - the proportion of C assimilated by the microbial community relative to C lost through respiration - is low (Cotrufo *et al.*, 2013; Sauvadet *et al.*, 2018; Kallenbach *et al.*,

2019). Furthermore, this suggests that the proportion of total respiration facilitated by the rhizosphere (roots and ECM) is larger further away from the tree (Figure 2.4), consistent with declining SOC stocks (and leaf litter inputs) at increasing distances from trees. Larger relative influence of the rhizosphere suggests an increase in root and microbial activity further away from trees, with potentially low CUE, as there is no change in root and hyphal production along transects. Increased peripheral activity may result from competition between root and hyphal networks associated with neighbouring trees (Boddy, 2000) and provides tantalising, although indirect, evidence for larger autotrophic contributions to respiration and potentially rhizosphere priming of the soil heterotrophic community (Figure 2.6) (Fontaine *et al.*, 2007; Wild *et al.*, 2016). The current study does not, however, allow us to allocate the respiration signal to various C sources originating from many potential C pools within the ecosystem, and therefore caution must be applied when interpreting results.

Key soil processes, such as C turnover and storage, relate to the diversity of morphotypes (ECM or ERM, inferred here based on above-ground vegetation being ericaceous shrubs or *Betula* spp.), growth forms and exploration types of the mycorrhizal community (Cornelissen *et al.*, 2001; Clemmensen *et al.*, 2013, 2015; Hazard & Johnson, 2018). Changes in soil microbial community composition along transects could be examined in more detail using appropriate sequencing techniques to identify microbial species present. Here we find that, although production rates of roots and hyphae remained consistently high up to 3 m from trees within the forest, there was a decline in the fungi:bacteria ratio, and therefore potential C storage (Bailey *et al.*, 2002). The apparent discrepancy between hyphal production and fungi:bacteria ratio may be explained by an inability of the hyphal production assay

to detect small changes in abundance detectable at a molecular level, or by variation in the abundance of bacteria or saprotrophic fungi, as the primers used are not ECM specific. This pattern may, in part, be caused by the dependence of saprotrophic fungi on C inputs in the form of birch tree litter, which decrease with increasing distance from trees (Appendices Figure 7.1) and are more labile than understorey litter (Parker *et al.*, 2018). Variation in standing litter is likely to cause spatial heterogeneity within the saprotrophic community not seen in mycorrhizal fungi communities, which receive plant photosynthates directly (Lindahl et al., 2007).

Our evidence for patchiness of SOC stocks in birch forests should be interpreted in the context of forest succession patterns, as varying SOC stocks and C accumulation in relation to individual trees may be linked to historical demographics and turnover (i.e. the forest seen today is merely a snapshot of a dynamic ecosystem (Callaghan *et al.*, 2002; Van Bogaert *et al.*, 2011). Without the on-going recruitment and deaths of trees, there might be net accumulation of SOC close to trees, and net loss of SOC further away from trees. However, with on-going turnover – tree death, and establishment of trees in forest gaps – the development, persistence and magnitude of spatial patterns in SOC stocks at contrasting distances from living trees will be related both to forest dynamics as well as soil organic matter turnover. Slow population turnover would lead to increasingly patchy SOC stocks driven by trees. However, establishment of trees in forest gaps on shorter time scales, could facilitate re-accumulation of lost SOC by new trees in forest gaps. In Fennoscandian Lapland, trees can be 200 years old (Tømmervik *et al.*, 2009), but age varies significantly (86±43 years (mean±SD); Millar (1980)) with a high density of mountain birch seedlings/saplings (~1800 ha^{-1} of ≤ 1.5 m height and ≤ 15 mm basal stem diameter; B. Dick, pers. comm.). Decaying tree stumps are also evident

and important as the influence of a single tree may be long lasting, given the microbial community it sustained whilst alive, and will influence future microbial communities after death (Clemmensen et al. 2013; Fernandez et al. 2016; Godbold et al. 2006; Varik et al. 2013). Furthermore, cyclical defoliating *Epirrita* moth outbreaks occur in this area on decadal timescales (Olsson et al. 2017; Tenow and Bylund 2000) causing tree loss or increased polycormy. Taken together, the timescales of forest dynamics may enable development of patchy SOC stocks related to individual trees, as found here. This further illustrates the complexity of soil C dynamics (Sulman *et al.*, 2018) and emphasises the importance of long-term measurements and integration of biological traits with soil processes for global change modelling and predicting Arctic responses to climate change at plot and vegetation levels (Shaver *et al.*, 2013; Wurzburger & Clemmensen, 2018).

Based on our measurements, we suggest that the influence of an individual mountain birch tree within these forests extends at least 3 m from the main stem, far exceeding the average crown diameter (167 ± 35 cm; Appendices Table 7.2). This influence includes continued high production rates of fine roots and ectomycorrhizas to at least 3 m away from trees, with the ectomycorrhizal network likely to extend further beyond that of the root network (Anderson and Cairney 2007; Simard and Durall 2004). The spatial distribution and range of ectomycorrhizal hyphae depends on fine root distribution (Pickles *et al.*, 2010) and mycorrhizal taxa (Agerer, 2001), but mycelia have been demonstrated to extend up to 9.6 cm away from mycorrhizal root tips, depending on exploration types (Weigt *et al.*, 2012). Although we lack direct evidence, the wide ranging influence and extent of the birch mycorrhizosphere and associated ectomycorrhizas in this area provides a tantalizing suggestion of the presence of a *wood-wide-web* and the potential for common mycelial networks

(CMNs) (Beiler et al. 2010; Johnson 2015; Peter 2006; Selosse et al. 2006). The ability of ectomycorrhizas to colonise multiple host plants, and thereby create a cytoplasmic link through which nutrients (He *et al.*, 2003; Selosse *et al.*, 2006) and signalling molecules (Babikova *et al.*, 2013b) can be exchanged, has been well documented in the lab (Arnebrant et al. 1993; Finlay and Read 1986; Pickles et al. 2017). However, there are very few studies of CMNs in the field (Deslippe and Simard 2011; Simard et al. 1997) and, to our knowledge, none on mountain birch trees in the Arctic. Deslippe et al. (2011) found transfer of C between conspecific pairs of dwarf birch (*Betula nana* L.) in the Alaskan Arctic and argued that the magnitude of this C transfer may alter competitive plant interactions in the arctic tundra. The implications of the potential presence of common mycelial networks in the Arctic, and in other regions, could be profound for forest health, C turnover and stocks, both above- and below-ground.

2.6 Conclusions

Here we have focussed on the influence of mountain birch trees within forests on key soil processes such as respiration, SOC stocks, root and mycorrhizal production and microbial community abundance in an ecotone forest in sub-Arctic Sweden. We conclude that the direction, strength and magnitude of spatial trends within the forest floor in relation to proximal trees vary greatly. Our results show spatial variation in C accumulation rates in relation to nearest trees through changes in SOC stocks and litter fall. Contrastingly, we find no trend in respiration with distance from trees, likely mediated by an extensive root and ectomycorrhizal network of the birch trees, which efficiently exploit resources throughout the forest. This is an early indication of a '*wood-wide-web*' in these forests. Further investigation is required, however, to

understand the extent and significance of such a network. Recognising the interactions between the above-ground vegetation and below-ground soil processes, and the soil C cycles that they mediate, is crucial in these globally important sub-Arctic ecosystems undergoing climate change.

Chapter 3:
The spatial ‘reach’ of tree mycorrhizospheres for nitrogen scavenging

3.1 Abstract

Nitrogen (N), acquired by mycorrhizal fungi and supplied to host plants via the mycorrhizosphere, is the most growth limiting nutrient at the sub-Arctic treeline. The ability of trees and shrubs at this ecotone to access N pools provides a competitive advantage and shapes community dynamics in these forests. Here I investigate the spatial distance (or ‘reach’) over which trees and shrubs can access soil N pools in treeline forests using a ^{15}N soil labelling approach. A ^{15}N label was injected into the soil rooting zone and foliar samples collected from trees 1 – 50 m away and shrubs 1 – 10 m away from the labelled soil. I found ^{15}N label in mountain birch trees up to 4.5 m away from the labelled soil, beyond half the mean maximum distance between trees (~3 m). Understorey shrubs accessed the ^{15}N label over shorter distances than trees and showed evidence of highly directional root and mycorrhizal systems. The difference in mycorrhizosphere exploration distances between mountain birch trees and understorey shrubs may confer competitive advantage to trees, which may alter community structures within these forests. This is particularly important in light of predicted climate driven tree and shrub expansion in sub-Arctic treeline regions, with likely consequences for ecosystem carbon cycling and storage.

3.2 Introduction

Plant-soil interactions are vital for ecosystem function, and form inter-dependent links between above-ground plant communities and below-ground microbial communities (Wardle *et al.*, 2004). The central link in plant-soil interactions is the rhizosphere and the maintenance of symbiotic relationships with mycorrhizal fungi which explore the soil for water and nutrients and, in turn, receive plant photosynthates (Anderson & Cairney, 2007; Smith & Read, 2010), also referred to as the mycorrhizosphere (Sommer *et al.*, 2017). These symbiotic relationships are found in >90% of all terrestrial plants (Cairney, 2000) and are increasingly recognised as key players in shaping ecosystem function (Hazard & Johnson, 2018), geographical patterns (Read & Perez-Moreno, 2003; Wurzburger *et al.*, 2017) and nutrient cycling (Zhang *et al.*, 2019). Mycorrhizal fungi fall into several functional types based on the structures that create the physical interactions with their host, the family of host plants with which they associate, and their ability to mobilise soil nutrients (Read, 1991; Read & Perez-Moreno, 2003). The three most common functional types are (i) ectomycorrhizas (ECM) which associate with many tree species and form sheaths over plant roots externally, (ii) arbuscular mycorrhizas (AM) which associate with many grassland species and form arbuscule structures within plant root cells, and (iii) ericoid mycorrhizas (ERM) which associate with ericaceous plants and form coils within plant root cells (Read, 1999).

Within mycorrhizal fungi, different species vary according to *exploration type* which determines how much the mycelia and hyphal networks of the mycorrhiza increase the surface area of soil exploration and the distance the mycorrhiza extend from the root tip (Agerer, 2001). Exploration types vary from short, contact-dependent, forms,

to cord-forming or long distance extra-radical mycelia (Agerer, 2001), which have been shown to extend substantial distances away from the root tip (Weigt *et al.*, 2012). The length of extra-radical mycelium of AM and ECM fungi varies considerably, with typical estimates ranging from 10 to 100 m hyphae g^{-1} soil, or even up to hundreds of metres of hyphae per metre of root length (Leake *et al.*, 2004). In addition to functional and exploration type, factors such as hyphal turnover, and hyphal uptake capacity and efficiency may influence mycorrhizal nutrient foraging (Chen *et al.*, 2018). Recent evidence suggests that mycorrhizal nitrogen (N) mining strategies can affect soil C (Sulman *et al.*, 2017; Wurzburger & Brookshire, 2017) and ecosystem responses to elevated CO_2 (Terrer *et al.*, 2018). One context within which it is important to understand soil exploration by mycorrhizal fungi, and how this can shape spatial patterns in soil C, is in the sub-Arctic regions, where climate change has been predicted to cause increased ecosystem productivity and a northward advance of treelines into tundra (Myers-Smith *et al.*, 2011; Epstein *et al.*, 2013; Hofgaard *et al.*, 2013; Bjorkman *et al.*, 2018). This may lead to the mixing or replacement of resident ERM mycorrhizal communities by new ECM-dominated communities (Collier & Bidartondo, 2009), with consequences for soil C storage and turnover (Hartley *et al.*, 2012; Parker *et al.*, 2015).

Root and mycorrhizal production in the open treeline mountain birch forest in sub-Arctic Sweden have recently been found to be consistently high up to 3 m away from the base of individual trees, well beyond the crown width and potentially spanning large forest gaps (Friggens et al. 2019, Chapter 2). These findings indicate the potential for the treeline forest floor to be extensively explored by both roots and ECMs for nutrient & water uptake. This in turn may suggest the presence of a so called *wood-wide-web* in these forests (Wiemken & Boller, 2002; Simard & Durall,

2004; Beiler *et al.*, 2010) whereby the entire forest floor is explored by hyphal and mycelial networks connected to multiple trees. Extensive exploration by the mycorrhizosphere in these forests suggests that nutrient foraging by mycorrhizas may occur over long distances.

The understorey of these treeline forests is made up of both ECM shrubs (*Betula nana* L.) and ERM shrubs (*Vaccinium vitis-idaea* L., *Vaccinium myrtillus* L. and *Empetrum nigrum* L.). ERM fungi have short exploration type growth forms, which contrasts with ECM fungi commonly associated with the canopy forming mountain birch trees which associate with ECMs and are capable of long distance exploration types. Due to this co-occurrence of ECM and ERM fungal species within the forest, and the variation in exploration types formed by the two different mycorrhizal types, there may be a difference in the range of nutrient foraging and extent of hyphal exploration between canopy and understorey species.

One of the main nutrients exchanged between fungus and host plant in mycorrhizal symbioses is nitrogen. Nitrogen, essential for plant growth and photosynthesis, is generally considered the most limiting nutrient in high-latitude sub-Arctic ecosystems (Wallenda & Kottke, 1998; Sjögersten & Wookey, 2005). Although N is a limiting nutrient in Arctic and boreal ecosystems other key nutrients such as phosphorus (P) and potassium (K) can be limiting (Shaver & Chapin, 1995). Furthermore, the interaction and combination of multiple mineral nutrients such as N and P can alter or alleviate plant nutrient limitation (Street *et al.*, 2018a). Here the focus will be on N limitation. N limitation may be maintained through high N retention by mycorrhizas at low N levels and greater fungus-plant N transfer at high N levels (Näsholm *et al.*, 2013; Franklin *et al.*, 2014). Plants can use N in both inorganic and organic forms, particularly in systems where N is limited (Schimel & Bennett, 2004). The uptake of

organic N is facilitated by mycorrhizal fungi deploying hyphal exploration networks to forage for N using extracellular enzymes to degrade and decompose complex organic material (Schimel & Bennett, 2004; Talbot *et al.*, 2008; Lindahl & Tunlid, 2015). This strategy is utilised by both ECM fungi which associate with *Betula* species (Read & Perez-Moreno, 2003; Lin *et al.*, 2017) and ERM fungi which associate with ericoid shrubs (Emmerton *et al.*, 2001). Mycorrhizas further compete for soil N with free living saprotrophic fungi which have a much larger arsenal of extracellular enzymes for the decomposition of soil organic matter (Read & Perez-Moreno, 2003). However, due to the supply of photosynthates that mycorrhizal fungi receive from their hosts, they are able to grow through areas of low C and N to find patches of high N concentration in the soil, which they then mine extensively (Bending & Read, 1995). The presence of a wood-wide-web in these forests may result in N foraging and transport over long distances as plants and mycorrhizas exploit the nutrient resources surrounding them.

Labelling techniques, using the stable isotope ^{15}N, have been used to measure available N and N flow through soil and plant pools in ecosystems (Schimel & Bennett, 2004). These techniques have been used extensively to investigate transfer of N from source materials to plant tissues mediated by mycorrhizas in controlled environments (Finlay *et al.*, 1988; Johansen *et al.*, 1992; Hogberg *et al.*, 1999; Taylor *et al.*, 2004; Wurzburger & Brookshire, 2017) and in field experiments (Clemmensen *et al.*, 2008; Högberg *et al.*, 2008; Jones *et al.*, 2009; Remy *et al.*, 2016). To my knowledge, however, they have not been applied to investigate N transfer over long distances within sub-Arctic treeline mountain birch forest ecosystems. Göttlicher *et al.* (2008) applied a ^{15}N tracer to examine N transfer in 10 m radius plots of coniferous boreal forest, revealing transport to distances up to 9.5 m from sources.

High latitude mountain birch forests, as opposed to the boreal forest studied by Göttlicher *et al.* (2008), are undergoing rapid and dynamic changes in primary productivity and species shifts driven by climate change (Myers-Smith *et al.*, 2011; Elmendorf *et al.*, 2012; Epstein *et al.*, 2012a; Bjorkman *et al.*, 2018). In light of this dynamic change, much knowledge is to be gained from studying the presence and strength of a wood-wide-web in mountain birch forests and the consequences of this on organic matter decomposition and nutrient cycling within these important ecosystems. To test the presence and spatial reach of a wood-wide-web and nutrient foraging hyphal networks in a sub-Arctic treeline forest, a nitrogen stable isotope (^{15}N) pulse-chase field experiment was set up, where ^{15}N was injected into the soil and traced into surrounding trees 1-50 m away and shrubs 1-11 m away. As roots and mycorrhizas explore extensively within open mountain birch forests I hypothesise that:

1. Ectomycorrhizal mountain birch trees can access soil nitrogen at least 3 m from a nitrogen source in the soil.
2. Ericoid mycorrhizal understorey species have a shorter range of nitrogen foraging than ectomycorrhizal mountain birch trees.

3.3 Materials and Methods

3.3.1 Site description and plot set-up

All studied plots were set up within a permafrost-free area (approx. 1 km^2) in the sub-Arctic treeline ecotone at Nissunsnuohkki, c. 4 km South of Abisko, Sweden (ca. 68°18'56.2"N 18°49'18.2"E), at ~600 m asl. The treeline forest is formed by mountain

birch (*Betula pubescens* Ehrh. ssp *czerepanovii* (Orlova) Hämet Ahti) and has an open canopy structure with an ericaceous understorey consisting of *Betula nana* L., *Vaccinium vitis-idaea* L., *V. myrtillus* L. and *Empetrum nigrum* L. ssp *hermaphroditum* (Hagerup) Böcher. Forest soils are microspodosols with a thin O horizon (< 5 cm) underlain by glacial till on a bedrock typically of hard-shale (Sjögersten & Wookey, 2002); soil pH in the organic horizon is 4.3 ± 0.1 (Parker et al., 2015). Within the open mountain birch forest, five plots with 50 m radius were selected (graphically presented in Figure 3.2). Care was taken to ensure a relatively homogeneous tree density and mixture of tree sizes within the plots. All plots were ≥50 m apart.

3.3.2 Soil Isotope Labelling

A 1 x 1 m square of ground was measured out at the centre of each plot for the ^{15}N label to be applied (Figure 3.1). 50 g of $^{15}NH_4Cl$ ≥98 atom% ^{15}N (Sigma-Aldrich, Dorset, UK) was dissolved in 5 L of deionised water resulting in a 10 g L^{-1} $^{15}NH_4Cl$ solution. One litre of the 10 g L^{-1} $^{15}NH_4Cl$ solution was dispensed in the 1 m^2 central labelling area (equivalent of 1 mm rainfall) as 100 x 10 ml soil injections. Soil injections were conducted as per Clemmensen *et al.* (2008) using a syringe inserted at 5 cm soil depth and pulling it up as the solution is dispensed so that the solution was applied within the top 5 cm of soil. The ^{15}N label was applied to all plots on the 10th June 2018.

3.3.3 Foliar sampling

Twelve trees at varying compass directions up to 20 m from the plot centre and three trees 20-50 m away from the plot centre were selected for sampling and their distances from the centre measured (Figure 3.1 & 3.2). 10-15 leaves from different

parts of the tree crown were sampled. This was conducted before any ^{15}N label was added to the plots (10/June/2018) and on days 5, 25 and 55 post labelling. The tree foliage was fully leafed out on day 0 and remained fresh with no sign of senescence up to day 55 post labelling. All foliar samples were transported to the lab within 4 hours of sampling and oven dried at 60°C for 72 hours then milled for homogenisation.

On day 53 post soil labelling foliar samples were taken from dominant understorey species; *Betula nana, Vaccinium vitis-idaea* and *Empetrum nigrum.* Foliar samples of each species were taken at four different distances (0.5 - 11 m) from the labelling area. Each of the four samples were taken at ordinal directions. Reference samples of each understorey species with natural abundance levels of ^{15}N were sampled ≥ 100 m from all ^{15}N labelling plots. Understorey samples were oven dried at 60°C for 72 hours and milled for homogenisation.

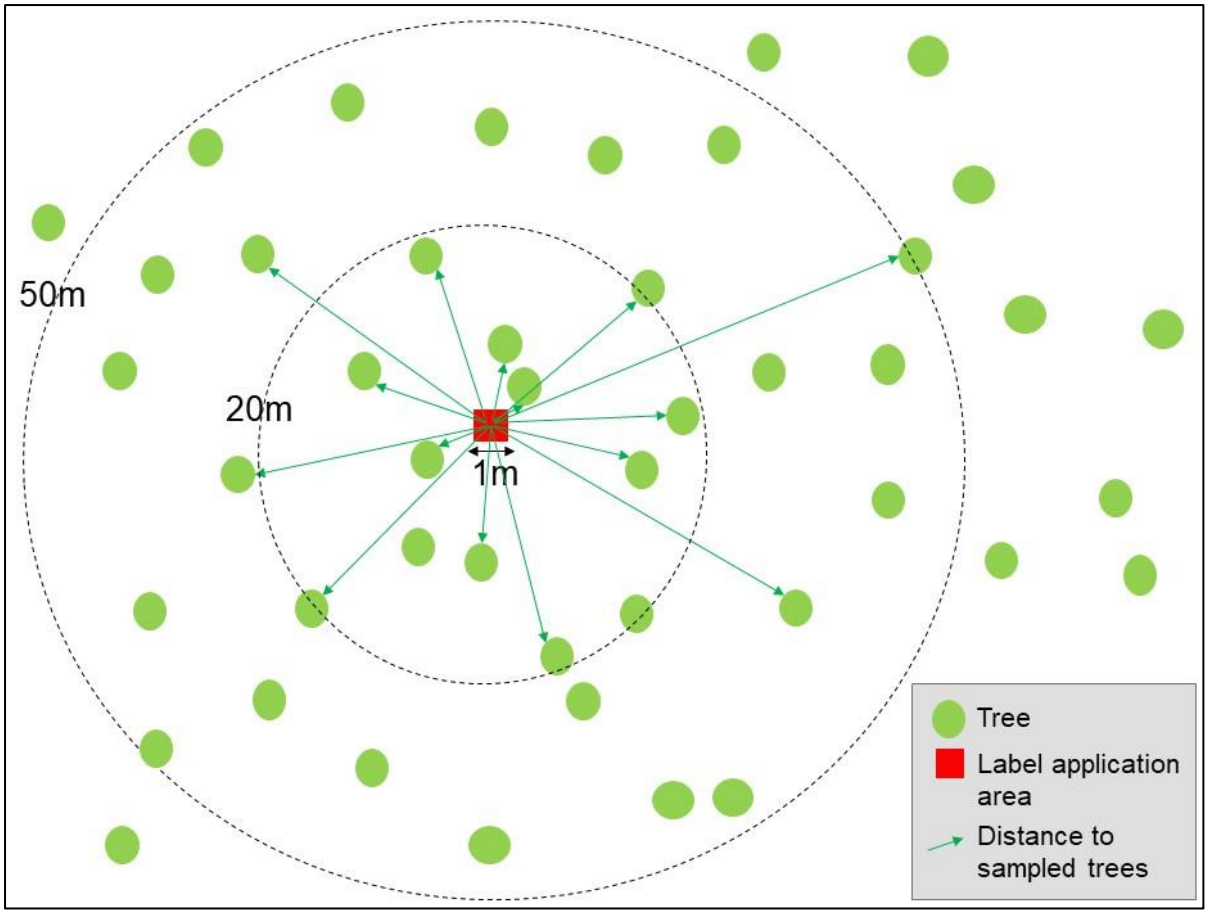

Figure 3.1. Plan view schematic diagram of plot set-up. The $^{15}NH_4Cl$ label was injected into the soil in a 1 m^2 area at the centre of each plot (red square). Foliar samples from 15 trees between 1 – 50 m away from the labelled area were harvested and analysed for their isotopic composition. Diagram not to scale.

3.3.4 Stable Isotope Analysis

Milled canopy samples (*Betula pubescens*) from days 0-55 were analysed at the NERC Life Science Mass Spectrometry Facility at the Centre for Ecology and Hydrology in Lancaster, UK, by EA-IRMS. Samples and standards were dried at 105°C for one hour in an oven then cooled and stored in a desiccator prior to analysis. A varying amount of each sample (enough to yield 100 µg nitrogen) was weighed using a high precision micro-balance, (Sartorius Ltd) and sealed into a 6 x 4 mm tin capsule (Elemental Microanalysis, Okehampton, UK). Samples were then

combusted using an automated Carlo Erba NA1500 Elemental Analyser coupled to a Dennis Leigh Technologies Isotope Ratio Mass-Spectrometer. Working standards of either natural abundance flour or ^{15}N-enriched flour were analysed after every twelfth sample, resulting in a maximum analytical precision of 0.32‰ for the natural abundance standard, and 3.10‰ for the ^{15}N-enriched flour (current mean value of 133.58‰). These standards are calibrated against the certified reference material IAEA-N1 (NIST number 8547, National Institute of Standards and Technology, Gaithersburg, USA). For duplicates analysed, standard deviation was a maximum of 0.33‰. Results are expressed in delta notation; i.e. $\delta^{15}N = [(R_{sample} / R_{standard}) - 1] \times 1000$ (‰) where R is the ratio of ^{15}N to ^{14}N in the sample and standard accordingly. All $\delta^{15}N$ results are expressed relative to the international standard of atmospheric air.

All understorey (*Betula nana, Vaccinium vitis-idaea*, *Empetrum nigrum*) were analysed at the UC Davis Stable Isotope Facility in California USA. Samples were analysed for ^{13}C and ^{15}N isotopes using an Elementar Micro Cube elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

During analysis, samples were interspersed with several replicates of at least four different laboratory reference materials. These reference materials have been previously calibrated against international reference materials, including: IAEA-600, USGS-40, USGS-41, USGS-42, USGS-43, USGS-61, USGS-64, and USGS-65 reference materials. A sample's provisional isotope ratio was measured relative to a reference gas peak analysed with each sample. These provisional values were finalised by correcting the values for the entire batch based on the known values of the included laboratory reference materials.

3.3.5 Statistical analyses

All statistical analyses were carried out using R Version 3.4.0 (R Core Team, 2017). The change in $\delta^{15}N$ enrichment of mountain birch leaves over distance was modelled using generalised additive models (Hastie, 2018) with distance from label as a fixed effect, grouped by sampling day and plot included as a random effect. The difference between sampling days compared using least-squares means (Lenth, 2018). The exponential decay of leaf N content over time in mountain birch trees was modelled using a self-starting non-linear asymptotic regression (stats::SSasymp; (R Core Team 2017). $\delta^{15}N$ enrichment of the understorey over distance from the label was analysed and presented as summarised raw data compared to natural abundance control samples plotted as a dashed line with standard deviation of the mean.

3.4 Results

In n=5 replicate 50 m radius plots a ^{15}N label was injected into the soil the central 1 m^2. Foliar samples were subsequently collected from mountain birch trees 1-50 m from the central labelled soil (Figure 3.2) and analysed for their N isotopic composition.

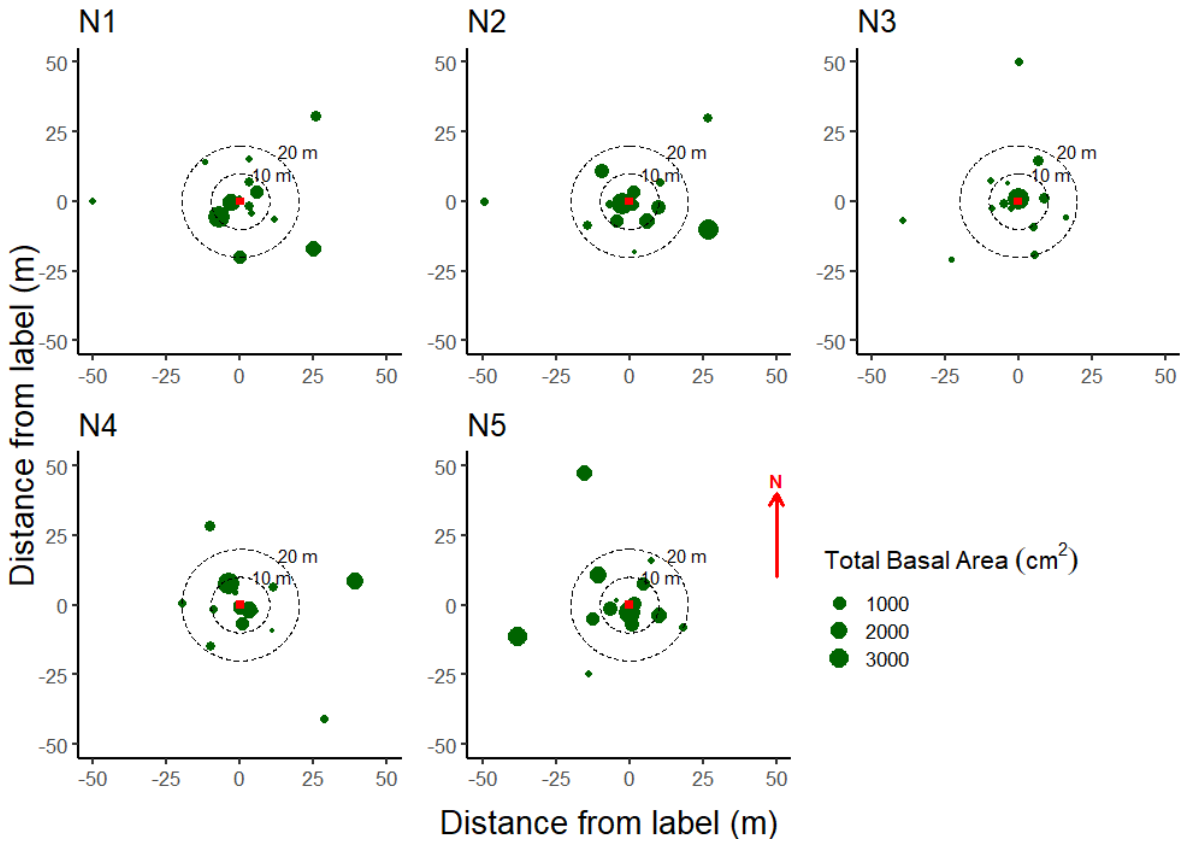

Figure 3.2. Layout of sampled trees in each plot (N1-5) relative to the central 1 m^2 of labelled soil (red square). Total Basal Area of trees is the sum of the basal area of any and all polycormic stems calculated individually and then summed.

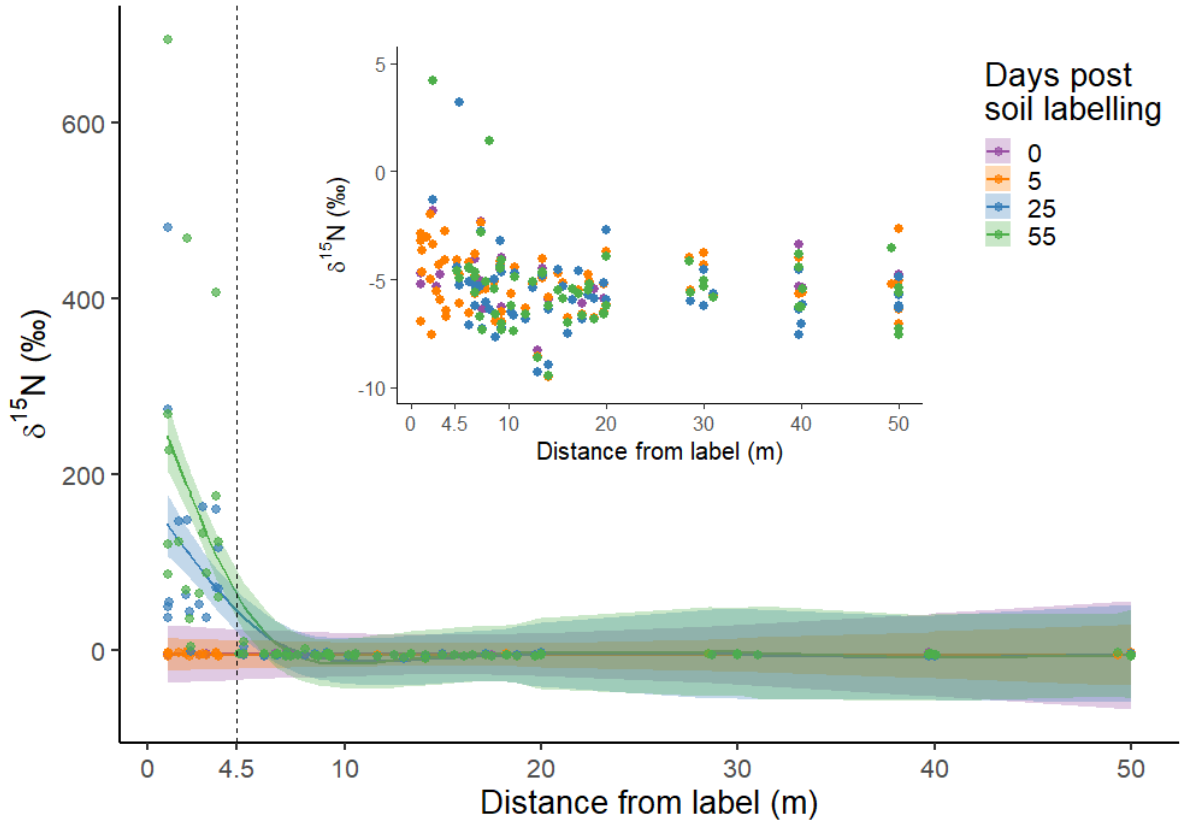

Figure 3.3. $\delta^{15}N$ enrichment of mountain birch leaves sampled from 1 – 50 m away from the labelled soil on days 0 to 55 post soil labelling. Day 0 is before any label is added and therefore natural abundance of ^{15}N. in the main figure lines were generated using a generalised additive model, error ribbons = ±1 standard error. Inset figure shows raw data with the y-axis narrowed around the natural abundance ^{15}N range to aid visualisation of data distribution from 1 – 50 m away from the labelled soil.

There is no difference in $\delta^{15}N$ enrichment between day 0 (natural abundance) and day 5 post soil labelling at any sampled distance (0 – 50 m) away from the labelled area (P = 0.98) (Figure 3.3). Foliar samples are significantly more enriched for $\delta^{15}N$ than natural abundance (day 0) samples on days 25 and 55 post labelling (P = 0.042 and 0.0012 respectively) (Figure 3.3). Significant $\delta^{15}N$ enrichment is found up to 4.5 m away from the labelled area. There is no difference in the levels of $\delta^{15}N$ enrichment beyond 4.5 m between days 25 and 55 (P = 0.1886).

The $\delta^{15}N$ enrichment declines with distance between 0 – 5 m from the labelled area on day 25 (P = 0.0487), but on day 55, this trend is not significant (P = 0.0643), when analysed separately using a linear model for this distance bracket. The difference in enrichment between day 25 and 55 from 0 - 5 m is not significant (P = 1.00).

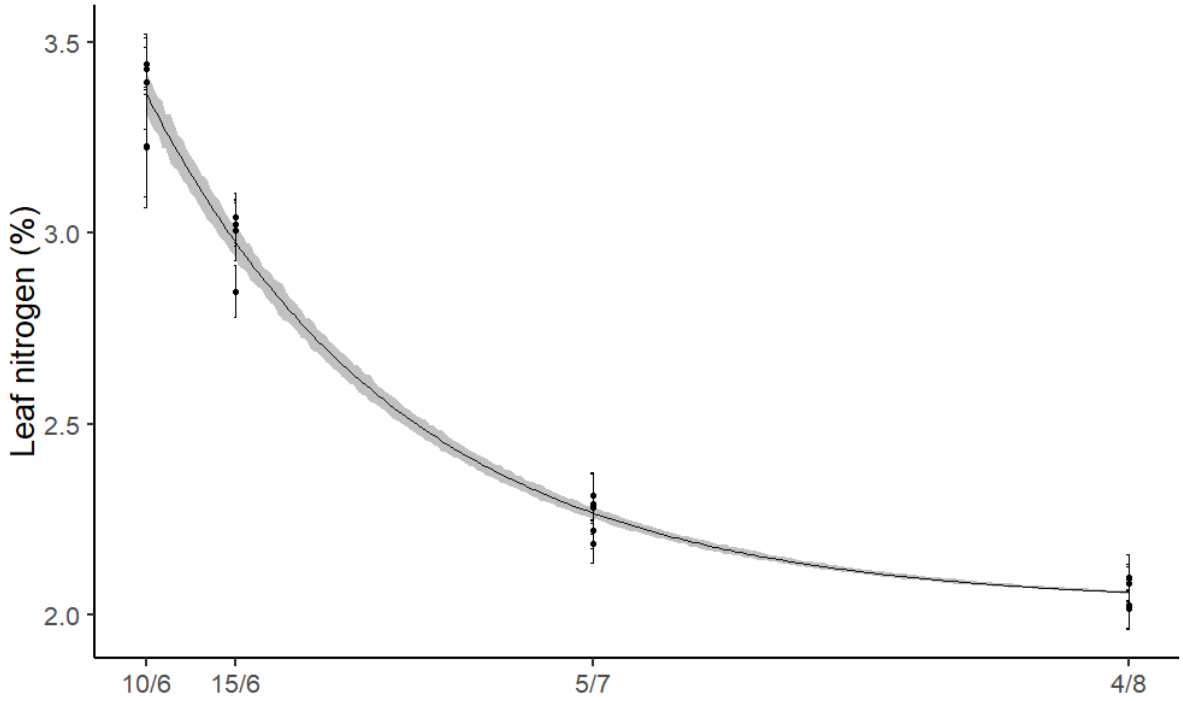

Figure 3.4. Nitrogen content (%) of mountain birch leaves sampled in the growing season of 2018. Points indicate plot averages (n=15). Line predicted from a nonlinear least squares model, error ribbon is ± 1 standard error.

The nitrogen content of sampled leaves declines throughout the growing season following an exponential decay pattern with an asymptote at 2.03% leaf nitrogen. The initial recorded mean percentage leaf nitrogen was 3.35% on 10/6 (day 0 of ^{15}N pulse), which had decreased by 10.8% by day 5, by 32.6% by day 25 and by 38.4% by day 55 (Figure 3.4).

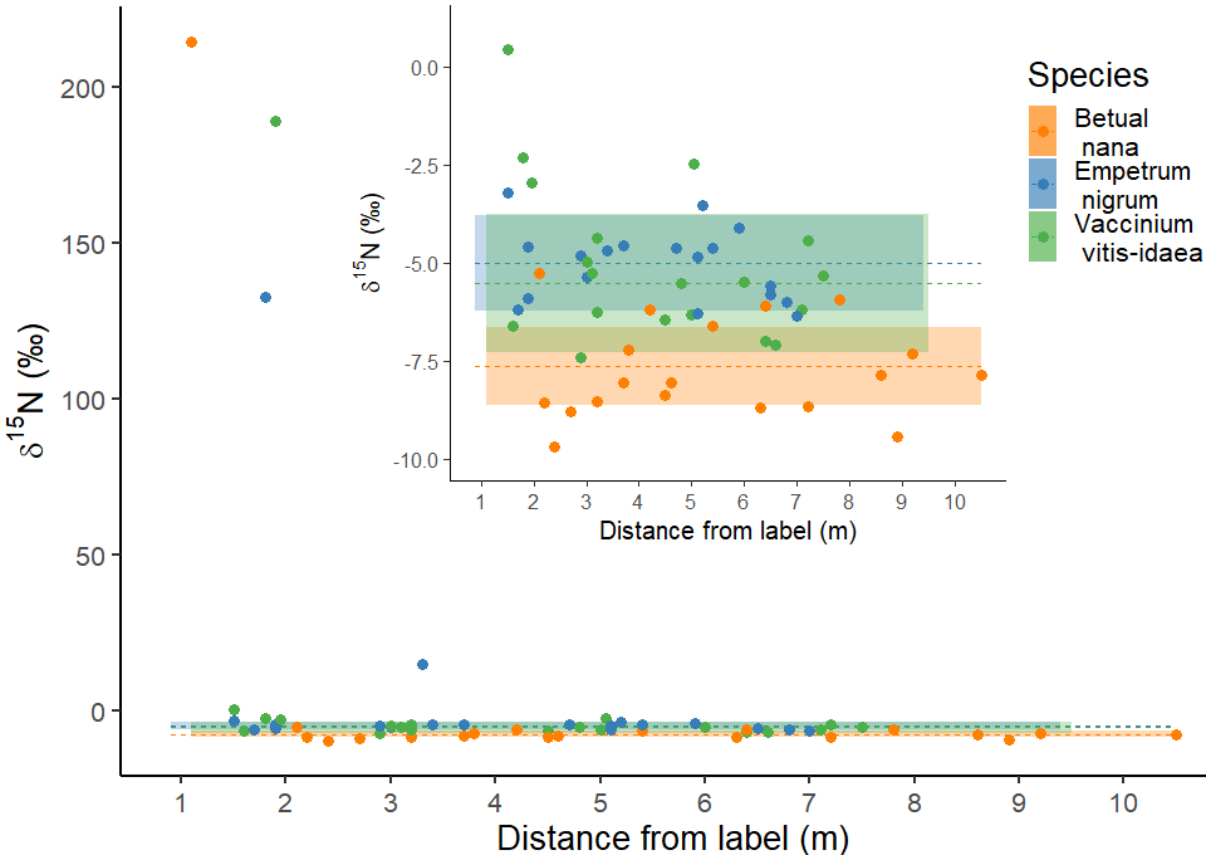

Figure 3.5. $\delta^{15}N$ enrichment of understorey shrub leaves sampled from 1 – 11 m away from the labelled soil on day 53 post soil labelling. Dashed lines and associated error ribbons (±1 standard deviation of the mean) are natural abundance $\delta^{15}N$ of control samples for each species sampled >100 m away from labelled soil. Inset figure narrows the y-axis around the natural abundance range to aid visualisation of data distribution from 1 – 11 m away from the labelled soil.

Enrichment was found only in some shrubs within 3.3 m of the label, whilst the majority showed no label incorporation within that radius (Figure 3.5). In *B.nana*, $\delta^{15}N$ enrichment can be found at 1.1 m away from the label but at no further distances. In *E.nigrum*, $\delta^{15}N$ enrichment can be found up to 3.3 m away from the label; however, not all samples between 1-3.3 m are enriched, some have $\delta^{15}N$ levels similar to natural abundance samples. In *V.vitis-idaea* $\delta^{15}N$ enrichment can be found up to 1.9 m away from the label; however, not all samples between 1-1.9 m are enriched, some have $\delta^{15}N$ levels similar to natural abundance samples.

3.5 Discussion

Using a ^{15}N stable isotope label, the uptake of N from the soil into mountain birch tree foliage was detectable up to 4.5 m from the central area of label injection into the soil (Figure 3.2). This finding supports Hypothesis 1; that mountain birch trees can access N at least 3 m away from the N source. Beyond 4.5 m away from the labelled soil area there is no significant uptake of the ^{15}N stable isotope label detectable in mountain birch tree foliage. The uptake of the ^{15}N stable isotope label up to 4.5 m away from the labelled soil is mediated by root and mycorrhizal exploration and scavenging for mineral nutrients which are passed to the mountain birch tree host in exchange for photosynthetic sugars (Anderson & Cairney, 2007; Smith & Read, 2010). In these sub-Arctic mountain birch treeline forests it has previously been demonstrated that both root and mycorrhizal production remained consistently high between 0.25 - 3 m away from the nearest tree (Friggens *et al.*, 2019, Chapter 2). The current results further support the finding of a large spatial 'reach' of the mountain birch tree mycorrhizosphere and provides new evidence of nutrient uptake and transfer by the mycorrhizosphere to the mountain birch tree hosts up to 4.5 m away from host trees. As half of the mean maximum distance between individual trees in these open canopy treeline forests is ~3 m (Friggens *et al.*, 2019, Chapter 2) the ability of the forest mycorrhizosphere to supply host trees with N from up to 4.5 m away implies that there are overlapping root and ECM networks in these forests.

The potential for overlapping mycorrhizopheres and ECM networks suggests that there is a possibility for the formation of common mycelial networks (CMNs) in these forests. CMNs are formed when one fungal individual colonises multiple host plants thereby connecting different plant individuals below-ground via mycorrhizal hyphal

and mycelial networks, thus creating a physical cytoplasmic link between host plants (Barto *et al.*, 2012; Simard *et al.*, 2012). Through this cytoplasmic link, carbon (Simard *et al.*, 1997a; Deslippe & Simard, 2011; Pickles *et al.*, 2017), nutrients such as N (Ek *et al.*, 1997; He *et al.*, 2005) and signalling molecules (Babikova *et al.*, 2013b; Song *et al.*, 2014) can be passed between plants. The spatial reach of mountain birch ECMs up to 4.5 m away from host trees and overlapping across forest gaps indicates a tantalising possibility for the formation of CMNs in these forests, which may alter competitive advantages and forest community structure (Deslippe & Simard, 2011), with consequences for tree and shrub encroachment onto tundra areas (Myers-Smith *et al.*, 2011; Hofgaard *et al.*, 2013; Bjorkman *et al.*, 2018; Raynolds *et al.*, 2019). This is the first study to explore the spatial reach and exploration of treeline mountain birch mycorrhizospheres. More work on N scavenging across the forest floor and how these properties affects competition is needed in light of growing evidence of CMNs and the vegetation changes predicted under future climate warming scenarios.

Overlapping root and ECM networks may provide a further strand of evidence for the existence of a so-called wood-wide-web in these forests. However, this only holds true up to a certain point, as there was evidence of N uptake across forest gaps but only up to 4.5 m away from host trees, not across the entire forest. Connectivity in the forest over larger distances may be possible through host tree associations with multiple fungal genets and the formation of multiple CMNs by each host tree. Using multi-locus microsatellite DNA analysis, the complex architecture of the wood-wide-web has been mapped in Douglas fir forests which shows that individual host trees can be linked via mycorrhizas to up to 47 other individuals in the forest through an association with eight different fungal genets (Beiler *et al.*, 2010). It has been

theorised that mycorrhizal plant hosts which form multiple CMNs may provide the basis for a relay system, through a chain of plants, indirectly connected via multiple CMNs whereby signals may be propagated over larger distances than a single mycorrhizosphere or CMN can reach (Babikova *et al.*, 2013a). However, this theory is more robust in the context of inter-plant herbivory signalling (as originally proposed by Babikova *et al.* (2013a)), as here there is a fitness and evolutionary benefit for both the fungus and plant host to relay the herbivory signal. However, it becomes much less likely when considering mineral N uptake, as here there is little or no fitness benefit for the plant to readily pass N, a limited resource, to neighbouring plants. That being said, this theory could be tested using ^{15}N stable isotope tracers, as in the current study, if the herbivory signal was a protein and could have the ^{15}N signal incorporated into its structure. However the nature of the herbivory signal is not currently known (Johnson & Gilbert, 2015).

The change in $\delta^{15}N$ enrichment is not detectable in mountain birch tree foliage at any distance from the central labelled area 5 days post soil ^{15}N labelling, and was only detectable on days 25 and 55 post labelling (Figure 3.3). This finding highlights the lag time for N transport in the mycorrhizosphere and tree phloem. In a similar field experiment (Göttlicher *et al.*, 2008) the detection of a ^{15}N tracer in Douglas fir trees up to 9.5 m away from the soil injection point was found between 37-56 days post soil injection. However they do not report on samples harvested before day 37 of their experiment. The reason(s) for the lag seen here may be a combination of several different factors. For example the hyphal proliferation in response to higher N concentrations (in 1 x 1 m patches) and subsequent acquisition of the ^{15}N by ECMs may be an initial lag. Once acquired the exchange of ^{15}N between ECMs and the

host tree could cause a further lag. Finally, the translocation of the ^{15}N throughout the host tree via phloem transport and into the foliage could cause further lag.

To further our understanding of CMN transport and the dynamics of N resource competition in ecotone forests undergoing climate driven changes requires the development of relevant stable isotope pulse-chase methods. Tracking the movement of the ^{15}N label over time and space through soil, fungi and host plant requires a more detailed sampling of the various components in the pathway, e.g. soil, ECM hyphae, host tree root and stem tissue. Furthermore, a sampling regime with more frequent time intervals than those here would be required to quantify the duration of ^{15}N flow through the soil-fungus-plant system. The spatial reach over which the ^{15}N label could be detected did not increase between days 25 and 55. This suggests that (a) the spatial detection limit of 4.5 m represents the effective reach of *B. pubescens* trees at this site over which nutrients are accessed, and (b) transport of N along this distance from soil to tree foliage takes between 5-25 days.

Mountain birch leaf N content declined exponentially through the growing season of 2018, from 3.35% in early June to 2.06% in early August (Figure 3.4). This decline may represent a decrease in total leaf N content or may be an artefact of increasing leaf carbon content, and therefore a change in the C:N ratio of the leaf, as the season progresses. A similar pattern was found in three broad-leafed species in Wisconsin, where the leaf N content increased from early May to mid-July after which it declined rapidly until early-October (Reich *et al.*, 1991). The difference in the seasonality between the current study and that of Reich *et al*. (1991) is likely due to the difference in latitude, and therefore growing season length. No data on the leaf N content of mountain birch trees in the very early season in Abisko (May - early June)

was collected and therefore an increase in leaf N content was not observed. However, field experiments from the Abisko area find high N availability in soils at the start of the growing season attributed to nutrient flushes from snowmelt (Weih, 1998) which may be reflected in foliar N content.

The ^{15}N label was detectable in the foliage of all three selected understorey species in the forest at distances from the labelled soil that were shorter than those found for mountain birch trees (Figure 3.5). This finding supports Hypothesis 2 that ericoid mycorrhizal understorey species have a more limited range of access to soil N than ECM mountain birch trees. The overall radius of shrub reach for N acquisition varied from 1.9 – 3.3 m away from the ^{15}N source. It is noteworthy however, that for each of the three understorey shrub species samples only a few plants within this radius showed evidence of uptake of the ^{15}N label and the majority of plants did not show any uptake of the label, even at short distances away from the source. This result indicates that the mycorrhizosphere networks associated with understorey shrubs in these forests are stochastic and not evenly distributed radially from any given shrub. Rather, it seems that shrub mycorrhizosphere systems are highly directional or asymmetric (Göttlicher *et al.*, 2008), which may be due to microtopography, hydrological barriers, rocks, or simply chance. Furthermore, this finding suggests that the mycorrhizosphere or hyphal networks of understorey shrubs are unlikely to be connected and form CMNs along which N can travel.

The exploration distances of ECM shrubs and trees are expected to be longer than ERM shrub mycorrhizospheres due to the potentially long-distance growth forms of their hyphal structures (Agerer, 2001). However, here it was found that of the understorey shrubs, the ERM species *E. nigrum* can access the ^{15}N from the furthest

distance away from the labelled soil area (Figure 3.5). The ability of *E. nigrum* to access the ^{15}N at greater distances than expected may be due to an extensive lateral root system and vegetative propagation across the forest floor linking patches of shrubs (Bell & Tallis, 1973) rather than exploration and N uptake by ERMs *per se*.

Given that N is the primary limiting nutrient in these forests (Wallenda & Kottke, 1998; Sjögersten & Wookey, 2005) and the current results show that the canopy forming mountain birch trees can access and take up N across larger spatial distances than the understorey shrub species, this may provide a competitive advantage for the mountain birch trees over the understorey in these forests. If such a competitive advantage, mediated by increased access to mineral nutrient uptake, does exist, then it may lead to altered community structures in these forests with mountain birch trees being more successful than understorey or tundra species. The dynamics and community composition of mountain birch trees and tundra species in the sub-Arctic regions is particularly important given recent predictions of tree and tall shrub expansion and encroachment into tundra heaths (Myers-Smith *et al.*, 2011; Pearson *et al.*, 2013; Raynolds *et al.*, 2019), which is likely to affect ecosystem carbon cycling and storage (Hartley *et al.*, 2012; Parker *et al.*, 2015) long term.

3.6 Conclusions

Using a ^{15}N soil labelling experiment, this work presents evidence of N uptake by mountain birch trees, facilitated by ECM networks over distances up to 4.5 m away from a central N source 25 and 55 days post soil labelling. This result indicates that the mycorrhizosphere networks in these forest are overlapping as they can facilitate N uptake beyond half the mean maximum distance between trees (~3 m), suggesting the potential for formation of CMNs connecting mountain birch trees.

Both ECM and ERM understorey shrubs have shorter distances over which they can scavenge this soil N pool, and show patters of stochastic and highly directional mycorrhizosphere systems. The difference in mycorrhizosphere exploration and nutrient uptake distance between the canopy forming mountain birch trees and the understorey shrubs may confer competitive advantage to the trees, given that N is a limiting resource, which may alter community structures within these forests. This is particularly important in light of predicted climate driven tree and shrub expansion in sub-Arctic regions, with likely consequences for ecosystem carbon cycling and storage.

Chapter 4:
Tree-to-tree C transfer via common mycelial networks in sub-Arctic birch forests

4.1 Abstract

Some mycorrhizal fungi are able to colonise multiple plant hosts and multiple plant species simultaneously to form complex common mycelial networks (CMNs). These CMNs can act as cytoplasmic links, facilitating the transport of carbon and mineral nutrients between plants below-ground. Recent evidence from a sub-Arctic treeline forest suggests that the mycorrhizas of mountain birch trees extend across large forest gaps, providing the potential for CMN formation. Here I investigate the presence and strength of both con- and hetero-specific CMNs in these mountain birch forests using a novel whole crown ^{13}C pulse-chase technique. Leaf samples were collected from neighbouring conspecific trees and heterospecific understorey shrubs on days 1-10 and 377 post crown labelling. I found no evidence of either con- or hetero-specific CMNs in these forest, but the method and sampling regime used here was unable to definitively rule out mountain birch tree CMNs in these forests. Further investigations are needed to understand fully the presence or absence of CMNs in these forests and how this might affect the population and community dynamics of tree encroachment into tundra.

4.2 Introduction

Symbiotic relationships with mycorrhizal fungi, which explore the soil for water and nutrients and, in turn, receive plant photosynthates (Anderson & Cairney, 2007; Smith & Read, 2010), are crucial for tree health and forest ecosystem function. The importance of these symbiotic relationships is well established, with >90% of all terrestrial plants forming mycorrhizal associations (Cairney, 2000) and a global distribution of trees with mycorrhizal associations (Steidinger *et al.*, 2019). Recently there has been increasing focus on the abilities of mycorrhizas to colonise multiple hosts and potentially form physical links between host trees to create common mycelial networks (CMNs) through which nutrients and signalling molecules can be passed (Johnson, 2015; Hazard & Johnson, 2018).

The nature of plant-fungi associations resulting in mycorrhizal symbiosis is often non-specific (Selosse *et al.*, 2006), which means that the same plant can associate with multiple fungal species as well as the same mycorrhizal fungus associating with multiple plant hosts (Bruns *et al.*, 2002; Smith & Read, 2010). In ectomycorrhizal (ECM) associations with tree species, the lack of specificity in tree-fungi associations may stem, in part, from the fact that both plants and fungi involved in ECM symbioses are polyphyletic with multiple origins of the symbiosis influenced by co-evolutionary events (Bruns *et al.*, 2002; Molina & Horton, 2015). Furthermore, both plants and fungi have large parts of their physical structures that are not associated directly with their fungal or plant host partner and are therefore free to simultaneously colonise and associate with other hosts, thereby creating CMNs between separate host plants (Bruns *et al.*, 2002). Not only can mycorrhizal fungal species associate with multiple hosts of the same species (i.e. con-specifically), but

mycorrhizal fungi can also associate with multiple hosts of different species (i.e. hetero-specifically). Although mycorrhizas have traditionally been divided into functional groups based on their host plants, new evidence is emerging that the same genet of mycorrhizal fungi can associate with different plant species commonly thought to host only specific mycorrhizal functional types (Grelet *et al.*, 2009, 2010; Leopold, 2016). Grelet *et al.* (2010) found that the mycorrhizal fungus *Meliniomyces variabilis* can simultaneously colonise *Pinus sylvestris* (Scots pine), an ECM tree species, and *Vaccinium vitis-idaea,* an ericoid mycorrhizal (ERM) understorey shrub species, but they found no evidence of the formation of large CMNs between the two.

The ability of fungi to colonise multiple plants and for plants to host multiple fungi leads to the possibility of CMNs. CMNs connect different plant individuals below-ground via mycorrhizal hyphal and mycelial networks, creating a physical cytoplasmic link between host plants (Barto *et al.*, 2012; Simard *et al.*, 2012). Furthermore, the spatial expansion and reach of CMNs may be rapidly increased as different mycorrhizal networks may become interconnected by means of hyphal fusions (Giovannetti et al. 2004). Combined, this results in a many-fold increase in the surface area by which trees and other host plants explore and scavenge the soil for nutrients, minerals and water, the importance of which is illustrated by CMNs being found in a wide range of terrestrial ecosystems (Simard *et al.*, 2012).

An important function of these cytoplasmic links between plants, facilitated by mycorrhizal fungi, is that they can act as below-ground avenues (Barto *et al.*, 2012) for the transfer of water, carbon (C) and other nutrient resources among plants within a community (Simard et al., 2012). The net transfer of C (Simard *et al.*, 1997a; Selosse *et al.*, 2006; Teste *et al.*, 2010; Deslippe & Simard, 2011; Babikova *et al.*,

2013b; Klein *et al.*, 2016; Pickles *et al.*, 2017), nitrogen (Ek *et al.*, 1997; He *et al.*, 2005), phosphorous (Ren *et al.*, 2013), water (Egerton-Warburton *et al.*, 2007) and signalling molecules (Babikova *et al.*, 2013b; Song *et al.*, 2014) between plants has been demonstrated using both lab and field experiments (Simard *et al.*, 2012). All of these studies found evidence for CMNs using pulse-chase experiments with isotope labelling of various eco-physiologically relevant structures of host plants. In the Arctic, the transfer of C through CMNs has been detected in the dwarf birch shrub *Betula nana* using a ^{13}C pulse-chase labelling experiment (Deslippe & Simard, 2011). Deslippe and Simard (2011) found that 10.7 ± 2.4 % of photosynthetic C was transferred between conspecific pairs of *B. nana*. In contrast, Botnen et al. (2014) report low CMN occurrence in a widespread Arctic *Dryas octopetala*, *Salix polaris* and *Bistorta vivipara* community, on the basis of a lack of spatial autocorrelation in fungal operational taxonomic units (OTUs). This emphasises that the prevalence and significance of CMNs in the Arctic remains largely unknown.

Nonetheless, CMNs are potentially important in the Arctic, which currently experiences rapid changes to plant communities (Kaplan & New, 2006; Bjorkman *et al.*, 2018), with inevitable consequences for below-ground microbial communities (Wardle *et al.*, 2004) and the links and interactions between the two. It has been suggested that the transfer of growth limiting nutrients and other key compounds between conspecifics may alter competitive plant interactions in the Arctic tundra (Deslippe & Simard, 2011). Furthermore, Deslippe and Simard (2011) found C transfer between conspecifics to be linked to ambient temperatures and suggest that this may lead to a positive feedback, increasing *B. nana* shrub monodominance with increasing temperatures within Arctic ecosystems affected by climate warming.

It must be remembered that, as well as benefits there is an energy cost associated with plants and fungi forming CMNs, and this energy cost may not be evenly distributed among different plant species in the CMN (Leake *et al.*, 2004). In some cases this results in asymmetry in the investment and return of resources to and from the CMN system (Walder *et al.*, 2012), which may in part explain the rarity of heterospecific CMNs. However, as well as facilitating transfer of resources, CMNs provide host plants with access to greater genotypic and species diversity of mycorrhiza which may provide increased resilience to environmental challenges such as climate warming (Hazard & Johnson, 2018). It is clear that there are both costs and benefits to both plants and fungi in CMNs, which may alter competitive interactions and provide resilience to ecosystems undergoing rapid climate driven change, such as in the Arctic. It is therefore important to understand more about the presence and strength of CMNs in the Arctic (and elsewhere) and how these might modulate responses to climate change.

Here I focus on the potential for CMNs to form in sub-Arctic treeline forests and the implications this might have in light of rapid climate driven changes in Arctic ecosystems (Kaplan & New, 2006; Bjorkman *et al.*, 2018). In a Swedish mountain birch (*Betula pubescens* Ehrh. ssp *czerepanovii*) treeline forest recent evidence suggests that mycorrhizal fungi extend 3-4.5 m away from tree bases (Chapters 2 & 3). I therefore sought to investigate the presence of CMNs in these forests using a whole-crown ^{13}C pulse-chase approach combined with targeted tree ‘trenching’; physically severing any root and mycorrhizal connections between trees. This experiment was designed to test the following hypotheses (graphically presented in Figure 4.1):

1. Carbon can be transferred between *Betula pubescens* trees via intact CMNs.

2. Carbon cannot be transferred from birch trees to heterospecific understorey shrub species.

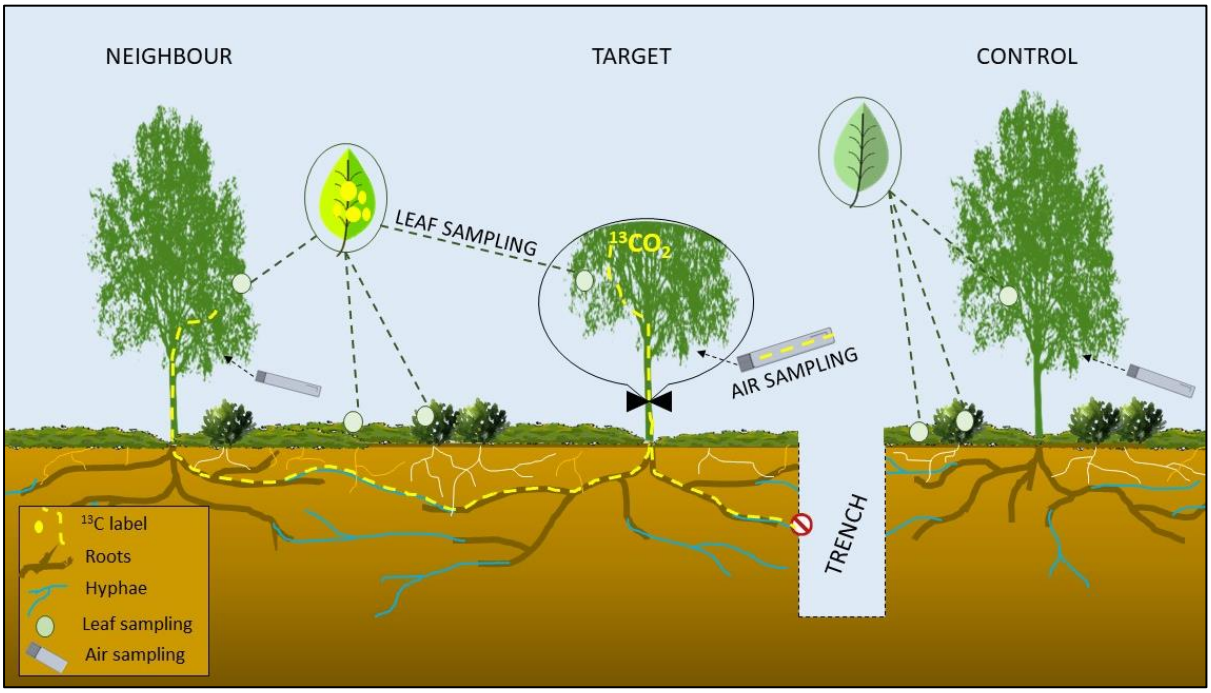

Figure 4.1. Experimental set-up showing target, neighbour and control trees relative to the trench with leaf sampling from canopy forming and understorey species either side of the trench. Hypothesis 1 is depicted as the yellow ^{13}C-label which theoretically can be transferred from the target to the neighbour tree via CMNs but cannot be transferred to the control tree as CMNs here have been severed by trenching. Hypothesis 2 addresses the transfer of ^{13}C label to dwarf shrubs located in the vicinity of 'target' and 'neighbour' trees, with samples taken from 'control' trees and shrubs as reference.

4.3 Materials and Methods

4.3.1 Site Description and Tree selection

All studied plots were set up within a permafrost-free area (approx. 1 km^2) in the sub-Arctic treeline ecotone at Nissunsnuohkki, South of Abisko, Sweden (ca. 68°18'56.2"N 18°49'18.2"E), ~600 m asl. The treeline forest is formed of mountain birch (*Betula pubescens* Ehrh. ssp *czerepanovii* (Orlova) Hämet Ahti) and has an

open canopy structure with an understorey of dwarf birch shrubs (*Betula nana* L.) and ericaceous shrubs V*accinium vitis-idaea* L., *V. myrtillus* L. and *Empetrum nigrum* L. ssp *hermaphroditum* (Hagerup) Böcher.

Forest soils are microspodosols with a thin O horizon (< 5 cm) underlain by glacial till on a bedrock typically of hard shale (Sjögersten & Wookey, 2002). Soil pH in the organic horizon is 4.3 ± 0.1 (Parker et al., 2015).

Five trees (to be ^{13}C labelled, henceforth referred to as 'target trees') were selected ≥ 50 m apart within the open mountain birch forest. Target trees were selected to have equidistant neighbours in a straight line (as far as possible within the natural variation of the forest). The average distance to the central target tree from selected neighbour trees was 384.5 ± 55.7 cm (mean ± SD) and the average difference in distance to the target tree between tree pairs was 41.0 ± 33.4 cm (mean ± SD). Plots were selected to have a representative understorey with three dominant species present, and a target tree with a single stem and compact crown to allow a tight seal of the crown bag (see below).

4.3.2 Plot set-up

The plot set-up is graphically summarised in Figure 4.1. An approximately 25 cm deep trench was cut in a semi-circle (enclosing one neighbour tree) at the mid-point between the target tree and one neighbour (this became the 'control tree', henceforth referred to as such), severing all root and mycorrhizal connections between them. The rhizosphere connections between the target tree and the other neighbour tree remained undisturbed.

Four *Betula nana* plants and four patches of *Vaccinium vitis-idaea* and *Empetrum nigrum* were marked for sampling and their distance to the target tree measured.

Two of each species were on the same side of the trench as the target tree and two of each species were on the opposite side of the trench to the target tree to serve as controls.

Leaf samples were collected from all selected trees and understorey plants before any isotope was introduced into the system, taken to represent natural abundance $\delta^{13}C$.

4.3.3 Isotope labelling

The crown of the target tree was covered in a large (140 x 178 x 230 cm) clear plastic bag (Gardener's Dream Ltd, Glasgow, UK) and sealed to the stem at the base of the crown. Inside the crown bag, two small battery operated fans (Mountain Warehouse, Victoria, London) were secured to branches to circulate air within the crown bag during labelling. A hand-held Traceable® probe (Fisher Scientific) was secured to a branch and shielded from direct solar radiation to monitor temperature inside the labelling bag throughout the labelling process.

Target trees were labelled using $^{13}CO_2$ produced, *in situ*, by adding 90 ml 2% HCl, in small aliquots (1-5 ml), to 2 g $Ca^{13}CO_3$ (99 atom % enriched with ^{13}C, Sigma-Aldrich Ltd, Dorset, UK) contained in a beaker outside the crown bag (Figure 4.2). The $^{13}CO_2$ was led through an EGM-4 infrared gas analyser (PP Systems International, Amesbury, MA, USA), to monitor the concentration of CO_2 produced, and pumped into the bag surrounding the tree crown. The concentration of CO_2 inside the crown bag was monitored using an EGM-5 infrared gas analyser (PP Systems International, Amesbury, MA, USA) connected to the crown bag with plastic tubing. The out-flow from the EGM-5 was connected to the beaker containing the $^{13}CO_2$ production, thereby creating a closed circuit system of gas flow through the beaker

and crown bag (Figure 4.2). Crown $^{13}CO_2$ labelling was conducted for approximately 1h15mins and until fully drawn down to below ambient concentrations.

Crown $^{13}CO_2$ labelling was carried out on all 5 target trees on 20/7/18 between 08:00 – 16:30. This day was chosen for crown labelling as it had diffuse high clouds allowing sufficient light for high photosynthetic rates and uptake of the label but without direct sunlight overheating the trees inside the crown bags (this could have caused stomatal closure and damage to the trees).

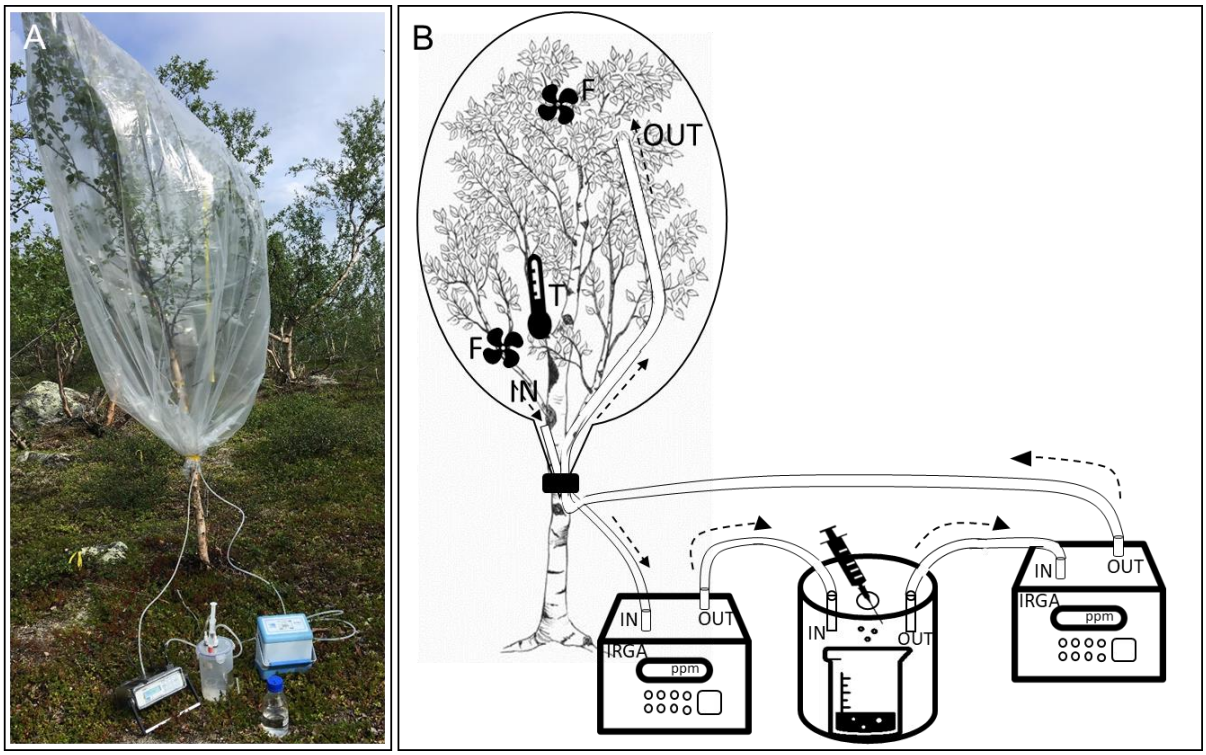

Figure 4.2. A) Picture of pulse labelling set up. B) Diagram of $^{13}CO_2$ pulse labelling set up. Clear crown labelling bag around target tree with two fans (F), one thermometer (T) in the crown space, and gas flow to and from the bag. Gas flows through two infrared gas analysers (IRGA) either side of $^{13}CO_2$ generated by adding HCl to $Ca^{13}CO_3$ using a syringe. Dashed lines indicate direction of cyclic gas flow.

4.3.4 Foliar sampling

Leaves were sampled from the selected birch trees (target, control and neighbour) on days 1, 3, 6, 10 and 377 post labelling. On each sampling occasion, 10-15 leaves were collected from across the whole crown. Leaf samples were also taken from three dominant understorey species (*Betula nana*, *Vaccinium vitis-idaea* and *Empetrum nigrum*) either side of the trench on the same days as mountain birch leaf sampling was conducted.

All foliar samples were frozen in the field within 1 hour of sampling to halt metabolic processes. All samples remained frozen at -20°C for at least 24h and were then dried at 60°C for 72 hours.

4.3.5 Air sampling

24 ml of air samples stored in 12 ml evacuated glass vials (Exetainer®, Labco Ltd, Ceredigon, UK) were taken from within the crown bag surrounding the target tree and directly adjacent to both the control and neighbour trees during ^{13}C-pulse labelling. After ^{13}C-pulse labelling, air samples were taken at the same time as leaf samples, directly adjacent to all three trees (target, neighbour and control) within a plot.

4.3.6 Stable isotope analysis

Crown leaf samples (*Betula pubescens*) and all air samples pre- and on days 1-10 post ^{13}C-pulse were analysed at the NERC Life Science Mass Spectrometry Facility at the Centre for Ecology and Hydrology in Lancaster, UK, by Elemental Analyser-Isotope Ratio Mass Spectrometry (EA-IRMS). Samples and standards were dried at 105°C for one hour in an oven, then cooled and stored in a desiccator prior to

analysis. A varying amount of each bulk sample (enough to yield 500 µg carbon) was sealed into 6 x 4 mm tin capsules (Elemental Microanalysis, Okehampton, UK). Samples were then combusted using an automated Carlo Erba NA1500 Elemental Analyser coupled to a Dennis Leigh Technologies Isotope Ratio Mass-Spectrometer. Isotope results are expressed in δ notation, where $\delta^{13}C = [(R_{sample} / R_{standard}) - 1] \times 1000$ (‰) where R is the ratio of ^{13}C to ^{12}C in the sample and standard accordingly. All $\delta^{13}C$ results are expressed relative to the international standard Pee Dee Belemnite (Sharp, 2017).

Working standards of flour and ^{13}C-labelled glutamic acid, which are calibrated against certified reference material Sucrose-ANU (NIST number 8542, Bureau of Analysed Samples Ltd, Middlesbrough, UK), were analysed after every twelfth sample, resulting in an analytical precision of 0.11‰. For duplicates analysed, standard deviation was a maximum of 0.28‰.

All understorey samples and crown samples from day 377 post ^{13}C-pulse were analysed at the Soil Microbial Ecology Lab at the University of Manchester. 4 mg of encapsulated samples were combusted in a Costech (Costech Analytical Technologies, Inc., California, USA) combustion module paired with a Picarro G2201-i CRDS (cavity ring down spectrometer; Piccaro, California, USA) isotopic analyser for isotopic CO_2/CH_4.

4.3.7 Statistical analyses

All analyses were carried out using R Version 3.4.0. The change in $\delta^{13}C$ enrichment over time was modelled using a linear model (R Core Team, 2017) for the control and neighbour trees, with sampling day as the sole covariate. The exponential decay of $\delta^{13}C$ enrichment over time in the target trees was modelled using a self-starting

non-linear asymptotic regression (stats::SSasymp; (R Core Team, 2017), with sampling day as the sole covariate. Pre-pulse data and day 377 data was analysed and presented as summarised raw data with standard deviation of the mean. $\delta^{13}C$ enrichment of the understorey over time was analysed and presented as summarised raw data with standard deviation of the mean.

4.4 Results

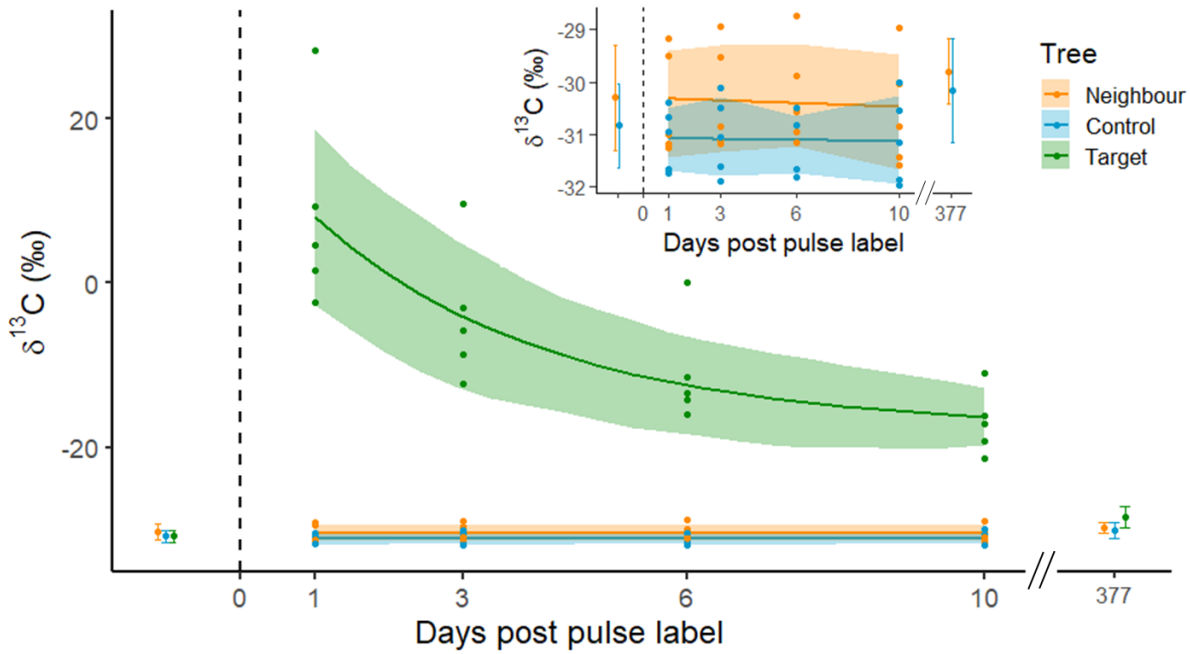

Figure 4.3. $\delta^{13}C$ of each treatment type (target, neighbour, control, n=5) pre-, 1-10 and 377 days post pulse labelling. The target tree received the label directly, the neighbour tree was adjacent to the target tree with roots and mycorrhizas intact and the control tree was adjacent to the target tree with roots and mycorrhizas severed. Pre-pulse samples are taken as natural abundance $\delta^{13}C$. Error bars and ribbons are ±1 standard deviation. The inset narrows the y-axis range for 'neighbour' and 'control' samples, to aid visualisation of differences between trees.

Leaves of the target tree are significantly more $\delta^{13}C$ enriched throughout the initial 10-day post pulse period than either the control or neighbour trees ($P < 0.001$) (Figure 4.3). The depletion of the $\delta^{13}C$ signal follows an exponential decay function with the formula: $y(t) \sim y_f + (y_0 - y_f)\, e^{-\alpha t}$, where the asymptote $y_f = -17.74$ and rate $\alpha = 0.316$. There is no difference in $\delta^{13}C$ levels between neighbour and control trees either pre- or post ^{13}C labelling ($P = 1.00$ on all sampled days). By day 377 post ^{13}C labelling the target tree is no longer significantly $\delta^{13}C$ enriched relative to both the control ($P = 0.143$) and the neighbour ($P = 0.079$) trees, which in turn do not differ significantly from each other ($P = 0.586$) (Figure 4.3). Furthermore, on day 377 post

^{13}C labelling, no leaf samples are different from the natural abundance $\delta^{13}C$ pre-pulse samples (P = 1.00).

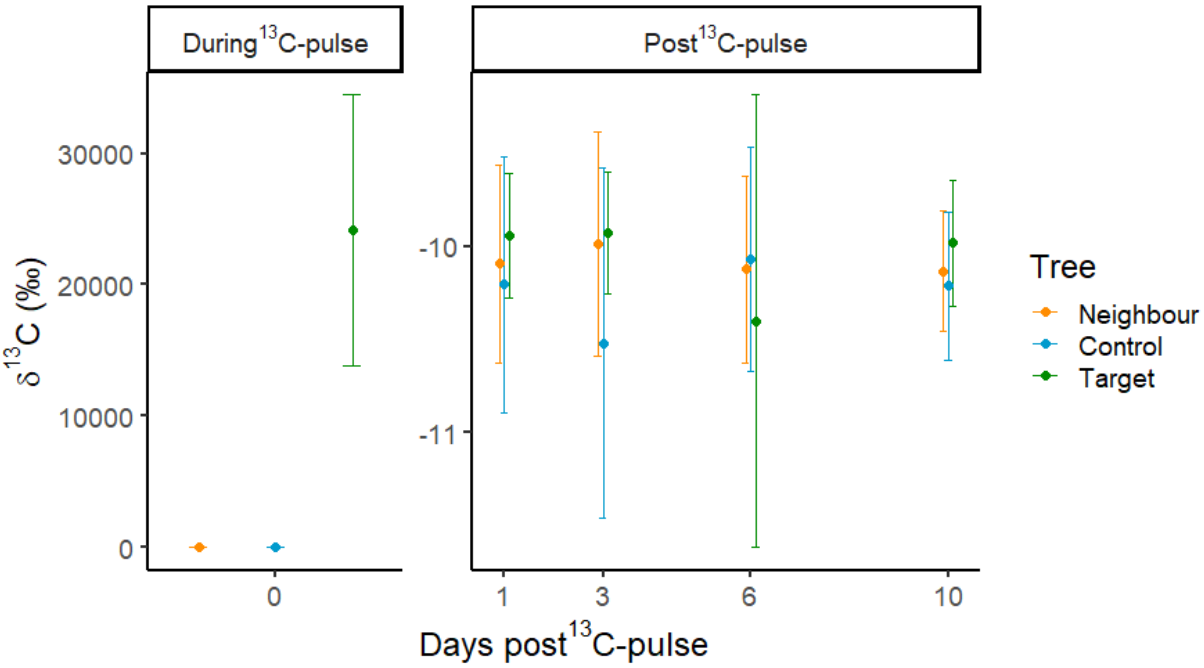

Figure 4.4. Mean (points) levels of $\delta^{13}C$ enrichment in air samples taken at all three trees (target, neighbour and control) during and after ^{13}C pulse-labelling. Error bars are ±1 standard deviation.

Air samples from inside the crown bag during the ^{13}C pulse of the target tree on day 0 show $\delta^{13}C$ is significantly higher than any other samples (P < 0.001) (Figure 4.4). All other samples (i.e. all controls, all neighbours and target trees on days 1-10) have similar levels of $\delta^{13}C$ (P = 1.00) (Figure 4.4).

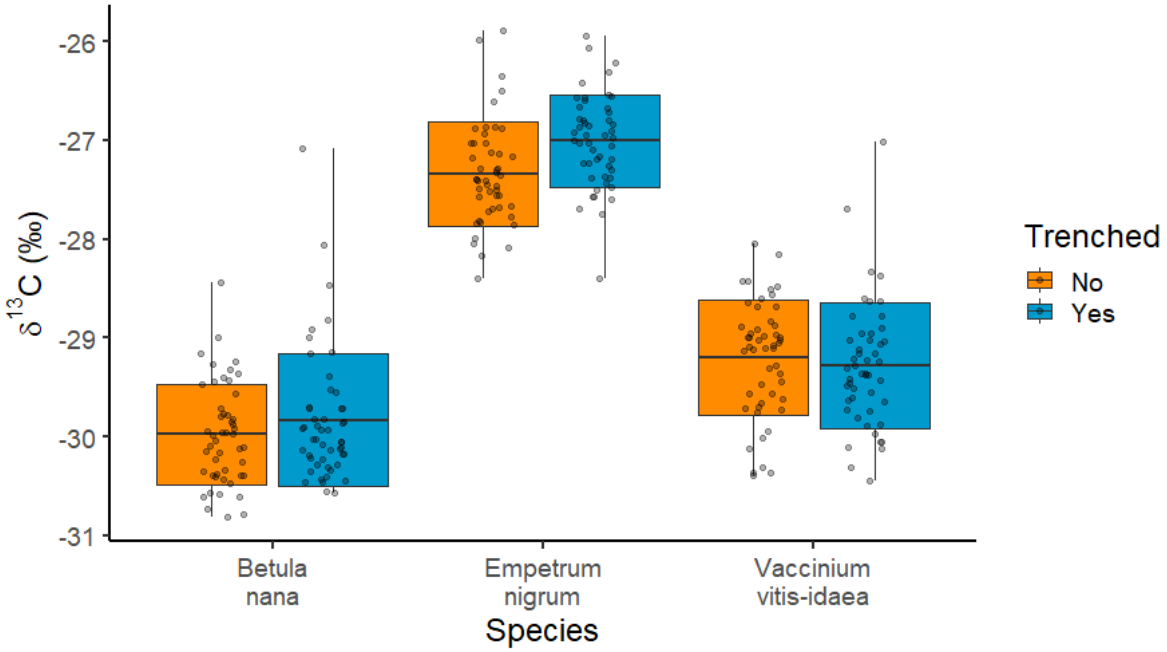

Figure 4.5. Level of $\delta^{13}C$ enrichment in understorey species sampled at varying distances from the labelled tree either side of the trench severing roots and mycorrhizas. Box shows mean ±1 standard deviation. Vertical lines indicate minimum and maximum (i.e. sample range).

There is no difference in $\delta^{13}C$ levels either side of the trench separating the pulse labelled tree in *B. nana* (P = 0.41) or *V. vitis-idaea* (P = 0.48) when all post labelling samples are pooled together (Figure 4.5). $\delta^{13}C$ levels are significantly higher in trenched *E. nigrum* samples (P = 0.012) when all post labelling samples are pooled together, however this is due to the pooling of samples from multiple days post ^{13}C labelling which is not apparent when comparing samples either side of the trench on each separate sampling day separately (Figure 4.6 & Table 1). All three understorey species have different levels of natural abundance $\delta^{13}C$ (P < 0.001).

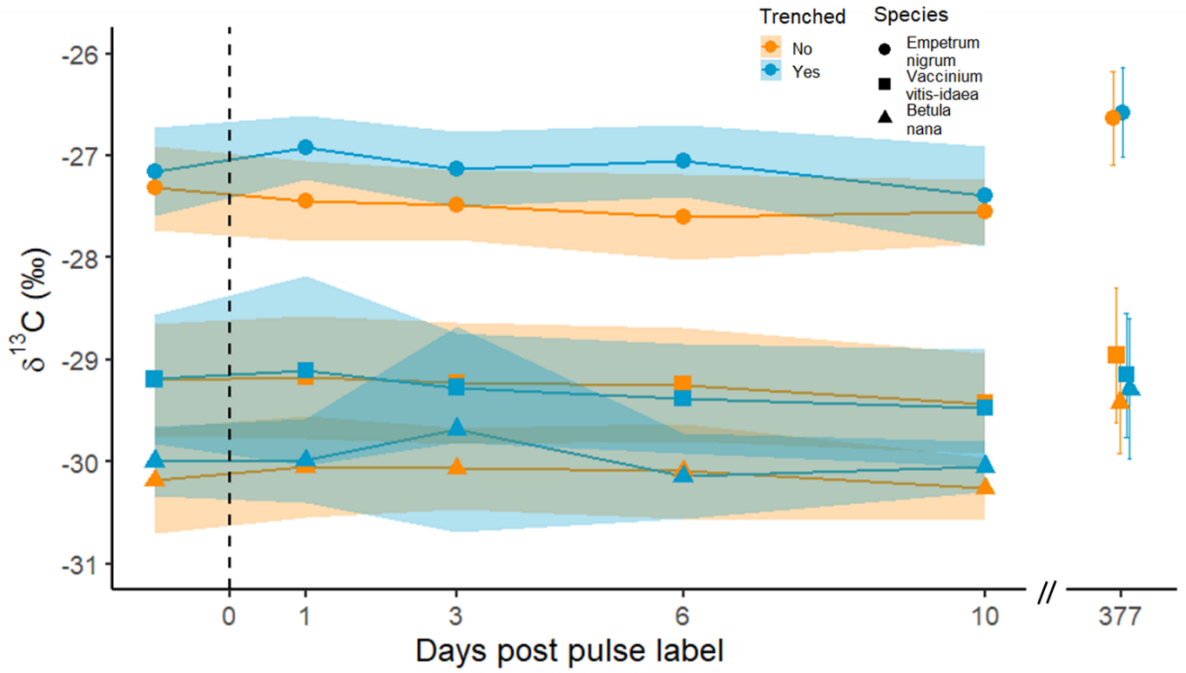

Figure 4.6. Mean level of $\delta^{13}C$ enrichment in understorey species up to ten and 377 days post pulse labelling of the target tree. Colour illustrates whether there was a trench severing roots and mycorrhizas between the pulse labelled tree and where the understorey was sampled. Error ribbon and bars are ±1 standard deviation.

There was no significant change in enrichment either side of the trench over time (Table 1).The levels of $\delta^{13}C$ on day 377 are significantly higher than on day 10 for all three species (*B. nana*: P <0.001, *E. nigrum*: P <0.001, *V. vitis-idaea*: P = 0.039) (Figure 4.6).

Table 4.1. P-values for levels of $\delta^{13}C$ enrichment in understorey samples taken either side of the trench before an isotope label was added (pre-pulse) and on days 1-10 and 377 post-pulse. Based on a t-test, paired for trenching treatment within each species.

	Day Pre-pulse	Day Post-pulse				
Species	**-1**	**1**	**3**	**6**	**10**	**377**
Betula nana	1.00	1.00	1.00	1.00	1.00	1.00
Empetrum nigrum	1.00	0.18	1.00	0.094	1.00	1.00
Vaccinium vitis-idaea	1.00	1.00	1.00	1.00	1.00	1.00

4.5 Discussion

Using a whole crown bag and $\delta^{13}C$ enriched $Ca^{13}CO_3$, I successfully labelled five replicate mountain birch trees *in situ* in a Swedish sub-Arctic treeline forest (Figure 4.3). The levels of $\delta^{13}C$ enrichment varied between trees but leaves of all five target trees had significantly higher $\delta^{13}C$ levels than pre-pulse natural abundance $\delta^{13}C$ samples and control tree samples, physically separated from the target tree by trenching. The $\delta^{13}C$ enrichment of target trees decreased over time following an exponential decay function by more than 3-fold in the first 10 days after the ^{13}C-pulse. This suggests that the majority of assimilated ^{13}C during the pulse-labelling period was rapidly re-located and/or respired by the target tree. Air sampling during the ^{13}C-pulse labelling shows very high $\delta^{13}C$ enrichment inside the crown bag but none outside the bag (Figure 4.4), importantly indicating that there was little or no $^{13}CO_2$ leaked from the bag. Air samples from the subsequent 10 days post ^{13}C-pulse show no enrichment at any of the trees (Figure 4.4) which means that no $^{13}CO_2$ respired from target trees was detectable. This also indicates that any leaf enrichment detected stemmed from the original ^{13}C-pulse, and not significantly from fixed and subsequently respired $^{13}CO_2$ from the target tree. The success of this novel method for pulse labelling whole trees with a stable isotopic label in the field is promising, with considerable scope for application in many other forest ecology contexts undergoing anthropogenic climate change. One application could be to investigate the effects of climate change driven Arctic ‘greening’, expansion of shrubs and northward advance of treelines (Myers-Smith *et al.*, 2011; Bjorkman *et al.*, 2018), which increases above-ground plant productivity and C inputs below-ground. It remains largely unknown how this will alter below-ground processes and affect the abundance of different species which allocate recently fixed C between

plant and soil pools differently (Street *et al.*, 2018b) potentially altering soil C sequestration (Wookey *et al.*, 2009; Parker *et al.*, 2016). This novel method of whole crown pulse labelling provides a tool to trace the fate of recently fix C from the atmosphere into various plant, mycorrhizal and soil pools and to investigate how this C input (likely to increase under future climate scenarios (Bjorkman *et al.*, 2018)) affects soil C turnover times in various pools once allocated. As well as having valuable applications in Arctic and sub-Arctic treeline forests, this novel method may also be applied in boreal and tropical contexts, although the small stature of trees (as found in Arctic treelines) did ease the process of attaching the crown bag.

Following the successful isotopic labelling of the target trees, I found no evidence of the ^{13}C label being incorporated into leaf tissues in neighbour trees (Figure 4.3). Therefore, there is no evidence of conspecific common mycelial networks (CMNs) between *B. pubescens* in these forests using this experimental field approach (Figure 4.1), and I (cautiously) reject the original Hypothesis 1.

The lack of evidence of CMNs in Arctic treeline *B. pubescens* trees is in contrast to a well-documented globally widespread occurrence of these networks in a range of terrestrial ecosystems (Simard *et al.*, 2012). However, there are limitations to this approach that must be addressed. This experiment, by its nature, is ‘proof-positive’. This means that, although there is no evidence supporting Hypothesis 1, and it is therefore reject, the null-hypothesis– that *B. pubescens* does not form CMNs in these forests – cannot be accepted as there is no empirical evidence of the absence of CMNs based on this experimental approach. Although I believe the experimental design to be robust, using plots representative of the wider forest community, the possibility that CMNs do occur in these forests cannot be ruled out. Furthermore, given the volume and strength of the literature finding evidence contrary to the

current study, albeit in other geographical regions, I accept that the failure to detect CMNs here may be due to limitations of the method used rather than strong evidence of the absence of CMNs.

A further limitation of this experiment lies in the post ^{13}C-pulse sampling regime. Foliar samples, of both trees and the understorey shrubs, were taken 1, 3, 6, 10 & 377 days post ^{13}C-pulse (Figures 4.3 & 4.6). The number of sampling days was constrained by the resources available for isotope determinations in this project. The timing of this sampling regime was selected based on evidence from experiments using ^{13}C pulse-chase methods which measured return respiration from trees via roots to the soil surface after 48h (Subke *et al.*, 2009), allocation to fine roots after 3 days (Endrulat *et al.*, 2010), and enrichment found in fungal PLFA biomarkers (Churchland *et al.*, 2012) and soil microbial cytoplasm 2-4 days post pulse (Högberg *et al.*, 2008). Furthermore, I assumed that transfer of recently fixed ^{13}C-containing compounds from the target to the neighbour tree via CMNs would take longer than return respiration or root and mycorrhizal allocation alone given the significantly more complex pathway of transfer via root and mycorrhizal transport and uptake by the neighbour tree. However, it is possible, given the sampling regime used here, that the isotopic signal in the neighbour tree was missed if the transfer of ^{13}C-containging compounds via CMNs occurred faster or slower than predicted and hence fell out-with the sampling days used here. Here foliage from adjacent con- and hetero-specifics was sampled, however this may not be where the ^{13}C-signal ends up. It is possible that C is transferred via CMNs but that it stays in the root or phloem tissues of the plant and was therefore not found by sampling foliage alone. Although sampling roots may have been more successful in locating the ^{13}C-signal, there is a

methodological issue with this approach as it would be difficult to identify which plant the sampled roots belonged to with any degree of confidence.

^{13}C was used for pulse labelling in order to investigate the transfer of compounds assimilated by the target tree directly, however using ^{15}N (as in Chapter 3) or a dual $^{13}C/^{15}N$ (Ek *et al.*, 1997) signal may have been more successful and is worth pursuing in future investigations of CMNs in treeline forests.

The absence of evidence of mountain birch tree CMNs here is surprising, given the recent finding that roots and mycorrhizas can extend 3-4.5 m away from the base of mountain birch trees in these forests (Chapters 2 & 3), and that CMNs have been found in other *Betula* species, e.g. *Betula papyrifera* (Simard *et al.*, 1997b) and *Betula nana* (Deslippe & Simard, 2011). One of the reasons that no evidence of CMNs between mountain birch trees was found here may be because the energy cost of entering into CMNs (Leake *et al.*, 2004) is too high in this harsh environment. However, CMNs have been found in other parts of the Arctic (Deslippe & Simard, 2011) with similarly harsh environments. Furthermore, high abiotic stress environments appear to increase ECM diversity on mountain birch roots (Ruotsalainen *et al.*, 2009), thereby increasing chances of CMN formation. In addition to this, one of the main benefits of CMNs for plant hosts is increased potential for soil nutrient scavenging including the mining and uptake of nitrogen (He *et al.*, 2003; Simard *et al.*, 2012). Nitrogen is a key nutrient, and limits plant growth in these treeline forests (Sjögersten & Wookey, 2005), so that extensive 'investment' in fungal networks constitutes a benefit that is likely to outweigh any energy costs to plant hosts.

In the Arctic, transfer of isotopically labelled C-containing compounds via CMNs has been found to occur between *B. nana* dwarf birch shrubs (Deslippe & Simard, 2011) but not mountain birch trees, as presented here. Deslippe and Simard (2011) suggest that the level of C transferred between individuals may confer a competitive advantage and thereby alter competitive interactions between plant species in the Arctic. Furthermore, they find that the magnitude of the C-transfer, between dwarf birch shrubs is temperature sensitive and may increase with warming, thereby acting as a positive feedback to ecosystem change as the climate warms. If transfer of C occurs between dwarf shrub individuals but not mountain birch trees, and this transfer of C via CMNs conveys competitive advantages which will likely increase with warming, then this may influence the dynamics of Arctic greening and shrub and tree expansion. In this scenario dwarf birch shrubs, which are already expanding (Myers-Smith *et al.*, 2011; Bjorkman *et al.*, 2018), will continue to do so onto tundra heath areas and increase in monodominance, which may leave the mountain birch trees at a competitive disadvantage. Furthermore, the potential difference in the presence of CMNs between mountain birch trees and dwarf birch shrubs means that the latter have access to greater mycorrhizal and fungal genetic diversity, which may provide greater resilience to environmental challenges (Hazard & Johnson, 2018) such as climate change. The continued and potentially accelerated expansion of dwarf birch shrubs onto soil C-rich tundra heathlands may have negative effects on the global climate, as recent evidence suggests that soil C turnover is faster beneath dwarf birch shrubs than adjacent tundra heath, which may lead to the net release of soil C into the atmosphere as CO_2 (Parker *et al.*, 2015).

Current results show that the ^{13}C label does not appear in either of the three dominant understorey species (*B. nana, E. nigrum* and *V. vitis-idaea*) sampled on

either side of the trench severing roots and mycorrhizas (Figure 4.5 & 4.6). The levels of $\delta^{13}C$ in the understorey remain unchanged on days 1-10 post ^{13}C-pulse of the target tree and are the same as natural abundance $\delta^{13}C$ samples taken pre ^{13}C-pulse (Figure 4.6 & Table 4.1). Based on these results there is no evidence for heterospecific CMNs occurring between mountain birch trees and any of the three dominant understorey species (both ECM and ERM) in these sub-Arctic treeline forests. Hypothesis 2 is therefore accepted. Although Hypothesis 2 is accepted, it is within the ecological context studied here and does not unequivocally exclude the possibility of heterospecific CMNs in other ecological contexts. The discovery of heterospecific CMNs represents a relatively new field of research, with the discovery of single fungal genets colonising both ECM tree and ERM shrub hosts (Grelet *et al.*, 2009, 2010; Villarreal-Ruiz *et al.*, 2012; Lukešová *et al.*, 2015a). For example; the mycorrhizal fungus *Meliniomyces variabilis* can simultaneously colonise Scots pine, an ECM tree species, and *Vaccinium vitis-idaea,* an ERM understorey shrub species (Grelet *et al.*, 2010) and several strains in the *Phialocephala fortinii s. l.—Acephala applanata* species complex (PAC) form both ECM intraradical structures in silver birch (*Betula pendula*) or Norway spruce (*Picea abies*) and ERM intraradical structures in *Vaccinium myrtillus* (Lukešová *et al.*, 2015b). Although these findings provide a theoretical basis for the existence of heterospecific CMNs between canopy forming tree species and ericaceous understorey shrubs, none have been found to date. The lack of heterospecific CMNs may, in part, be explained by the imbalance in the energy cost to host species and asymmetry in the investment and return of resources to and from the CMN system (Walder *et al.*, 2012).

4.6 Conclusions

Presented here is the successful ^{13}C-labelling of mountain birch trees using a whole crown bag in a Swedish sub-Arctic treeline forest. This pulse-chase technique was used to investigate the transfer of C-containing compounds from a central target tree to neighbouring conspecific trees and heterospecific understorey shrubs via CMNs. However, the ^{13}C isotopic label was not detected in neighbour trees or understorey shrubs, with intact root and mycorrhizospheres, 1-10 or 377 days post ^{13}C-pulse, and there is therefore no evidence of either con- or hetero-specific CMNs in these forests. Although this result does not unequivocally exclude the possibility of mountain birch trees forming CMNs, it suggests that there may be an altered competitive advantage between mountain birch trees and dwarf birch shrubs, which can form CMNs (within certain ecological contexts at least), with potential consequences for dwarf birch dominance and expansion onto tundra heaths altering soil C turnover dynamics.

Chapter 5:
Tree planting in organic soils does not result in net carbon sequestration on decadal timescales

Note:
This chapter has been submitted to *Global Change Biology* co-authored by:
Alison J. Hester, Ruth J. Mitchell, Thomas C. Parker, Jens-Arne Subke & Philip A. Wookey

5.1 Abstract

Tree planting is increasingly being proposed as a strategy to combat climate change through carbon (C) sequestration in tree biomass. However, total ecosystem C storage that includes soil organic C (SOC) must be considered to determine whether planting trees for climate change mitigation results in increased C storage. We show that planting two native tree species (*Betula pubescens* and *Pinus sylvestris*) of widespread Eurasian distribution onto heather (*Calluna vulgaris*) moorland with podzolic and peaty podzolic soils in Scotland did not lead to an increase in net ecosystem C stock 12 or 39 years after planting. Plots with trees had greater soil respiration and significantly lower SOC in organic soil horizons than heather control plots. We found a net ecosystem C loss in one of four sites with deciduous *B. pubescens* stands, and no net increase in ecosystem C at three sites planted with *B. pubescens* and an additional stand of evergreen *P. sylvestris* at one site. Therefore, at all four sites there is no gain in ecosystem C stocks 12-39 years after afforestation. We hypothesise that altered mycorrhizal communities and autotrophic C inputs have led to positive 'priming' of soil organic matter, resulting in SOC loss, constraining the benefits of tree planting for ecosystem C sequestration. The results

are of direct relevance to current policies, which promote tree planting on the assumption that this will increase net ecosystem C storage and contribute to climate change mitigation. Ecosystem-level biogeochemistry and C fluxes must be better quantified and understood before we can assure that large scale tree planting in regions with considerable pre-existing SOC stocks have the intended policy and climate change mitigation outcomes.

5.2 Introduction

Anthropogenic climate change has been described as the greatest current threat to ecosystems and all that depends on them (Nolan *et al.*, 2018). World-wide strategies to mitigate climate change have therefore been proposed (Paris Agreement, 2015). Notable among these is the growing international momentum behind tree planting, and the extensive afforestation of areas with future climates potentially suitable for forest cover (UNEP, 2011; “New York Declaration of Forests,” 2014; Bastin *et al.*, 2019; Lewis *et al.*, 2019). These proposed mitigation steps rely on sequestration of carbon dioxide (CO_2) by the production of tree biomass, but rarely consider storage of C in soils. Soil C storage is critically important, however, as more C is stored in soil globally than in vegetation and the atmosphere combined (Averill *et al.*, 2014). Furthermore, a large proportion of this is stored in high latitude regions (De Deyn *et al.*, 2008; Wookey *et al.*, 2009; Köchy *et al.*, 2015) and is vulnerable to loss through climate warming (Karhu *et al.*, 2014). Across humid temperate, boreal and sub-Arctic regions of the northern hemisphere, high densities of C are found in organic soils of uplands and tundra (Bradley *et al.*, 2005; Hartley *et al.*, 2012; Crowther *et al.*, 2019). The persistence of these significant C reserves depends in part on climatic conditions, but also significantly on land use and vegetation cover (Bradley *et al.*, 2005; Karhu *et al.*, 2014). At high latitudes, significantly greater ecosystem C-stores

are associated with low-stature, non-woody vegetation (tundra and ericaceous heathland vegetation), rather than with forests (Hartley *et al.*, 2012; Parker *et al.*, 2015). Similarly, trends of soil C loss following afforestation have also been reported in the context of forest plantations on grasslands (Guo & Gifford, 2002; Zerva *et al.*, 2005). Changes in land use and vegetation cover thus have the potential to influence biological and biogeochemical processes that can reduce soil and hence ecosystem C storage, resulting in a net C source to the atmosphere.

Planting trees in previously un-forested areas (or areas which have been deforested for centuries) creates profound changes to above-ground plant communities. This ultimately affects below-ground microbial communities, resulting in a reshaping of the ecosystem with consequences for stored soil C (Wardle *et al.*, 2004; Kyaschenko *et al.*, 2017a; Wurzburger *et al.*, 2017). These consequences remain poorly quantified and understood. Both above- and below-ground organisms play an important role in C sequestration (Wardle *et al.*, 2004), and it is vital to understand the combined responses of these communities to climate warming, and how they might be managed to facilitate climate mitigation (Amundson & Biardeau, 2018; Luyssaert *et al.*, 2018). Although afforestation has potential positive effects on C sequestration through the generation of plant biomass, it may have variable effects on soil C depending on tree and associated mycorrhizal species (Craig *et al.*, 2018), forest management (Kyaschenko *et al.*, 2017b), land use practices prior to afforestation (Guo & Gifford, 2002; Zerva *et al.*, 2005) and underlying soil characteristics (Jandl *et al.*, 2006). In light of the increasing drive to plant trees as a climate mitigation strategy, and the high levels of planting already occurring (UNEP, 2011; “New York Declaration of Forests,” 2014; Paris Agreement, 2015; Scottish Government, 2018; Scottish Forestry, 2019), it remains critically important to

understand the consequences for whole ecosystem C stocks, both above- and below-ground. This is also important in the context of climate driven tree encroachment onto C-rich soils in northern boreal and low-Arctic tundra regions (Tømmervik *et al.*, 2009; Hagedorn *et al.*, 2014; Reichle *et al.*, 2018) around the circumpolar north, which may increasingly attract the attention of policy-makers and ecosystem managers in coming decades.

Using four sites, each with a paired-plot experimental design of planted native tree species (*Pinus sylvestris* L. (Scots pine) and/or (*Betula pubescens* Ehrh. (Downy birch)), and associated un-forested heather (*Calluna vulgaris* (L.) Hull) 'control' plots (MOORCO, 2018), we investigate how woodland expansion onto heather moorland (a form of ericaceous heath) affects net ecosystem C.

5.3 Methods

5.3.1 Site description and experimental design

The sites used in this study form part of the Moorland Colonisation Project (MOORCO, 2018). The four sites used; Ballogie, Craggan, Delnalyne and Kerrow, are located across Northern Scotland (Figure 5.1). Stands of the native tree species downy birch (*Betula pubescens* Ehrh.), silver birch (*Betula pendula* Roth), or Scots pine (*Pinus sylvestris* L.) were planted at each site in a replicated block design onto *Calluna vulgaris* (L.) Hull dominated heather moorlands 12 or 39 years previously (Table 5.1). Tree planting and heather control treatments were randomly assigned to plots within blocks, following measurement of baseline soil parameters (bulk density,

organic horizon depth and %C) to ensure no underlying systematic bias between plots (Appendices Table 7.3).

Table 5.1. Summary information for the four experimental sites. DBH: Diameter at breast height.

Site	Location	Elevation (m asl)	Soil type	O_h-horizon depth (mean ± SD cm)†	Forest stand age (y)	Species planted	Measurements in current study
Ballogie	Lamahip Hill, Aberdeenshire : 57°01'53.5"N, 2°43'53.5"W	230	Peaty podzol	7.9±2.0	12	*Betula pubescens, Pinus sylvestris*	SOC, soil respiration, hyphal & root production, understorey biomass, tree height & diameter*
Craggan	Ballindalloch, Moray: 57°22'31.0"N 3°20'14.0"W	206	Humus-iron podzol	38.7±13.9	39	*Betula pubescens*	SOC stocks, tree DBH*
Delnalyne	Lagganvoulin, Glen Livet: 57°14'27.0"N 3°20'38.0"W	433	Humus-iron podzol	17.2±3.2	39	*Betula pubescens*	SOC stocks, tree DBH*
Kerrow	Fasnakyle, Beauly: 57°19'48.0"N 4°45'55.0"W	379	Humus-iron podzol	7.9±1.7	39	*Betula pubescens, B. pendula*	SOC stocks, tree DBH*

† Data for heather control plots only, measured in 2018-2019. O-horizon depth here refers to depth following removal of the L (litter) layer.
*Used to calculate tree biomass using allometric equations in supplementary Table S2.

The Ballogie experiment was established in 2005 (site map in Figure 5.1) and consists of three fenced blocks each containing three plots (18 m x 15 m). Treatments of birch (*Betula pubescens*) and Scots pine (*Pinus sylvestris*), both planted with 1 m spacing, and heather-dominated (*Calluna vulgaris*) controls were randomly assigned to the plots. Planting was done by spade, with minimal disturbance to the soil profile, using 20 – 40 cm high Scots pine saplings and 40 – 60 cm high birch saplings, both of local provenance. The birch plot in block 1 failed to

establish and was therefore omitted from this study. Because of this, a fourth unfenced block, with the same treatments as the fenced blocks, was used for carbon stock measurements but not soil respiration measurements. The presence/absence of fencing was accounted for in the data analysis and block design. Within each planted and heather control plot (not applicable to Block 4), three 2 x 2 m sub-plots have been established and systematically weeded every year. The three weeding treatments were removal of: 1) ericoid species (WR), 2) graminoid species (WM), and 3) understorey species predicted to be dominant in future forest successional stages (WD) e.g. *Vaccinium myrtillus* and various grasses as described in Figure 1 of Hester *et al.* (1991). Weeding treatments were applied in order to investigate the effects of understorey vegetation change on soil C cycling during forest succession.

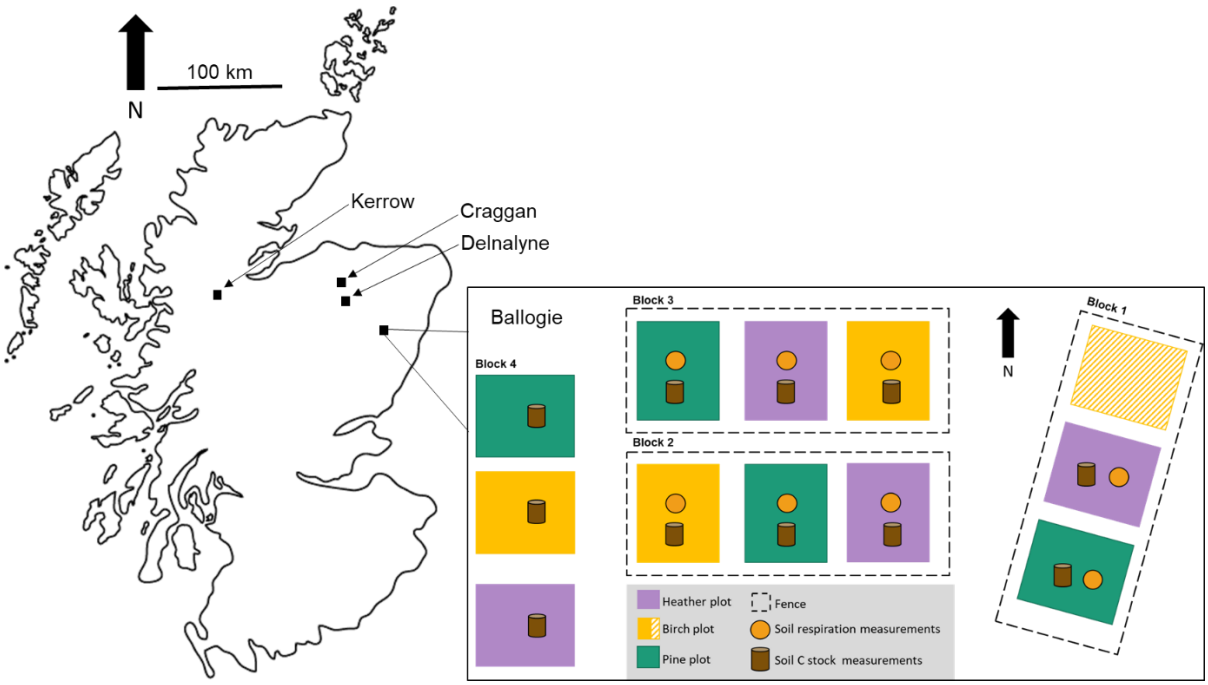

Figure 5.1. Map of experimental sites used across Northern Scotland, with detailed site map of Ballogie, showing block and plot layout (not to scale). Plots and blocks used for soil respiration and C stock measurements are indicated with separate icons (key in figure). Note that the placement of these icons does not equate to the position of the relevant measurements, merely that these measurements were taken at the plot in question. The hashed birch plot in Block 1 indicates failed tree growth. The detailed plot layout at other sites differed (see text).

At Delnalyne and Craggan twelve plots were established on heather moorland in a paired plot design, with one of each pair randomly assigned to a *Betula pubescens* treatment. The experimental design at Kerrow had six spatial blocks with three treatments: heather control, planted with *Betula pubescens*, and planted with *Betula pendula* (silver birch). Seedlings for all three sites were grown in pots at the Centre for Ecology and Hydrology (CEH) Edinburgh from a local seed source (John Miles letters, recorded by RJM). Tree saplings were panted at 0.5 m spacing between 1997 and 1981 and all three sites were fenced from grazing by large herbivores. See Mitchell *et al.* (2007) for further details of experiment design. At Kerrow, the establishment of an overhead power line through the site in 2012 led to trees being felled in 8 of the 12 birch plots (felled plots were excluded from analyses). Of the four planted plots remaining, two were *B. pubescens* and two were *B. pendula*, and both were used for carbon stock sampling. No difference in SOC stocks was found between the two species (P = 1.00), consistent with previous findings from the same plots (Mitchell *et al.*, 2007).

5.3.2 Soil respiration measurements

Soil respiration (Ballogie only) was measured at the edge and 4.5 m inside plots from the N,W,S and E edges of each plot (pooled by plot during data analysis) using a portable EGM-4 infrared gas analyser with a darkened CPY-4 chamber (PP Systems International, Amesbury, MA, USA). Respiration rates were calculated from the rate of CO_2 increase within the closed system over a period of 96 seconds. Respiration was measured from 15 cm diameter and 5 cm high PVC collars which were secured to the soil surface using non-setting plumber's putty (Plumber's Mait®, Bostik Ltd,

Stafford, UK) in order to minimise disturbance of the soil and prevent severing of any roots or fungal hyphae (Parker *et al.*, 2015). All vegetation was excluded from the collars; therefore respiration in this study is defined as the sum of microbial and root respiration within the chamber, and represents forest soil respiration. Respiration measurements at all collars were taken 12 times between 24th May 2017 and 16th October 2018. Soil temperature and moisture were measured at 5 cm soil depth at each collar using a hand-held Traceable® probe (Fisher Scientific) and a HH1 ThetaMeter (Delta-T Devices, Cambridge, England) at the same time as the respiration measurements were made.

5.3.3 Carbon stocks: Sampling and analysis

Carbon in the organic (O) horizon (hereafter referred to as soil organic carbon (SOC)) was inventoried in all plots at all sites by taking soil cores using a stratified random approach within the plots (n = 4 per plot at Ballogie and Kerrow, n = 2 per plot at Craggan and Delnalyne). Where n = 4 cores, the location was randomised within each quarter of the plot, and where n = 2 cores, the location was randomised within each half of the plot. O-horizon depth (excluding litter layer, but including fermentation and humus layers) was recorded and cores were oven dried for 96 h at 50°C. Mineral soil C was not inventoried, therefore references to 'ecosystem C stocks' do not include mineral soil C. Soil organic matter (SOM) content for each sample was determined by loss on ignition in a furnace at 550 °C for 4 h (Ball, 1964) and a subset of samples (n=3 for each soil layer from each plot type from each site; 66 samples in total) was analysed for C content using a FLASH SMART elemental analyser (ThermoFisher Scientific, Waltham, MA, USA). SOM was converted to soil organic C (SOC) using a standard curve: SOC (kg m^{-2}) = SOM (kg m^{-2}) x 0.5291, (R^2 = 0.95), based on elemental analysis results. As it was not possible to excavate full

tree root systems in these permanent experimental plots tree root C was estimated as 35% of aboveground tree C as found by Renou-Wilson *et al.* (2010) in plots of afforested peaty soils of applicable age classes. Ground flora C (including shrubs, forbs and grasses) at Ballogie was inventoried by destructive harvest of three 50 x 50 cm quadrats within each plot, of all fresh biomass above the moss layer (if present), air dried for 14 days and oven dried for 24 h at 60°C. Above-ground biomass of shrub, forb and grass dry weight was converted into C stock using conversion factors; 0.48 for shrubs (Allen *et al.*, 2013) and 0.45 for forbs and grasses (Vogt, 1991). Tree carbon was inventoried using allometric equations (Appendices Table 7.4). Tree size (height and girth) was measured in 2 m radius subplots (n = 4 per plot at Ballogie and n = 2 per plot at Kerrow, Craggan and Delnalyne), centred on the location from which the soil core was taken, within each planted plot. At Craggan, Delnalyne and Kerrow tree girth was DBH (diameter at breast height), and at Ballogie diameter at 10% height was measured (instead of DBH due to the large variation in tree height with many shorter than 1.3 m (standard 'breast height')). Tree aboveground biomass was converted into C stock using a conversion factor of 0.54 (Renou-Wilson *et al.*, 2010).

5.3.4 Root and mycorrhizal hyphae production

At Ballogie, root and hyphal production was assayed using in-growth techniques. For hyphae, four sand-filled 5 cm x 5 cm bags of 41 µm mesh were deployed at 5 cm depth within each plot from May to October 2017 (181 days). Upon harvest, in-growth bags were freeze-dried for 72 h and hyphae were extracted by suspending 1.5 g sand in deionised water and sonicating for 10 minutes before filtering onto glass microfiber filters (Whatman TM) and analysing C content using a FLASH

SMART elemental analyser (ThermoFisher Scientific, Waltham, MA, USA). Five laboratory blank samples were processed as controls. For roots, four organic soil (from Ballogie)-filled bags of 2 mm plastic mesh with height = 5 cm and plan view area = 2.95 cm^2 were deployed at 5 cm soil depth in each plot from May to October 2018 (147 days). All roots were plucked and washed within 24 hours of harvest and dried for 72 h at 50°C.

5.3.5 Data analysis

All analyses were carried out using R Version 3.4.0 (R Core Team, 2017). Variation in soil respiration, soil and vegetation C stocks, root production and hyphal production was investigated using nested ANOVA following a linear mixed effects model (Pinheiro *et al.*, 2012). If interactions between fixed effects were not significant ($P > 0.05$), they were removed in order to maximise degrees of freedom (Crawley, 2007). Covariates that did not significantly improve the model fit, as measured by Akaike Information Criterion (AIC) (Akaike, 1998) values, were removed from the model. In the soil respiration model, treatment (birch, pine or heather control), soil temperature and moisture were included as fixed effects and block and collar (physical point where respiration measurement was taken) assigned as random effects, accounting for variation between block sampling dates (Harrison *et al.*, 2018). Tree basal area was removed from the soil respiration model as it did not significantly improve the model fit. In the C stock model, treatment (birch, pine or heather control) was included as a fixed effect and block as a random effect. In the root and hyphal production models, treatment (birch, pine or heather control) was included as a fixed effect and block and compass orientation as random effects. Variation in soil respiration through time was modelled using a generalised additive model with a mixed effects structure (Pedersen *et al.*, 2019) as described above.

5.4 Results

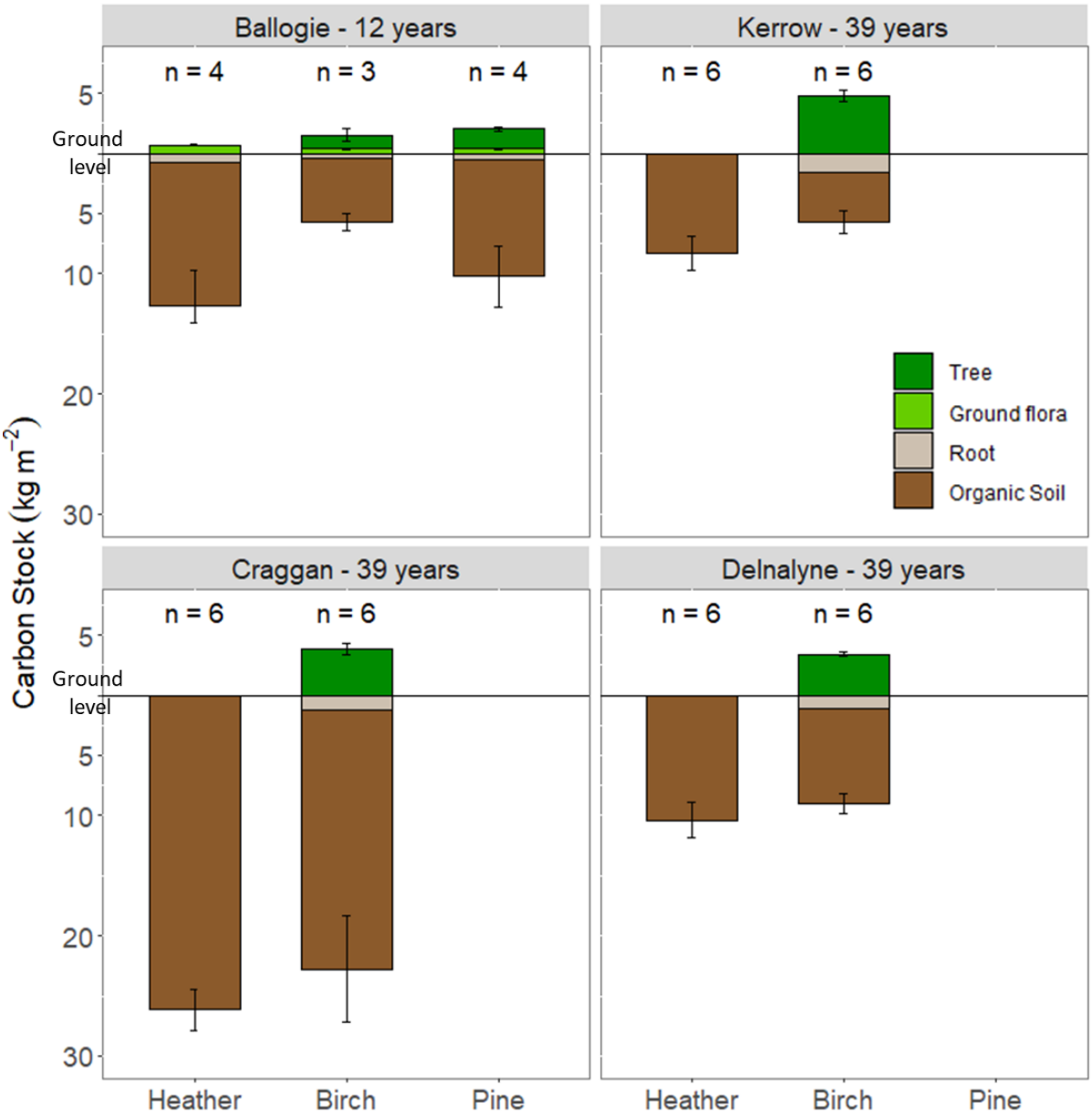

Figure 5.2. Mean ecosystem C stocks from different sites across Northern Scotland. Roots and organic horizon C stocks are represented beneath the zero-line on the y-axis and tree and ground flora above the line in planted birch and pine plots as well as in un-planted heather moorland ("Heather") control plots. Soil organic C (SOC) stocks based on O-horizon %C, depth and bulk density. Tree biomass estimations from allometric equations in Supplementary Table S2. Ground flora not inventoried at Craggan, Delnalyne and Kerrow as this does not significantly contribute to ecosystem C stocks at Ballogie (P= 0.51). Error-bars are standard error.

At Ballogie, SOC stocks in birch plots were 58% less (P = 0.02) than in the un-planted heather control plots, but no difference in SOC stocks was evident between

control and pine plots (P = 0.48) 12 years after planting (Figure 5.2). At Ballogie, the loss of soil C from organic horizons in birch plots was not compensated for by C in above-ground tree and shrub biomass as the combined above- and below-ground C (ecosystem C stock) in the birch plots was lower than the total C in the heather control plots (P = 0.012). No significant change in SOC stocks was seen in the Scots pine plots (Ballogie) 12 years after planting (P = 0.48), and in these plots a substantial amount of C has been sequestered as tree biomass (Figure 5.2). The ecosystem C stock was not significantly different (P=0.64) between pine and heather control plots 12 years after planting. At Kerrow, SOC stocks in birch plots were 50% less (P = 0.03) than in the un-planted heather control plots whilst ecosystem C stock (excluding understorey vegetation C) was not significantly different in birch plots relative to heather controls after 39 years (P = 0.26; Figure 5.2). At both the Craggan and Delnalyne sites, there was no significant difference in SOC (P = 0.34 and P = 0.18 respectively) or ecosystem C stock (P = 0.93 and P = 0.31 respectively; Figure 5.2) between birch and heather control plots 39 years after planting. At all sites tree planting was associated with a loss of SOC trend, however this was only significant at two of four sites.

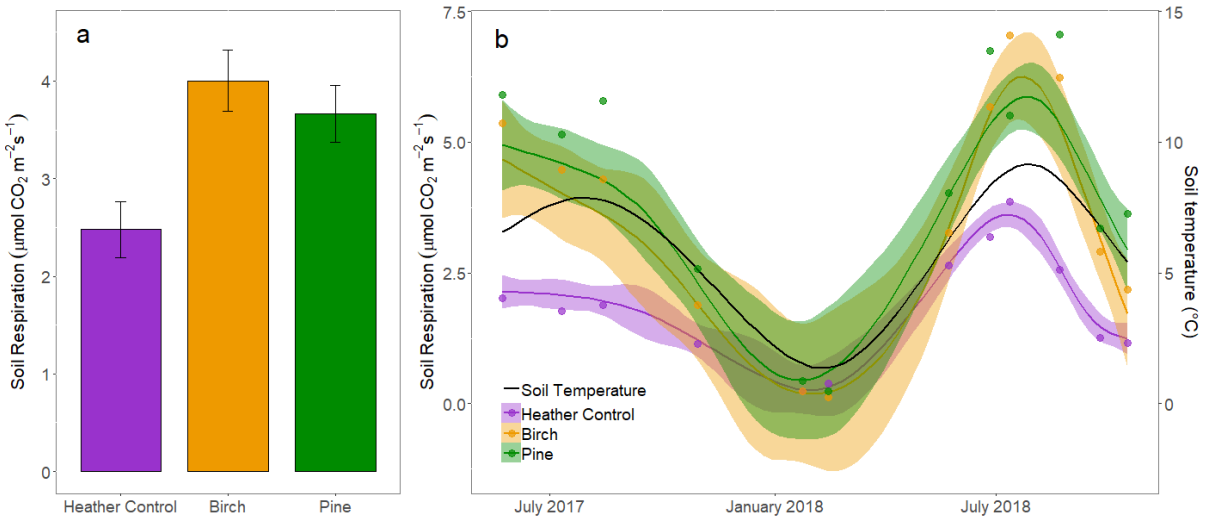

Figure 5.3. Modelled mean soil respiration in planted birch and pine plots and heather moorland ("Heather") control plots based on measurements conducted over 2017-2018 at the Ballogie site. a) Modelled mean for total measurement period from mixed effects models with block and plot as random effects, tree size, soil moisture and temperature as fixed effects and an interaction term between date and plot. b) Modelled mean over time with soil temperature averaged across all plots, together with measured data (points) using a generalised additive model with a mixed effects structure as in a). Output Error-bars/ribbons are standard error (n = 3).

Soil respiration was significantly ($P < 0.01$) higher in the birch and pine stands compared to the heather control plots across the measurement period at the Ballogie site, 12 years after planting (Figure 5.3a). Soil respiration showed a seasonal pattern, with apparent differences between the planted and control plots May-September ($P < 0.05$) and no difference between planted and control plots October-April ($P > 0.1$) (Figure 5.3a). The seasonal pattern corresponded with soil temperature variations (Figure 5.3b). There was no significant difference in the amount of roots produced in the heather control plots and planted birch ($P= 0.69$) or planted pine ($P= 0.11$) plots over one growing season (Appendices Figure 7.3). There was also no significant difference in the amount of mycorrhizal hyphae produced in the heather control plots and planted birch ($P= 0.37$) or planted pine ($P= 0.72$) plots over one growing season.

At Ballogie, where subplots were systematically weeded, none of the weeding treatments were associated with significantly altered soil respiration compared to un-weeded controls in Heather (WD: P = 0.93, WM: P= 0.53, WR: P = 0.50), birch (WD: P = 0.17, WM: P = 0.18 , WR: P = 0.16) or pine (WD: P = 0.19, WM: P= 0.21, WR: P = 0.25) plots (Appendices Figure 7.5).

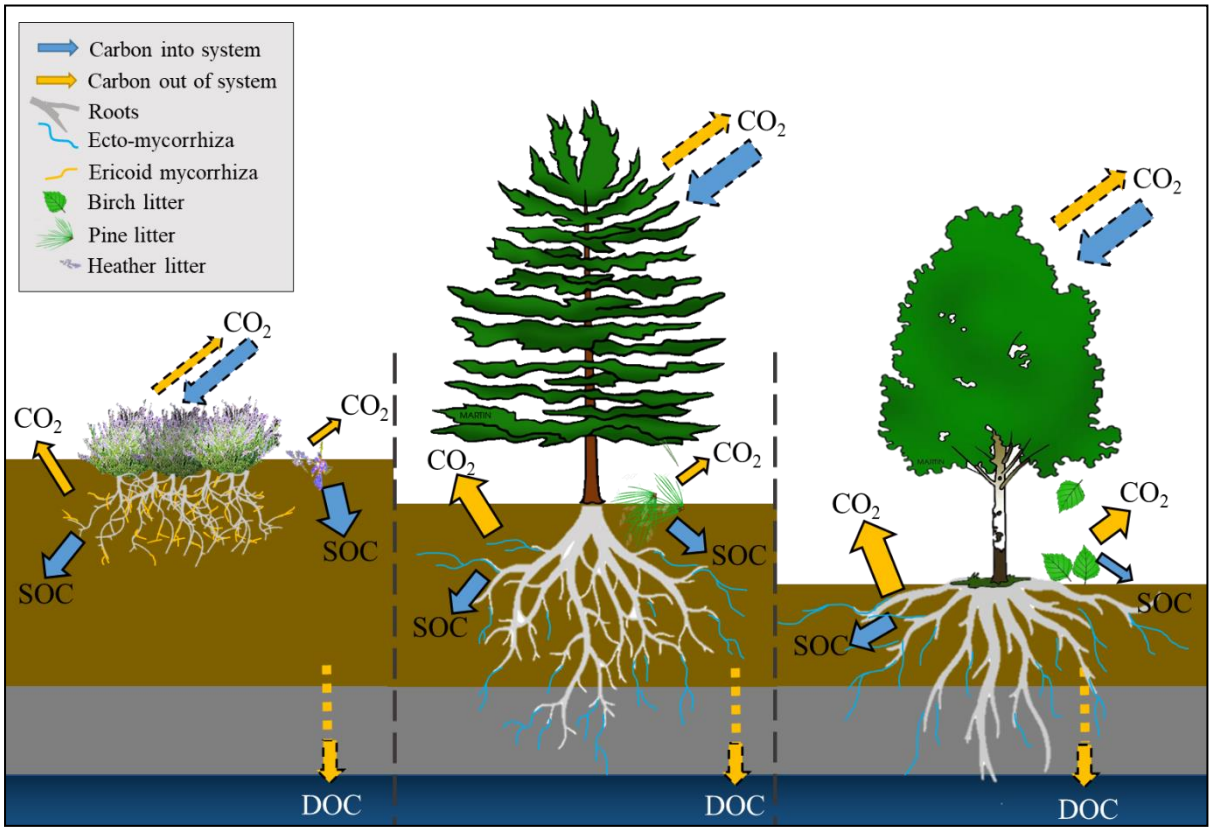

Figure 5.4. Conceptual diagram representing the relative differences in magnitude (represented by arrow width) of carbon fluxes into and out of the ecosystem in birch, pine and heather moorland plots. Carbon dioxide (CO_2) is fixed by plants and released back into the atmosphere through plant and soil (root, mycorrhiza and decomposer) respiration. Plant litter decomposition and root exudations are influxes of carbon into the soil and lead to soil organic carbon (SOC) formation, an unknown fraction of which is leached from the system in the form of dissolved organic carbon (DOC). Litter inputs may also, however, lead to accelerated decomposition ('priming') of pre-existing SOC. Dashed arrows indicate fluxes not quantified in this study. Organic and mineral soil horizons represented by brown (scaled to SOC stocks found at Ballogie in Figure 2) and grey boxes respectively.

5.5 Discussion

Planting replicated stands of two native tree species onto heather moorlands was associated with significantly lower O-horizon soil organic C (SOC) stocks at two sites, or no change in stocks at two other sites (Figure 5.2). Despite increased above-ground C associated with tree biomass, the loss of SOC in planted plots results in no significant increase in ecosystem C stock at any site over the duration of the experiment, and a net loss at one site (Figure 5.2). When considering both above- and below-ground C stocks, these data show no net gain in whole ecosystem C stock as a result of planting trees, over the decadal timescales observed here. At one site, Kerrow, above-ground tree C gains fully offset the significant SOC losses associated with tree planting (Figure 5.2), indicating that initial loss of SOC associated with planting trees may be compensated for by tree C gains after 39 years. As the birch stands continue to age and grow they may eventually lead to net ecosystem C sequestration after >39 years, assuming no further SOC loss. Our results show that afforestation of organic-rich soils will not result in net ecosystem C sequestration in the first 12-39 years but might do so after >39 years. Differences in SOC between heather and birch plots may be explained by different rates of C lost as CO_2 (Figure 5.3a) or as dissolved organic carbon (DOC, Figure 5.4). We did not quantify the latter, but early evidence from similar experimental plots suggests that DOC loss is 43% greater from planted birch plots than adjacent heather moorlands (Dawson *et al.*, 2007). The assessment of the extent to which organic materials - originating from leaf and root litter, rhizodeposition, and O-horizon SOM - are transferred to mineral horizons was beyond the scope of the current study, although we note the potential value of this for future research at these plots.

Plots planted with birch 39 years previously showed a significant decline in SOC stocks from O-horizons in planted birch stands relative to adjacent heather moorlands at one of three sites (Kerrow, Figure 5.2). Furthermore, there was no evidence of significant net ecosystem C accumulation in forested plots at any of the sites investigated (Kerrow, Craggan and Delnalyne). These results mirror those found when these three sites were surveyed in 2007 (Smith *et al.*, 2007). As these experimental plots are more than three times older than those at Ballogie, there may be regeneration of SOC stocks as forest stands age. However, at Kerrow, which is the same age as Craggan and Delnalyne (39 years), SOC stocks from organic horizons declined by 50% in planted birch plots relative to heather control plots (Figure 5.2). The direction of this trend is similar to Ballogie, and, although there is a slowing of SOC loss (4.8% $year^{-1}$ at Ballogie vs 1.3% $year^{-1}$ at Kerrow), this is still a highly significant loss of SOC from the system. This may, in part, be explained by a reduction in mycorrhizal C use efficiency as forest stands age and N availability decreases (Hagenbo *et al.*, 2019). A similar study (using some of the sites studied in the current work) found a decrease of SOC stocks by 20.6% in 20 year old birch plantations relative to heather moorland (Mitchell *et al.*, 2007), and declines in organic matter in the top 5 cm of soil were reported in birch forests (compared to adjacent heather moorland) of 21, 27, 38 and 50% in 18, 26, 38, and 90 year old forests (Miles, 1981), respectively. These results suggest high rates of organic matter decline in young forests, which slow as forest stands age. Using a modelling approach, Poeplau *et al.* (2011) estimate that afforestation of temperate grasslands results in SOC loss in the first 50 years after plantation but then gradually leads to SOC gain in the forest floor. Although the specific land use change explored in that paper is different from the current work, the change in the direction of the SOC trend

over time may be relevant in the current context. We found lower SOC stocks in birch forests than in adjacent heather moorlands in two sites, but the magnitude of this change was different, with younger stands depleting SOC stocks more.

As all plots (within site) showed no significant difference in baseline soil parameters prior to tree planting, the differences in stocks between planted and control plots found here are likely driven by the presence of planted trees. However, we note that there are significant differences in SOC stocks between sites, despite being established at the same time and using the same method (Figure 5.2). This suggests that abiotic factors, such as moisture, topography/aspect, and pre-existing soil conditions can affect the absolute magnitude of the change in SOC stock following planting of birch trees onto heather moorlands. Combined, this evidence shows that: (a) careful consideration must be taken when choosing sites for future tree planting schemes; and (b) a more nuanced approach to an evaluation of SOC stocks prior to planting, rather than simply whether peat horizons are less than or greater than 50 cm deep (Forestry Commission Scotland, 2016), is warranted.

Consistent with the inventories of SOC (Figure 5.2), planting two native tree species onto heather moorlands resulted in greater release of CO_2 from the soil (soil respiration) relative to the unplanted heather moorlands 12 years post planting (Figure 5.3a). This increase in soil respiration in planted plots relative to heather control plots occurred despite similar rates of root and mycorrhizal hyphae production between planted plots and heather control plots (Appendices Figure 7.3); i.e. root respiration potential is similar. Combined, this may indicate positive soil ‘priming’, whereby recent C inputs into the soil, mediated by carbon assimilation

above-ground, stimulate the soil microbial community, enabling decomposition of pre-existing soil C stores and release of CO_2 into the atmosphere (Fontaine *et al.*, 2007). This phenomenon has been detected from temperate peatlands (Walker *et al.*, 2016) to arctic permafrost soils (Street *et al.* (in press); Wild *et al.*, 2016) and may become a more prevalent mechanism, causing increased CO_2 release and soil C loss in regions with large soil C pools (Hartley *et al.*, 2012) affected by climate driven changes in plant communities. The loss of organic horizon SOC in moorland with tree planting coincides with a shift in the dominance of mycorrhizal type from ericoid mycorrhizal (ERM) fungi to ectomycorrhizal (ECM) fungi. This key difference may lead to faster hyphae turnover (Clemmensen *et al.*, 2015), and less diverse extracellular enzymes (Read & Perez-Moreno, 2003) but with potentially higher expression (Sterkenburg *et al.*, 2018), especially SOM-degrading peroxidases that may be used to liberate N from complex organic matter (Bödeker *et al.*, 2014). Priming of organic matter may be particularly important for trees as they colonise uplands and tundra as the N that they require for growth is typically bound to organic matter (Shaver *et al.*, 1992). A key question remains concerning the role of mycorrhizal fungi in priming of this soil and more widely across other ecosystems (Frey, 2019; Zak *et al.*, 2019). It should also be noted that many soils in these regions may be particularly vulnerable to the direct effects of warming (Karhu *et al.*, 2014). Higher soil respiration rates in the planted plots relative to the unplanted heather moorland controls were seasonal, with apparent differences in the spring and summer months (May-September; Figure 5.3b) and no difference between planted and unplanted plots in the autumn and winter months (October-April; Figure 5.3b). This seasonality corresponds with higher temperatures and photosynthetic activity leading to more C resources being allocated below-ground by the plants. Soil

moisture also exhibited clear seasonality across all treatments, with wetter soils in the autumn and winter months, followed by drying during spring and summer; however there was no difference between plot types (Appendices Figure 7.4, $P > 0.05$), possibly due to the relatively small scale of the experimental plantings and the likely importance of lateral soil water recharge at these sites. However, at the landscape scale, large-scale afforestation is likely to impact the ecosystem water balance, affecting soil moisture substantially (Roberts, 1999). In organic-rich soils, significant soil drying together with rhizosphere priming, may accentuate rates of SOC loss further (Birch, 1958; Fontaine *et al.*, 2007), both to the atmosphere and to ground- and surface-waters.

Although we find similar rates of root production between planted and unplanted treatments (Appendices Figure 7.3a), the increase in soil respiration seen in planted plots may represent the rapid cycling of recently fixed C back to the atmosphere via root respiration, which can be the fate of a large fraction of fixed C (Högberg *et al.*, 2001; Ryan & Law, 2005; Pumpanen *et al.*, 2009). The contribution of understorey species to this CO_2 efflux, assessed through systematic weeding treatments with sustained removal of key understorey species from sub-plots within all plots, is not significant (Appendices Figure 7.5). This result is similar to that found by Kritzler *et al.* (2016), where prevention of photosynthate allocation to the rhizosphere (through the implementation of 'girdling'; the removal of phloem tissues around stems) in *Calluna vulgaris* did not alter soil CO_2 efflux. Furthermore, it has recently been found that mountain birch and associated fungi are responsible for the majority of soil respiration in peak season in a sub-Arctic treeline forest (Parker *et al.*, 2020). Taken together, these results indicate that the canopy-forming tree species have a larger net contribution to the return flux of C (soil respiration) than understorey species,

although rhizosphere processes associated with the latter are too poorly understood at present to reach firm conclusions.

The difference in soil respiration and SOC stocks between birch and heather control plots is likely driven by contrasting mycorrhizal types (Figure 5.4), from the ERM heathland shrubs, with recalcitrant litter, slow hyphal turnover and suppression of saprotrophic decomposers, to the ECM birch and pine trees, with faster hyphal turnover and more generalist saprotrophic decomposers (Read & Perez-Moreno, 2003; Clemmensen *et al.*, 2015; Hazard & Johnson, 2018). The difference in SOC accumulation between birch and pine plots found here may also be explained by differences in quality of leaf litter (Dorrepaal *et al.*, 2005; Epps *et al.*, 2007; Brovkin *et al.*, 2012; Parker *et al.*, 2018) and root exudates (Smith, 1976), resulting in slower C turnover in the coniferous pine stands relative to the deciduous birch stands (Melvin *et al.*, 2015) (Figure 5.4). The combined above- and below-ground C stocks in the pine plots were similar to heather control plots 12 years after planting, indicating that planting pine trees onto heather moorlands may lead to little change in ecosystem C sequestration in the short- to medium-term (~12 years).

5.6 Conclusions

This study contributes to the debate on scenarios of change in soil and ecosystem C stocks in northern circumpolar boreal and low arctic ecosystems with similar plant functional types and controls on SOM dynamics. Indeed, model analyses (Pearson *et al.*, 2013) indicate that substantial regions of the ~1.63 million km^2 of circum-polar arctic vegetation communities which currently have sedge, shrub and moss-dominated vegetation have the potential to shift to forest (Raynolds *et al.*, 2019) and

have recently been identified as areas for potential tree restoration/afforestation (Bastin *et al.*, 2019). These are also systems where SOC densities are remarkably high (Hugelius *et al.*, 2013) and potentially vulnerable to both the direct (Karhu *et al.*, 2014) and indirect effects of warming. This long-term planting experiment, with ECM trees growing on former ERM heather moorland, provides the most informative empirical evidence to date for the potential effects of tree establishment in ericaceous heathlands, including tundra heaths. In the Scottish context, our data suggest that the current policy not permitting afforestation on peats >50 cm deep (Forestry Commission Scotland, 2016) should be reviewed and tightened; recommendations (to the Scottish Government's Woodland Expansion Advisory Group) that 34% of Scotland's land area may have potential for woodland expansion (Sing *et al.*, 2013) risk jeopardising soil (and ecosystem) C stocks on the extensive heather moorlands and heathlands with organic horizons of <50 cm depth. Ecosystem-level biogeochemistry and C fluxes must be better quantified and understood before we can be assured that large scale tree planting in these regions, with their massive pre-existing SOC stocks, will have the intended policy and climate outcomes.

Chapter 6:
General Discussion

The research presented in chapters 2 – 5 investigates key aspects of the forest mycorrhizosphere mediating plant-soil interactions and their effects on soil C turnover and storage in sub-Arctic and high latitude boreal ecosystems. The effects of plant-soil interactions on soil C cycling and storage are gaining prominence in a changing world under pressure to urgently combat the effects of climate warming both locally and globally (IPCC, 2018). The net zero CO_2 targets proposed by many countries, including the UK by 2050 (Committee on Climate Change, 2019) and Scotland by 2045 (Scottish Government, 2019), will not only require drastic reductions in CO_2 emissions from anthropogenic sources, but will also rely heavily on the uptake and sequestration of CO_2 by natural and managed ecosystems. Given the magnitude of this challenge and the urgency with which society now needs to act (IPCC, 2018) it is important to present and report on scientific evidence of ecosystem C cycling and sequestration in the context of global challenges and climate change mitigation. This chapter puts the work presented in previous chapters into the context of a changing world and the challenges and potential solutions.

6.1 Treeline forest encroachment onto tundra heathlands in the sub-Arctic

Climate warming in the Arctic has resulted in large areas of tundra becoming more productive (Epstein *et al.*, 2012b) with shrubs and trees increasing in both cover and height (Myers-Smith *et al.*, 2011; Elmendorf *et al.*, 2012; Bjorkman *et al.*, 2018). Climate warming driven shifts in Arctic and sub-Arctic vegetation from tundra classes to forest classes (Raynolds *et al.*, 2019) have been predicted to result in a 52%

increase in woody cover across the Arctic by 2050 (Pearson *et al.*, 2013). This change in above-ground vegetation may result in a loss of ecosystem C stocks based on space-for-time substitution estimates which have found significantly less ecosystem C in treeline forests and areas of woody shrubs compared with tundra ecosystems (Hartley *et al.*, 2012; Parker *et al.*, 2015), owing to C losses below-ground.

Previous work on the effects of tree and shrub expansion onto tundra heathlands in the sub-Arctic has focussed on forests, shrub-lands and tundra heathlands at the scale of vegetation types. However, in the current work I have found that soil C dynamics are not uniform within the forest (Chapter 2). Furthermore, I find that networks of symbiotic mycorrhizas may mediate competitive interactions between mountain birch trees, dwarf birch shrubs and ericaceous tundra plants (Chapters 3 & 4), and thereby influence treeline encroachment onto tundra heathlands.

6.1.1 Trees as drivers of soil C patterns in the treeline forest

Mountain birch trees within the sub-Arctic treeline forest may influence key soil C processes in a multitude of ways. These include C inputs in the form of root exudates, litter from roots, mycorrhizas and leaves (Clemmensen *et al.*, 2013; Cotrufo *et al.*, 2013), C loss from root respiration, C decomposition through associations with mycorrhizas and the priming of the saprotrophic community promoting decomposition of C stores (Fontaine *et al.*, 2007; Hartley *et al.*, 2012). In Chapter 2, I show that the patterns of soil C dynamics vary within the treeline forest and that the degree of homogeneity or heterogeneity of soil C dynamics varies with the specific process in question. My results show that soil CO_2 fluxes and both root and mycorrhiza production do not vary systematically along transects spanning half

the mean maximum distance between trees; thus I show consistency (but not homogeneity) of these processes throughout the forest floor (Figures 2.2 & 2.3). My result indicate that SOC stocks, standing litter and fungi:bacteria ratios do vary along the same transects and therefore result in patchiness across the forest floor (Table 2.1, Figure 2.5b & Appendix figure 7.1). These results indicate that the control on certain soil properties (SOC stocks, litter fall and F:B ratios) are dominated by proximity to individual trees within the forest, while other soil processes (CO_2 flux and mycorrhizosphere production) are the result of the contribution of multiple trees within the forest. Therefore, when predicting the effects on key soil processes of shrub and tree encroachment onto tundra heaths, the homogeneity of these effects varies depending on the process in question. It is important to note that these patterns hold true in the Abisko mountain birch treeline forest, with open canopies and low tree density, which is common for many Arctic treelines across North America and Eurasia (Payette & Lavoie, 1994; Kullman & Öberg, 2009). However, the patterns described here are likely dependent on tree density and rates of population turnover and may therefore be different in treeline forests of higher or lower density.

Understanding how soil processes vary spatially within treeline forests, based on empirical field measurements, will help guide selection of the appropriate resolution for up-scaling and model predictions. Although there is often a call for more data to inform global change models and facilitate more accurate predictions (Clark *et al.*, 2001), understanding the spatial scale at which such data is required is important in order to maximise data collection efforts for processes where high spatial resolution is needed. Fine scale spatial studies such as this, in combination with larger scale landscape studies, can help guide sampling effort by identifying processes where

high spatial resolution is needed. Such a framework would facilitate the most beneficial ratio of data collection efforts to model accuracy gains, which would provide much needed efficiency within the global change science community in light of the urgency of climate action.

6.1.2 Forest networks' influence on treeline encroachment onto tundra heathlands

The predicted vegetation shifts in the Arctic and treeline encroachment onto tundra heathlands have been linked to climate warming (Elmendorf *et al.*, 2012; Pearson *et al.*, 2013), but there are many other biotic and abiotic factors which may control these predicted shifts. As well as temperature, abiotic controls include soil development and hydrology, the latter of which may prevent tree encroachment into tundra heathlands in some areas due to the formation of bogs as a consequence of permafrost thaw (Skre *et al.*, 2002). Biotic factors include evolutionary history (Skre *et al.*, 2002), grazing pressure from reindeer and other herbivores (Olofsson *et al.*, 2009), as well as controls on seedling establishment such as competition (Hobbie & Chapin, 1998). One competitive advantage may be mycorrhizal status. Although it has long been recognised that mycorrhizal symbioses provide hosts plants with fitness benefits (Harley & Smith, 1983), it has more recently been proposed that the ability of certain mycorrhizas and host plants to form complex networks (Simard *et al.*, 2012) can provide both competitive advantages (Deslippe & Simard, 2011) and resilience to environmental change (Hazard & Johnson, 2018).

Evidence from Chapter 2 shows that mycorrhizas in the Abisko treeline forests have consistently high productivity ≤ 3 m away from the nearest tree (half the mean maximum distance between trees in these forests, Figure 2.3 & Appendices Table 7.1). Based on this, I used a stable isotope pluse-chase experiments in order to

investigate just how far away from trees mycorrhizal networks extend and whether common mycelial networks (CMNs) are formed in these forests (Chapters 3 & 4). The results show that mountain birch trees and their associated mycorrhizosphere can access soil nitrogen up to 4.5 m away but not over larger distances (Figure 3.3). This N scavenging distance for the ECM trees is greater than that found for the ERM and ECM understorey shrubs (1.1-3.3 m, Figure 3.5) and therefore may allow the trees access to a larger soil N pool (due to larger spatial reach) which provides a competitive advantage for the trees over the understorey shrubs. As evidence from Chapters 2 & 3 indicate that mountain birch tree mycorrhizas extend beyond the mean maximum distance between trees in these forests with the potential to form CMNs, in Chapter 4, I investigate the presence of both con- and hetero-specific CMNs in the mountain birch forest. Although CMNs have been found in dwarf birch shrubs in Arctic environments previously (Deslippe & Simard, 2011), no evidence was found in the current work of either con- or hetero-specific CMNs formed by mountain birch trees and their associated mycorrhizas in these forests (Figures 4.3 & 4.6). Even though these experiments cannot rule out the presence of mountain birch tree CMNs in these forests, one interpretation of this result could be that dwarf birch shrubs CMNs may be more prevalent than mountain birch tree CMNs. This dynamic, and the ability for C and mineral nutrients to be transported to other plants via CMNs (Simard *et al.*, 2012), may provide a competitive advantage to the dwarf birch shrubs over the mountain birch trees, which may lead to an increase in dwarf birch monodominance (Deslippe & Simard, 2011). This result is important in light of predicted tree and shrub expansion in the Arctic as dwarf birch is one of the main species observed to be expanding rapidly (Myers-Smith *et al.*, 2011).

The establishment of dwarf birch shrubs and mountain birch trees, which are ECM plants, onto tundra heathland made up primarily of ERM plants causes a shift in the dominant mycorrhizal type from ECM to ERM. These two systems function and cycle C in fundamentally different ways (Figure 6.1).

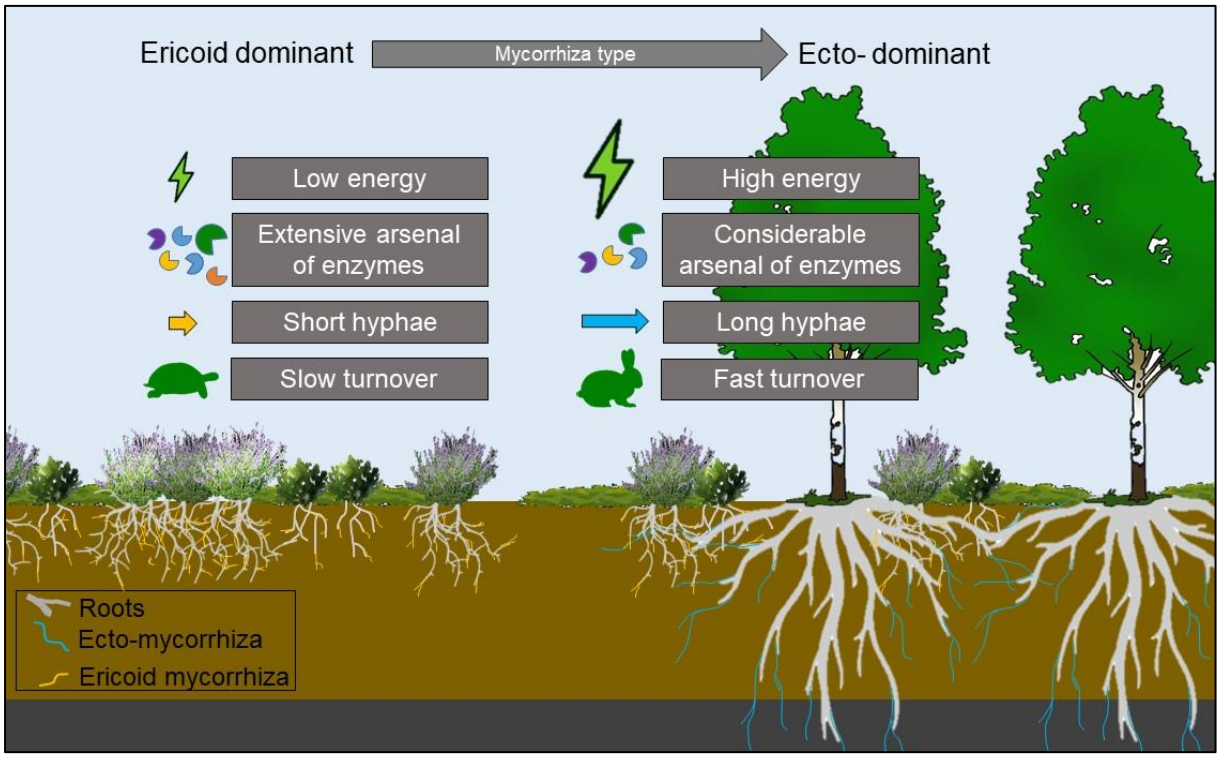

Figure 6.1. Schematic representation of the transition from an ERM dominant heathland to an ECM dominant forest. The ERM dominant heathland is a low energy system with slow C turnover and the ECM dominant forest is a high energy system with rapid C turnover.

The ERM dominant system has low primary productivity, short melanised hyphae and slow turnover of soil C leading to the build-up of SOM whereas the ECM dominant forest has high primary productivity, and ECM fungi that form long and readily decomposable hyphae which rapidly cycles soil C, leading to the loss of SOM (Read & Perez-Moreno, 2003; Clemmensen *et al.*, 2015). This transition from ERM to ECM dominant systems has been attributed as one of the main drivers of the lower soil C stocks found in the forest and shrub lands relative to tundra heathlands

(Hartley *et al.*, 2012; Parker *et al.*, 2015) and is likely to play an important role in the consequences of shrub and tree encroachment onto heathlands for global C cycles.

When considering the mechanism of this encroachment, it requires the successful establishment of seedlings in tundra ecosystems within pre-existing plant and microbial communities. A range of factors affect seedling establishment, such as temperature, water availability, snow cover, nutrients, light, surrounding vegetation and grazing pressure (Lett & Dorrepaal, 2018). In a detailed experiment investigating the interaction between temperature and surrounding vegetation on seedling establishment, Lett et al. (2017) found that mountain birch trees performed better in the presence of bryophytes than in bryophyte free soil, a response which the authors attribute to high available organic nitrogen of the bryophyte species. Given this important link between temperature, nitrogen availability and seedling establishment (Lett *et al.*, 2017) and the finding in Chapter 3 that ECM tree species have greater access to soil nitrogen sources than the ERM tundra species (Figure 3.3 & 3.5), mycorrhizal type might provide a further competitive advantage to tree species facilitating encroachment onto tundra heathlands. Furthermore, if CMNs facilitate C and nutrient exchange between dwarf birch shrubs (Deslippe & Simard, 2011), but not mountain birch trees (Chapter 4), then this may provide a mechanism through which dwarf birch shrubs can access larger soil N pools and thereby successfully expand into tundra heath areas.

More evidence of the presence and strength of CMNs in treeline forests, as well as the below-ground transfer of nutrients such as N and P between plants, is required in order to further the understanding of the population and community dynamics of trees, dwarf shrubs and ericaceous tundra plants in this ecotone and how they might respond to climate warming.

6.2 Planting trees onto ericaceous heathlands changes the dominant mycorrhizal type

Urgent action on climate change is required to limit global warming to 1.5°C (IPCC, 2018). A recurring theme in the action proposed by international governing bodies focusses on planting trees to sequester atmospheric CO_2 (UNEP, 2011; “New York Declaration of Forests,” 2014). The growing international momentum behind planting trees as a climate change mitigation strategy is in part driven by recent scientific publications which promote global forest restoration as the “best” and “most effective” climate solution (Bastin *et al.*, 2019; Lewis *et al.*, 2019). Bastin et al. (2019) identify large areas of the circum-polar north as having high potential for tree restoration, but these same areas include ericaceous heathlands (Raynolds *et al.*, 2019) with large soil C stocks vulnerable to being lost to the atmosphere if trees were to be planted on them (Hartley *et al.*, 2012; Karhu *et al.*, 2014; Parker *et al.*, 2015). Furthermore, planting trees in these areas would change the dominant mycorrhizal type from ERM to ECM, causing fundamental changes to C cycling in these ecosystems (Figure 6.1). It is therefore critical to understand the effects of planting trees onto soil C rich ericaceous heathlands for the whole ecosystem C cycle, both above and below-ground.

6.2.1 Planting trees in Scotland

Action on climate change at the national level is a requirement of the Paris Agreement, which requests each country to declare its Nationally Determined Contributions and post-2020 climate actions (Paris Agreement, 2015). The Scottish Government published its Climate Change Plan in February 2018 (Scottish

Government, 2018) outlining proposals and policies to mitigate climate change, and its impacts, between 2018-2032. In the section on land use change and forestry they propose to increase woodland creation targets from 10,000 ha $year^{-1}$ to 15,000 ha $year^{-1}$ by 2024 (Scottish Government, 2018) and in 2019 woodland creation targets were not only met but exceeded in Scotland (Scottish Forestry, 2019). These proposed mitigation steps focus and rely on the sequestration of CO_2 by the generation of tree biomass but do not consider storage of C in soils. In a paper commissioned to inform the Scottish Government's Woodland Expansion Advisory Group on which types of land in Scotland are best suited for planting, it was reported that 34% of Scotland's land area may have potential for woodland expansion (Sing *et al.*, 2013). Areas were deemed unsuitable for planting if they had peat deeper than 0.5 m (8% land area), but peat and peaty soils with <0.5 m depth, which may still have large C stores in the top 0.5 m, will have been considered potentially appropriate for planting.

In Chapter 5 I explored the effects of planting trees onto ericaceous heathlands in Scotland using a unique large scale planting experimental platform. I found that planting trees does not lead to net ecosystem C sequestration and in some cases causes to a net loss of C from organic soil to the atmosphere 12 and 39 years after tree planting (Figure 5.2). This result is of critical relevance to current policies, which promote tree planting based on the assumption that it will increase net ecosystem C storage.

Ericaceous heathlands are important soil C stores in Scotland (Lilly & Chapman, 2015) with ~292 t SOC ha^{-1} based on all four plots inventoried in Chapter 5. If this C density is applied to the 9.2% of *Calluna vulgaris* heathland land cover in Scotland (Rowland *et al.*, 2017), this equates to 212 Tg C stored in *C. vulgaris* heathland

organic soil. Assuming the same proportional loss of SOC as found in Chapter 5, (33.57%), planting birch trees on all of Scotland's *C. vulgaris* heathlands could result in ≈71 Tg of C lost from organic soil, not compensated for by above-ground biomass gains. Although this is a crude estimate, if linked back to the explicit consideration of climate change mitigation, this is equivalent to CO_2 emissions from more than 900,000 transatlantic flights (Glasgow-New York)("ICAO Carbon Emissions Calculator," 2016; DEFRA, 2019). These numbers are for Scotland alone, but I argue that the trends found here (Chapter 5) are relevant across northern circumpolar boreal and low Arctic ecosystems where soils with high C contents are being, or have the potential to become, colonized by trees due to climate change, leading to potential net ecosystem C loss.

My results clearly indicate the need for nuance in the global debate on tree planting. There is evidence from both sub-Arctic (Hartley *et al.*, 2012; Parker *et al.*, 2015) and boreal ecosystems (Chapter 5) that trees growing on ericaceous heathlands change the dominant mycorrhizal type and do not result in net ecosystem C sequestration; indeed in some cases colonisation by (or planting of) trees causes ecosystem C loss. Therefore, careful consideration must be given to the location of proposed tree planting, taking into account both biotic and abiotic factors, such as mycorrhizal status, existing soil C stocks and hydrology, in order for tree planting to be a robust climate change mitigation strategy.

6.2.2 Tree planting as a climate change mitigation strategy

For tree planting to become an effective climate solution leading to net sequestration of atmospheric CO_2 by forest ecosystems it must first be understood where tree

planting will lead to net ecosystem C sequestration and where it will lead to net ecosystem C loss. In Chapter 5 I found that planting trees onto ericaceous heathlands is not a robust climate change mitigation strategy, but tree planting may result in net ecosystem C sequestration in other ecosystems. In order to provide the best information to policy makers and foresters on where to plant trees for effective ecosystem C sequestration, data needs to be gathered from a wide range of ecosystems with varying soil types, existing vegetation and hydrology on the effects of planting trees on whole ecosystem C stocks. Such data gathering could lead to information of the effect of planting trees on ecosystem C sequestration along a continuum of soil C stocks (Figure 6.2).

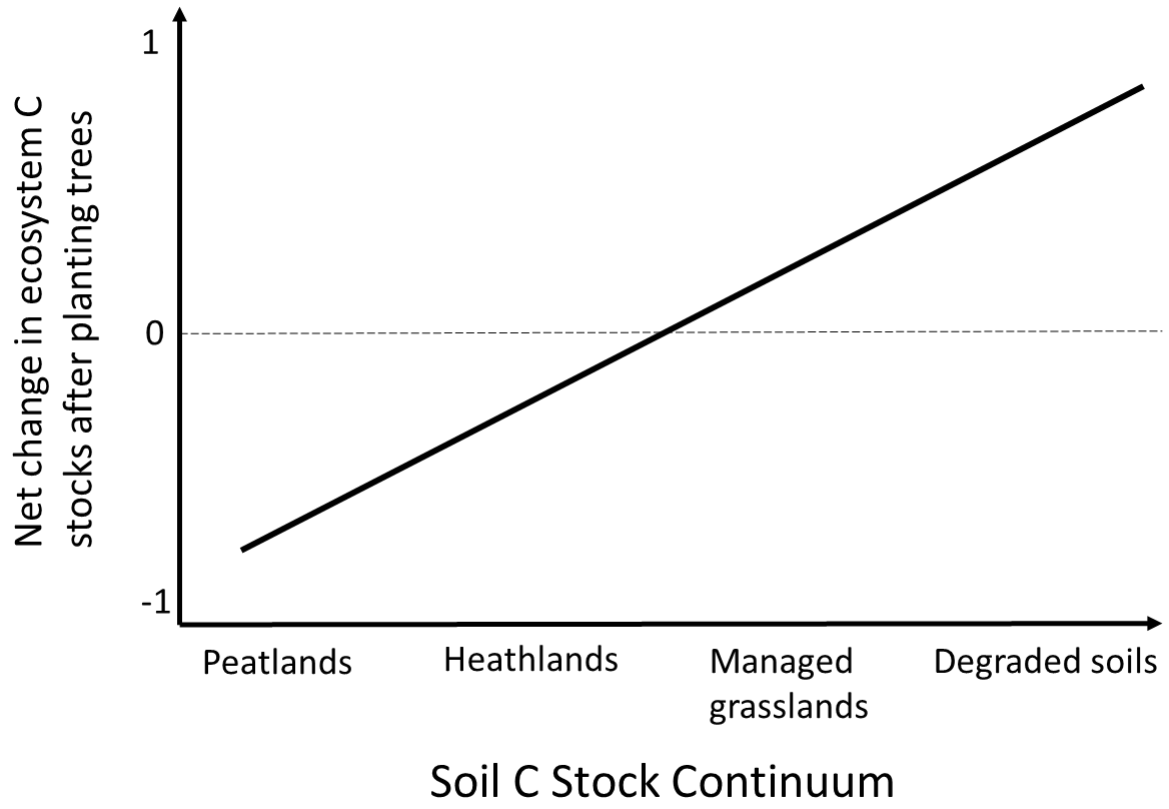

Figure 6.2. Theoretical net change in ecosystem C stocks after tree planting along a continuum of pre-existing soil C stocks. The units on the y-axis are relative.

Although tree planting is not an effective C sequestration strategy in ecosystems with large existing soil C stocks such as peatlands (Brække & Finér, 1991; Cannell *et al.*, 1993) and ericaceous heathlands (Hartley *et al.*, 2012; Parker *et al.*, 2015), it is likely that in ecosystems with lower existing soil C stocks planting trees may lead to net ecosystem C sequestration (Macedo *et al.*, 2008; Sang *et al.*, 2013). In ecosystems with lower soil quality and C stocks, the above-ground biomass gained by tree planting is more likely to outweigh any C lost from the soil and may even lead to an improvement of soil quality and a build-up of soil C stocks due to tree C inputs to the soil such as root exudates (Pausch & Kuzyakov, 2018), mycorrhizal necromass and leaf litter (Binkley, 2006; Cotrufo *et al.*, 2013).

Understanding the effect of planting trees in a wide range of ecosystems could be achieved through large scale tree planting experiments, which could be well replicated and controlled but would require large funds and take many years to yield meaningful results. Alternatively, this could be achieved through space-for-time substitutions, which, although less well controlled, may be more cost effective and yield results on a much more realistic time scale. Once spatially and temporally replicated data on the effects of tree planting in various ecosystems are obtained, the next steps would be to develop a framework for policy makers and foresters advising which ecosystems are best suited for tree planting for ecosystem C sequestration and which are not suitable for tree planting for ecosystem C sequestration. Although there are databases for land use and C stocks that enable such analyses (Tóth *et al.*, 2013) which have been effectively used to determine European-wide forest and grassland topsoil carbon stocks (Cotrufo *et al.*, 2019), more data is needed on the whole ecosystem C stock and how this changes, specifically after tree planting.

Trees contribute to C sequestration in above-ground biomass, however their interactions with soil C cycling and turnover must be better characterised in order to understand the effects of planting trees on whole ecosystem C stocks. The results presented here show the need for nuance in the global tree planting debate and that if terrestrial ecosystems are to be successfully managed for C capture – this does not always mean planting trees.

6.3 Plant–soil interactions driving soil C dynamics

Plant–soil interactions are strong drivers in shaping ecosystem function and responses to global change (Bardgett & Wardle, 2010; van der Putten *et al.*, 2016). Plant–soil interactions affect plant community dynamics (Bennett *et al.*, 2017) as well as ecosystem C and nutrient cycling (Guo & Gifford, 2002; Ehrenfeld *et al.*, 2005; Lee *et al.*, 2012). In the work presented here, I have examined various ways in which plants interact with soil via mycorrhizal fungi and how these plant-fungi-soil interactions drive soil C dynamics.

6.3.1 Ericaceous shrubs as drivers of SOM formation

The large stocks of soil C found in the Arctic and boreal regions (Köchy *et al.*, 2015) are linked with low vegetation C stocks (Crowther *et al.*, 2019). The ratio of soil:vegetation C in these regions is therefore strongly weighted towards soil C. Large areas of the circumpolar north are covered by ericaceous tundra heathlands (Raynolds *et al.*, 2019) associated with low stature shrubs, low stocks of C above-ground and large stocks of soil C below-ground. Ericaceous shrubs have been

identified as playing a key role in driving the long-term build-up of SOM through several pathways. Leaf litter from ericaceous shrubs is recalcitrant and takes a long time to decompose (Parker *et al.*, 2018), thereby contributing to SOM formation from the surface. ERMs that associate with ericaceous shrubs have melanised hyphae, the necromass of which is resistant to decomposition which contributes to the long term build-up of SOM (Clemmensen *et al.*, 2015) mediated by below-ground C inputs. Further contributing to the stabilisation of these below-ground C inputs is the complex chemical nature of ericaceous shrub roots and their ability to promote tannin complexes which stabilises fungal necromass and soil C (Adamczyk *et al.*, 2016, 2019). The identification of ericaceous shrubs and their associated mycorrhizas as drivers of SOM formation emphasises the importance of ericaceous heathland ecosystems and the predominantly ericaceous shrub understorey in boreal forests as playing a key role in ecosystem C sequestration globally.

The next steps in understanding the important role of ericaceous shrubs as drivers of SOM formation will be investigating which of the different pools of SOM are influenced by ericaceous shrub C inputs and the underlying mechanisms. The need to understand this comes from recent important advances in the field of SOM research which has identified that soil C stabilises into two main pools of SOM; i.e. particulate organic matter (POM) and mineral associated organic matter (MAOM) that are fundamentally different in terms of their formation, persistence, and functioning (Cotrufo *et al.*, 2013, 2015, 2019; Lavallee *et al.*, 2019). This paradigm shifting work has challenged the traditional view of recalcitrance through the finding that labile organic matter can be stabilised into the soil matrix as microbial products bound in mineral soil, and is an important pathway of SOM formation (Cotrufo *et al.*, 2013). It is therefore important that soil science disciplines, particularly those

centring around plant-soil interactions, adopt these newly proposed models and pathways of SOM formation. In a recent paper Lavallee et al. (2019) propose a framework that will allow soil scientists to move beyond simply studying bulk SOM, but to separate SOM into the POM and MAOM fractions. Lavallee et al. (2019) suggest that this new framework is the best way to understand and predict broad-scale SOM dynamics in the context of global change challenges and to provide the necessary recommendations to land managers and policy makers.

6.3.2 Ecosystem effects of changes to the dominant mycorrhizal type

If ericaceous shrubs and their associated mycorrhizal fungi drive stable SOM formation these ecosystems become highly important in terrestrial C sequestration for climate change mitigation. Based on my results, I argue that tree encroachment or planting onto ericaceous heathlands will alter the dominant mycorrhizal type from ERM to ECM (Figure 6.1). This changes the ecosystem functioning from a low energy input, slow soil C turnover system to a much higher energy system with faster soil C turnover with consequences for ecosystem C stocks (Read & Perez-Moreno, 2003; Clemmensen *et al.*, 2015). By investigating the effects of the forest mycorrhizosphere, through sub-Arctic treeline advance and planting of trees onto ericaceous heathlands, I have found that changing the dominant mycorrhizal type from ERM to ECM leads to a loss of soil C. Furthermore, I find that the spatial patterns of this soil C loss are driven by individual trees and their associated mycorrhizosphere networks within treeline forests. These findings are highly relevant in a changing world, and highlight the importance of whole ecosystem approaches, both above and below-ground, when devising solutions to mitigate and combat these growing environmental challenges.

Chapter 7: Appendices

Appendices for Chapter 2

Table 7.1 Mean (x̄) physical metrics of the 5 nearest neighbouring trees and their distance to the main studied trees (x̄ ± SD).

Block	Tree	x̄ Distance from main tree (cm)	x̄ Number of stems	x̄ Diameter at 50 cm (cm)
1	1	700.0±120.7	4.0±1.9	5.18±2.53
	2	520.0±109.5	3.8±3.6	6.68±3.57
	3	366.0±163.6	5.2±1.9	6.87±1.52
2	1	460.0±183.9	1.4±0.5	5.85±4.06
	2	579.4±263.3	1.6±1.3	4.64±1.20
	3	552.0±130.4	4.2±4.9	5.79±2.93
3	1	252.6±140.3	1.4±0.9	5.50±4.21
	2	514.8±121.6	1.4±0.9	8.15±6.11
	3	398.2±76.5	3.4±4.8	5.09±2.18
	Total x̄	482.6±187.4	2.9±2.9	5.98±3.29

Table 7.2 Physical metrics of studied trees. Total crown width is an average of the distance from the outer most crown foliage in N-S and E-W orientations. Half crown width is a measure of the crown from the tree stem to the outer most crown foliage.

Block	Tree	Number of Stems	Total diameter at 50 cm (cm)	Total Crown Width (cm)	Half Crown Width (cm)
1	1	1	7.64	215.0	107.5
	2	15	99.31	445.0	222.5
	3	4	8.28	405.0	202.5
2	1	4	6.37	285.0	142.5
	2	3	8.59	330.0	165.0
	3	4	6.45	322.5	161.3
3	1	1	11.78	285.0	142.5
	2	1	9.39	390.0	195.0
	3	3	7.64	327.5	163.8
	Mean	**4**	**18.38**	**333.9**	**166.9**
	SD	**4.33**	**30.39**	**70.4**	**35.2**

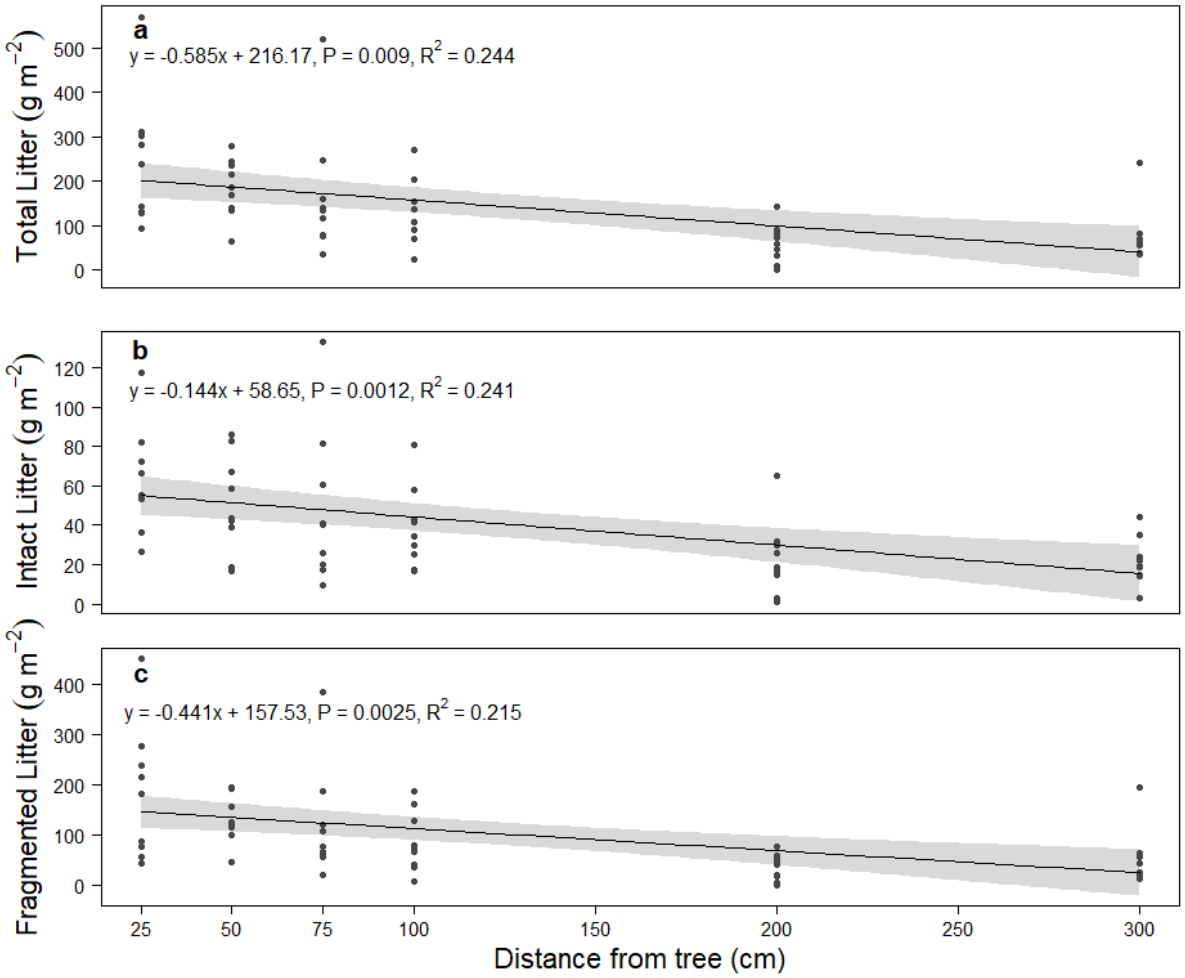

Figure 7.1 Leaf litter collected along transect extending 3 m away from individual birch trees. Total (a) collected litter was split into intact (b) and fragmented (c) litter. Solid line is predicted litter mass based on a linear mixed effects model. Grey polygon indicates 97.5% confidence interval of the predicted line. All three measures decrease significantly with increasing distance away from the tree.

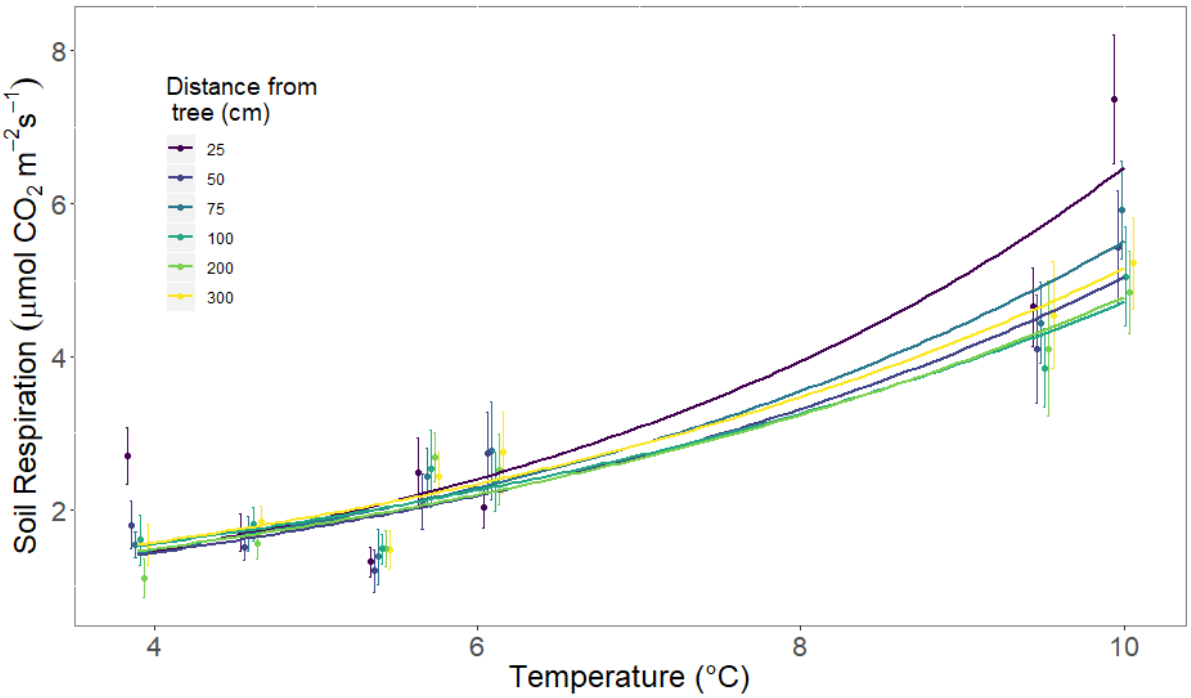

Figure 7.2 Soil respiration temperature response curves calculated for respective distances from tree bases. Points are temperature per measurement day with mean soil respiration and standard deviation error bars; lines are exponential regressions following SR = Rate0 $Q_{10}^{(T/10)}$, as defined in Methods of Chapter 2.

Appendices for Chapter 5

Baseline soil data

Soil carbon stocks were assessed prior to tree planting at the four sites of this study. Protocols used differed beteen the sampling at sites planted between 1977 and 1981 (Craggan, Delnalyne and Kerrow), the site at Ballogie (planted in 2005), and the soil sampling carried out in 2018/2019. Whilst this means that no direct comparison of C stocks between all sites is possible (e.g. between stocks in Figure 5.2 and Table 7.3 below), we present here the findings of relevat parameters to clarify that underlying soil conditions did not differ between treatents at the time of tree planting. Planting

treatments and controls were allocated randomly following baseline measurement, and following a blocked design.

Soil sampling protocols for baseline soil C stocks

Sites Craggan, Delnalyne and Kerrow (planted 1977 – 1981): O_l and O_f horizons were removed, and soil cores extracted to a constant depth of 15 cm, including O_h as well as mineral soil material. The depth of organic horizon (O_h) was not recorded. Bulk density and soil C content determined for the entire soil core.

Ballogie (planted 2005): O_l and O_f horizons were removed, and soil cores extracted to a constant depth of 5 cm, including O_h as well as mineral soil material. The depth of organic horizon (O_h) was recorded, and bulk density and soil C content determined for the entire soil core.

Table 7.3. Baseline soil parameters measured prior to tree planting. Mean ± 1 standard deviation are presented. P-values refer to pairwise combinations within sites.

Site Year	Treatment	% C	Bulk density (g cm^3)	Depth of Organic horizon (cm)	P-values
Ballogie	Heather control	29.5±4.9	0.37±0.06	3.1±0.7	%C: >0.1, BD: >0.1,
2005	Planted Birch	27.1±4.7	0.38±0.04	3.9±2.3	OD: >0.1
	Planted Pine	24.0±7.9	0.40±0.10	3.4±0.8	
Craggan	Heather control	38.6±14.4	0.27±0.13		%C: 0.79, BD: 0.81
1978	Planted Birch	37.3±9.0	0.26±0.05		
Delnalyne	Heather control	25.1±8.7	0.44±0.13		%C: 0.11, BD: 0.07
1977	Planted Birch	18.6±8.0	0.55±0.16		
Kerrow	Heather control	9.3±2.8	0.40±0.14		%C: 0.46, BD: 0.24
1981	Planted Birch	10.4±3.7	0.47±0.09		

Table 7.4. Allometric equations used to calculate tree biomass (kg). At Ballogie diameter at 10% height was used instead of DBH due to the large variation in tree height with many shorter than 1.3 m (standard 'breast height').

Species	Tree component	Equation	Authors
Birch	Crown	$log(Biomass) = -3.4 + 10.3 * \left(\frac{DBH}{DBH + 10}\right)$	(Marklund, L., 1988)
Birch	Stem	$Biomass = -1.5 + 0.92 * log(DBH^2 * H)$	(Mälkönen, 1977)
Pine	Total tree (Above-ground)	$log(Biomass) = 0.98 * \left(2.29 * (log(\pi * DBH))\right)$	(Lim & Cousens, 1986)

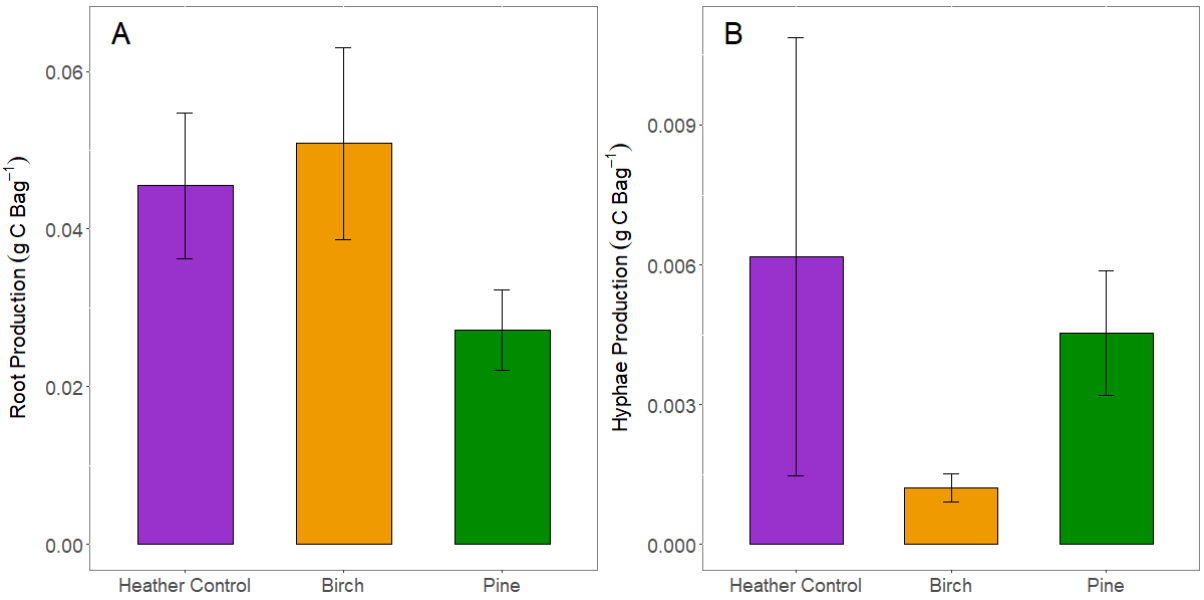

Figure 7.3. Root (A) and hyphal (B) production in one growing season in planted birch and pine plots as well as in un-planted heather control plots, at Ballogie. Error-bars are standard error. There were no statistically significant comparisons (P > 0.05) in the amount of roots or mycorrhizal hyphae produced in the heather control plots, planted birch and planted pine over one growing season.

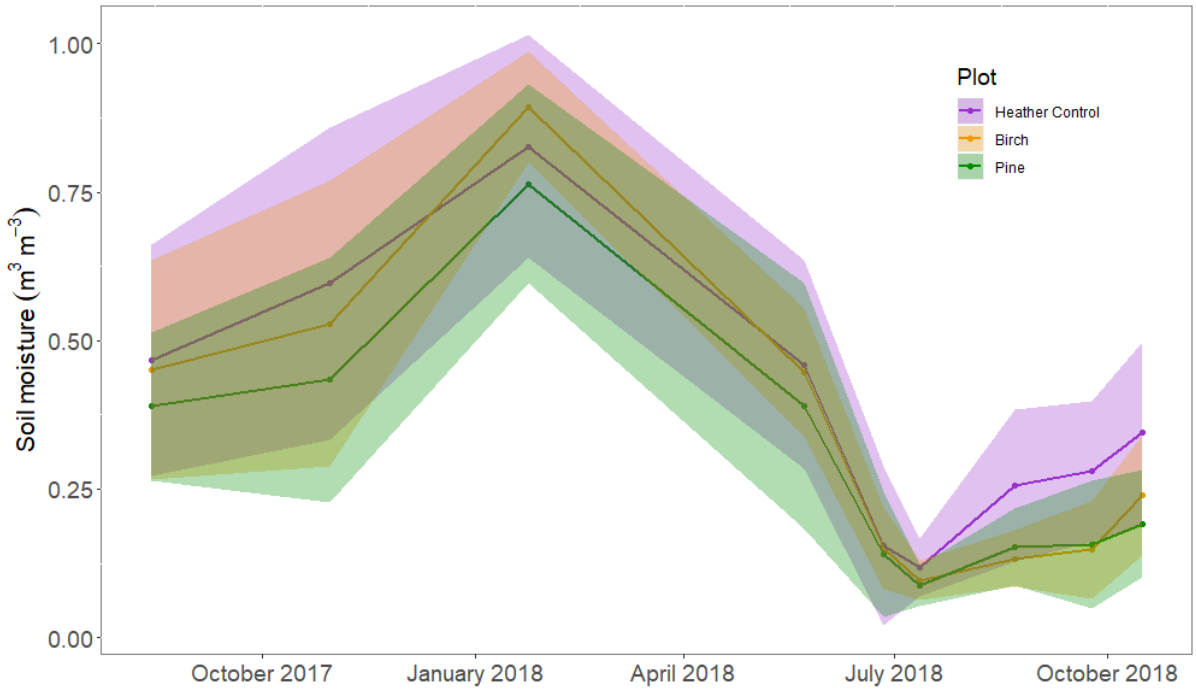

Figure 7.4. Mean (points) soil moisture at 5 cm depth in planted birch and pine plots as well as in un-planted heather control plots, at Ballogie, over time. There were no significant differences in soil moisture between plots over the measurement period ($P > 0.05$). Error-ribbon is ±1 standard deviation of the mean.

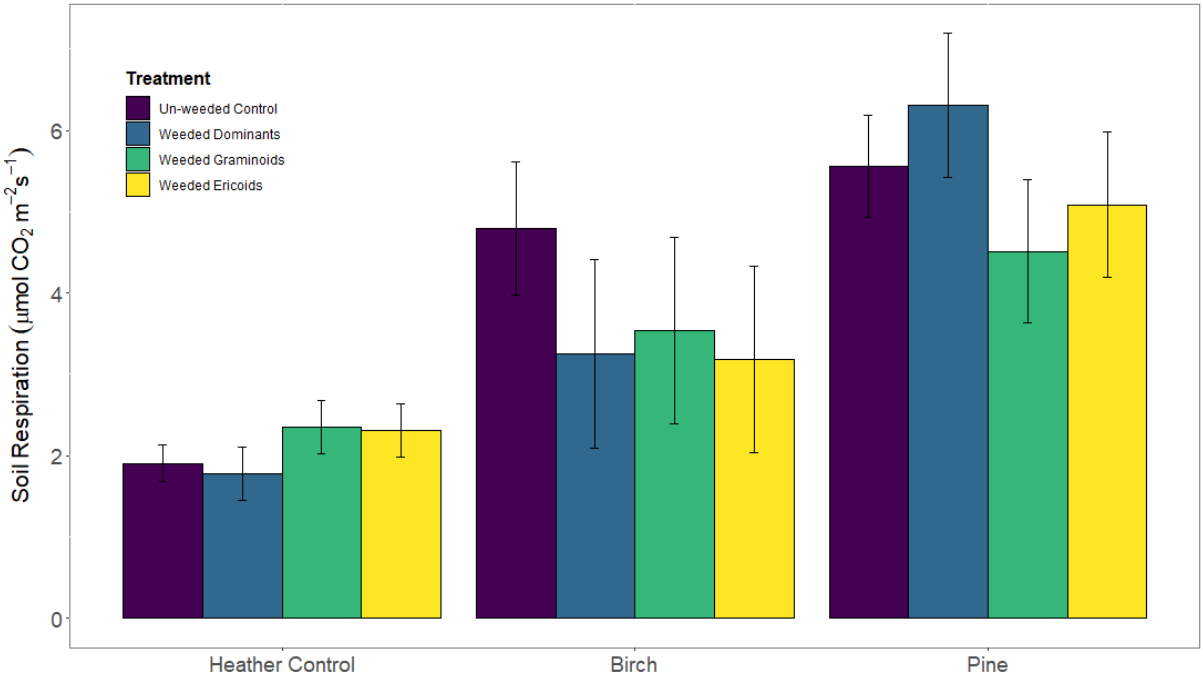

Figure 7.5. Mean Soil respiration from weeded sub-plots and un-weeded controls in planted Pine and Birch plots as well as in un-planted heather control plots, at Ballogie, May-October 2017. Weeding treatments include removal of future dominant understorey species (WD), graminoid (WM) or ericoid species (WR). Output from linear mixed effects models with block and plot as random effects. Error-bars are standard error of the mean.

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