Ecological Controls of Rhizosphere Processes and Soil Organic Matter Dynamics at a Sub-arctic Treeline

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Thesis abstract

Rapid climate change in the Arctic and Sub-Arctic is causing vegetation change across large areas of tundra. Shrubs and trees are undergoing range expansions as part of an over-all trend of 'greening' of the tundra. This is of importance because northern peatlands contain around half of total soil carbon (C) and there is a potential for productive vegetation to interact with this C in a number of ways: (1) Ectomycorrhizal fungi (ECM) in symbiosis with trees and shrubs could potentially stimulate decomposition through extracellular enzyme production whilst extracting nitrogen (N) for their hosts; (2) deep snow, trapped by tall vegetation insulates the soil, resulting in higher winter-time microbial activity and has potential to influence growing season microbial activity; (3) the biochemistry of litter and decomposition environment associated with more productive vegetation could result in accelerated mass loss of litter and stimulate decomposition of older soil C.

This thesis investigates how productive sub-arctic plant species in Northern Sweden interact with soil C by using 'space-for-time' transitions from forests (*Betula pubescens*), through intermediate shrub vegetation (*Betula, Salix*), to tundra heath (*Empetrum nigrum*). This was to test how ECM fungi, winter snow accumulation, defoliation events and litter input influence C cycling. C stocks, respiration rates and ECM growth rates were measured across these ecotones. It was found that birch forests and shrub stands had significantly lower soil C storage and higher respiration rates than adjacent heaths. This is contrary to the predictions of earth system models. Higher ECM growth rates at plots with low C storage and high

cycling rates implied that they had an important role in the stimulation of C decomposition.

To test whether snow cover in forests over winter had an important effect on C cycling, soils were transplanted between forest and heath (different snow cover), and respiration rates were measured over summer. It was found that deep snow cover over winter increases microbial activity in summer due to a warmer, more stable winter environment; this is hypothesised to be due to the environmental selection of a more active assemblage of decomposing microbes. A defoliation event of part of the birch forest by caterpillars allowed for a natural 'experiment'. Trees with different degrees of defoliation were compared in their influence over soil C cycling processes. Defoliated plots shifted to slower-cycling states through a shift in the ECM community. This further implied that ECM fungi have an important role to play in rapid cycling of C in forests. A decomposition experiment using the litter of significant plant species in forest, shrub and heath communities was carried out by transplanting them between these key environments. This work showed that rapid decomposition of litter in the forest is driven by an interaction between carbohydrate-rich litter input and an effective decomposer community. This work addresses the relationship between vegetation productivity and C storage in the soil. This theme runs through every experiment as they test specific interactions between different plant groups and the soil. The results from this thesis suggest that increasing productivity and shrub expansion in the Arctic will stimulate decomposition of soil C via a number of pathways. Plant-soil interactions are clearly of importance in determining the fate of C in ecosystems and will play a key part in the balance of C in the future.

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Chapter 1: Introduction

1.1. Climate change in the Arctic

Greenhouse gases (GHGs) warm the atmosphere because of their capacity to absorb and reemit infrared wavelengths of energy, causing a 'greenhouse effect' (IPCC, 2013). The most important of these gases are water vapour (H_2O), carbon dioxide (CO_2) and methane (CH_4). CO_2 concentrations in the atmosphere are increasing due to anthropogenic combustion of fossil fuel reserves and land use change (IPCC, 2013). As a result, the climate is warming. Natural fluxes of carbon (C) from ecosystems (photosynthesis and respiration in biological systems) are over 18 times larger than the production of CO_2 from anthropogenic sources. However, increasing CO_2 concentrations since the industrial revolution in 1750 and associated warming may interact with the 'natural' carbon cycle and causing feedbacks and further warming (IPCC, 2013).

Over the last century the global climate has warmed by, on average, 0.78 °C (IPCC, 2013). This change in temperature has been forced primarily by greenhouse gas emissions from anthropogenic sources with little or no influence from 'natural' forcings (IPCC, 2013). If the level of human activity and release of GHGs continues at the current rate, there could be an overall planetary warming of up to 4 °C by 2100 (IPCC, 2013). Northern high latitudes (above 60 °N) have warmed by between 1-4 °C in the last 50 years, proceeding at a rate that is far higher than the planetary mean (ACIA, 2005; Serreze & Barry, 2011). By the end of the 21st century, if GHG emissions follow their current trajectory the Arctic may warm by up to 11 °C (IPCC, 2013). The disproportionate increase in temperature recorded in

northern latitudes compared to the global mean is known as 'Arctic Amplification' (Serreze & Francis, 2006; Serreze *et al.*, 2009; Serreze & Barry, 2011; Cohen *et al.*, 2014). Arctic amplification is driven by a number of interacting physical processes relating to energy fluxes and weather patterns, but primarily by sea ice loss (Serreze & Barry, 2011). Loss of sea ice, due to climate warming, decreases albedo and results in increased solar radiation absorption into the ocean then energy transfer back to the atmosphere (Serreze *et al.*, 2009). This leads to negative feedbacks with ever thinner layers of seasonal sea ice which take less energy to melt; leading to a rapid decrease in sea ice cover in the last 50 years (Stroeve *et al.*, 2007). If the current trend holds, summer sea ice over the Arctic Ocean could be completely lost by 2050, therefore driving the highest regional temperature increases anywhere on Earth (IPCC, 2013).

1.2. The response of the arctic carbon cycle to climate change

The global terrestrial pool of carbon is estimated to store between 1500 and 2400 Pg C (Schimel, 1995; IPCC, 2013), of which approximately 1580 Pg is stored in the soil; around 2.5 times more than that stored in vegetation (610 Pg C) (Schimel, 1995). There is considerably less confidence in estimates of the terrestrial C pool which has shown more variation it its capacity to sequester C than the oceanic pool (Cox *et al.*, 2000; Le Quere *et al.*, 2009). Feedbacks are predicted to occur with future warming as contrasting components (broadly, photosynthesis and respiration) of the terrestrial C cycle respond differentially both to warming and to increases in atmospheric CO_2 concentrations (Cramer *et al.*, 2001). There remain critical uncertainties in the magnitude, and even sign, of these feedbacks due to limitations

in our understanding of key plant and soil processes (Cox *et al.*, 2000; Todd-Brown *et al.*, 2013; Zhang *et al.*, 2014).

From a terrestrial biological perspective, the Arctic has been defined as areas poleward of the arctic treeline, where tundra vegetation dominates (Hustich, 1979; Walker, 2000; Callaghan *et al.*, 2002). It has been estimated to hold up to half of the total soil organic C on Earth, making it the most important store of soil carbon of any biome (Tarnocai *et al.*, 2009). The majority of this carbon is locked in permafrost (continually frozen ground) (Tarnocai *et al.*, 2009) which hold approximately twice the amount of C present in the atmosphere (Schuur *et al.*, 2013). Peatlands have a pan-arctic coverage (CAFF 2010) and despite their small global coverage, store between 15-30 % the world's soil carbon (Limpens *et al*. 2008). Factors such as slowly-decomposing litter input, low annual temperatures, prevalence of anoxic conditions, and low fire frequency, have, over geological timescales, made northern peatlands a strong sink of C (Hobbie *et al.*, 2000; McGuire *et al.*, 2009), despite very low net primary productivity (NPP) (Cao & Woodward, 1998).

As temperature increases over the Arctic at rates which far surpass the global mean (Serreze & Barry, 2011), models suggest that increases in respiration and disturbance by fire may be weakening the C sink-strength of the system (Hayes *et al.*, 2011). This corroborates with observations which have large variation in estimates of sink strength of arctic tundra, ranging from strong to very weak (Cahoon *et al.*, 2012; McGuire *et al.*, 2012) and predictions that respiration may increase exponentially with temperature increase (Davidson *et al.*, 2006). In

addition to this, C stored in permafrost is potentially vulnerable to climate warming, with possible feedbacks to the climate system through the release of biogenic C to the atmosphere (Schuur *et al.*, 2008). Experimental warming of peatland underlain with permafrost showed that ecosystem respiration (ER) rates increased persistently over an eight year period and a significant proportion of the respired C was derived from old C stores (Dorrepaal *et al.*, 2009). The response of ER to temperature increases has been shown to be due to microbial communities in tundra soils with high soil C storage that are exceptionally responsive to temperature increase, more so than more southerly ecosystems (Karhu *et al.*, 2014). Due to their storage of the largest pool of soil carbon globally (Tarnocai *et al.*, 2009), research has focussed on responses of arctic soils to climate warming; but there is considerable uncertainty as to whether increases in ER will shift their sink-source status.

1.3. Changes in productivity

Since satellites have been able to measure vegetation greenness from space there has been an abundance of data and studies showing that arctic terrestrial vegetation has increased in productivity. The first to measure normalised difference vegetation index (NDVI) were Myneni *et al.* (1997) who observed large increases in productivity (up to 50% increase) between 1981-1991 up to 70 °N which they linked to lengthening of the growing season. In recent decades this has been snow-free seasons have lengthened on average at a rate up to 6.3 days per decade, with this pattern driven by regional climate warming (Zeng *et al.*, 2011). This process is thought to be linked to sea ice decline (Bhatt *et al.*, 2010) and therefore part of the feedbacks related to 'polar amplification' (Serreze & Barry, 2011). This pattern was further observed over Alaska up to 2001 and strong correlation found between

NDVI and above-ground biomass in a 'ground-truthing' study (Jia *et al.*, 2003). These finer scale measurements were made across Canada and Alaska to re-affirm the trend across the arctic tundra (Goetz *et al.*, 2005; Verbyla, 2008). NDVI studies in Siberia mirrored what was observed in the North American tundra and that greening was driven by warming of the climate (Forbes *et al.*, 2010; Blok, Daan *et al.*, 2011). However, other work highlighted human activity through reindeer herding and development as other important factors, more so than climate change in some areas (Walker *et al.*, 2009). Latest multi-sensor studies up to 2008 have shown the greening trend in large areas across the Arctic (Guay *et al.*, 2014) and also further established a link between sea-ice decline, warming temperatures and productivity increase (Bhatt *et al.*, 2010; Fraser *et al.*, 2014).

Recent work has confirmed that the increases in productivity in the Arctic observed from space have resulted in sequestration of C in plant biomass with a 20 % increase in productivity equating to a 0.4 Pg C increase in biomass in 30 years (See Figure 1.1(Epstein *et al.*, 2012)). This increase in biomass was most pronounced in lower latitudes of the tundra, in ecosystems where graminoids, dwarf shrubs and herbs dominate, down to the sourtherly limit of the tundra, where erect shrubs in genera such as *Betula*, *Salix* and *Alnus* constitute the majority of vascular vegetation and trees encroach in riparian areas (for detailed definitions of tundra climate zones, see Walker *et al*. (2002)). These areas with the warmest summer temperatures and highest standing biomass have undergone increases in plant biomass of up to 25 % (Epstein *et al.*, 2012). In contrast, polar deserts and areas of the High Arctic have only undergone modest increase in biomass (of 2-6 %) (Epstein *et al.*, 2012). Long-term studies on the ground have supported the notion that the Arctic is becoming

more productive (Elmendorf *et al.*, 2012b) and that climate warming is responsible for these changes.

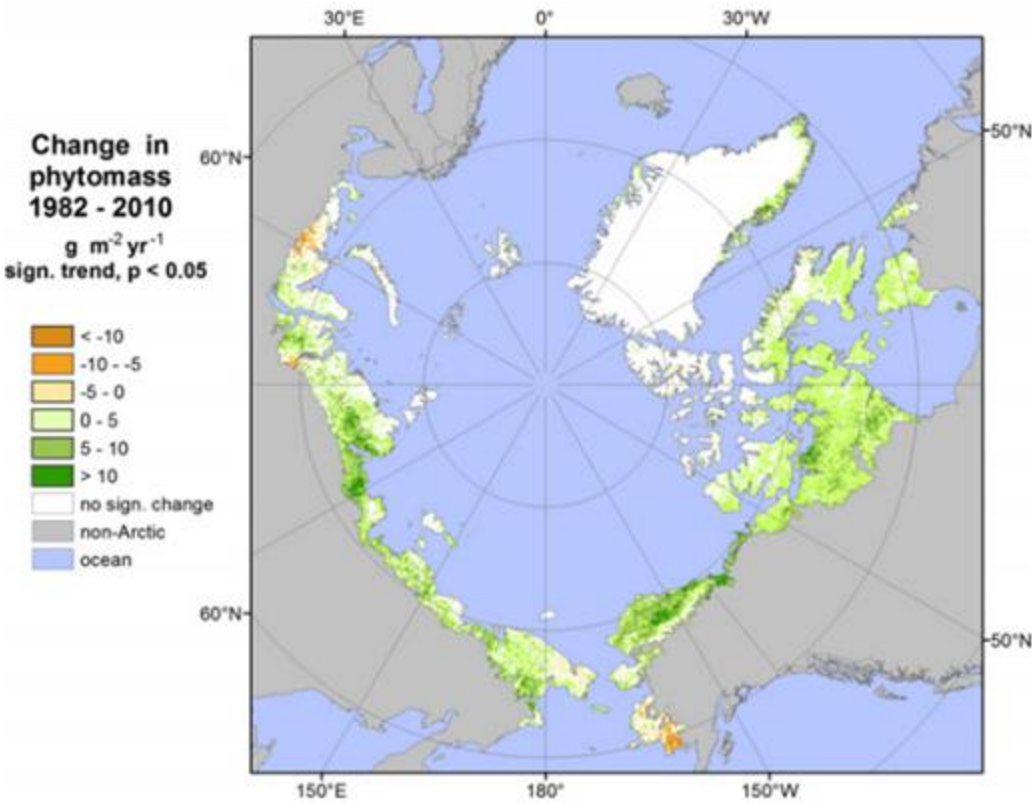

Figure 1.1: Changes in aboveground phytomass (calculated from changes in NDVI) in tundra ecosystems above the treeline. Taken from Epstein *et al.* (2012).

Earth system models (ESMs) have predicted that increases in atmospheric CO_2 will out-weight increases in temperature-related increases in respiration and stimulate increases in C sequestration in arctic ecosystems over the next 100 years (Cramer *et al.*, 2001; Qian *et al.*, 2010; Todd-Brown *et al.*, 2013). This predicted increase in C storage is driven by increases in NPP (Epstein *et al.*, 2012) and therefore an increase in litter-fall and below-ground biomass production. ESMs therefore predict that climate change will increase the sink capacity of the Arctic (Cramer *et al.*, 2001; Qian *et al.*, 2010; Todd-Brown *et al.*, 2013).

1.4. Change in productivity in boreal regions

In sub-arctic and boreal ecosystems, the opposite of the greening phenomenon has been occurring over a similar timeframe; where forests have experienced reductions in productivity (Goetz *et al.*, 2005; Verbyla, 2008; Koven, 2013; Bjerke *et al.*, 2014). Climate warming in northern forests (ACIA, 2005) has increased drought stress of trees which, along-side pest outbreaks, has resulted in reduction in productivity across North-American boreal forests (Goetz *et al.*, 2005; Verbyla, 2008). At the same time, climate warming in Northern Scandinavia has increased the range and severity of insect outbreaks (Jepsen *et al.*, 2008) to cause large decreases in NPP in some forests (Goetz *et al.*, 2005; Bjerke *et al.*, 2014). In sub-arctic regions, where greening of tundra (Rundqvist *et al.*, 2011) and browning of forest (Bjerke *et al.*, 2014) is occurring, it is important to consider both effects for the future carbon balance for the ecosystem.

1.5. Shrub expansion in the Arctic

1.5.1. Observations

Shrub expansion is considered an important component of the widely-observed increases in NDVI (Tape *et al.*, 2006). Increases in temperatures and a longer growing season (Zeng *et al.*, 2011) have not only increased the productivity of species already present but also allowed the expansion of more productive species into what was previously low canopy tundra (for an example, see Fig. 1.2(Myers-Smith *et al.*, 2011a; Myers-Smith *et al.*, 2011b)). This was first documented in riparian zones in Alaska by Sturm *et al.* (2001b) and then more extensively by Tape *et al.* (2006), comparing contemporary photos of shrub stands with photos of the same sites around 50 years later. As well as Alaska, shrubs have been observed to be expanding in Siberia (Forbes *et al.*, 2010; Frost & Epstein, 2014), North-West Territories of Canada (Fraser *et al.*, 2014), Quebec (Ropars & Boudreau, 2012) and sub-arctic Sweden (Rundqvist *et al.*, 2011). The connection between temperature increases and growth and expansion of shrubs has further been shown using dendroecology and analysis of age structure of shrub stands (Forbes *et al.*, 2010; Hallinger *et al.*, 2010; Blok *et al.*, 2011). Further, it was found that arctic dwarf shrubs show 'bursts' of recruitment on Greenland after warm periods of climate (Büntgen *et al.*, 2014). Genera that are most commonly observed to be responding to warming and lengthening growing season by expanding into less productive systems are deciduous shrubs *Betula, Salix, Populus* and *Alnus* (Myers-Smith *et al.*,

2011a). ESMs calculate that by 2050, woody cover may increase by up to 52 % along with other widespread vegetation changes (Pearson *et al.*, 2013).

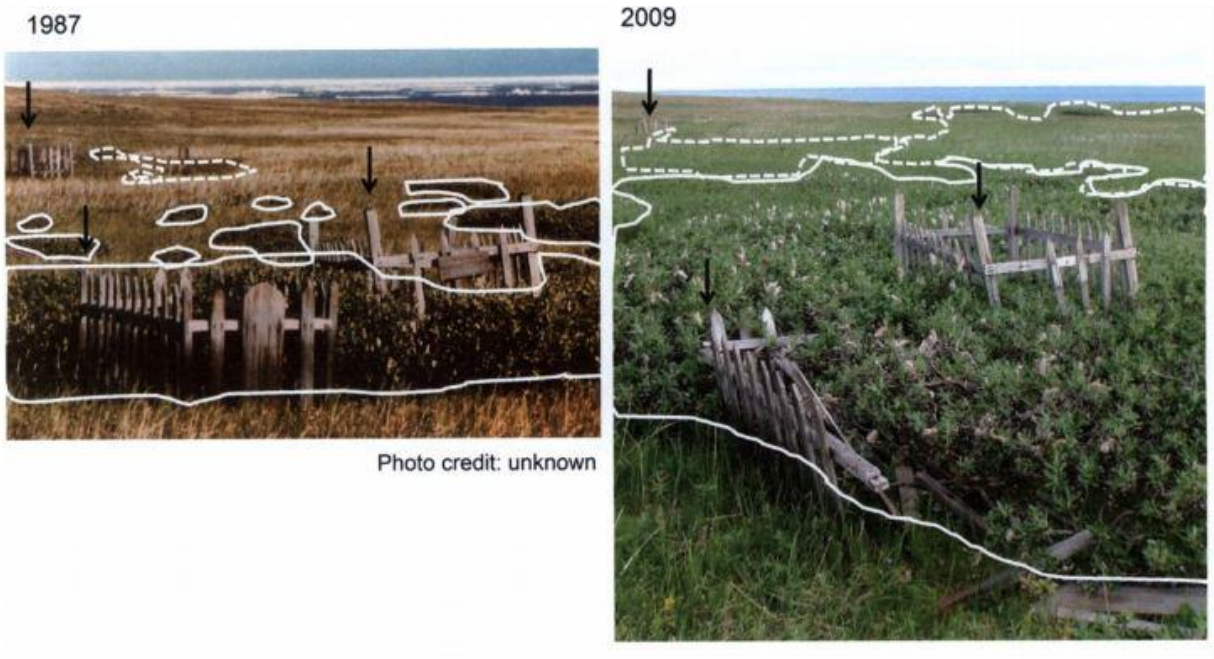

Figure 1.2: Repeat photography from 1987 and 2009 showing expansion of shrub patches *Salix richardsonii* on Herschel Island, Canada, from Myer-Smith *et al.* (2011b),

1.5.2. Warming experiments

Passive warming treatments in the International Tundra Experiment (ITEX), using open top chambers (OTCs), have been particularly informative in terms of explaining observed deciduous shrub increase in the Arctic and predicting how vegetation communities will further change in the future (Walker *et al.*, 2006; Elmendorf *et al.*, 2012a). They warm air temperatures by 1-3°C in arctic, sub-arctic and alpine tundra systems across the world (Henry & Molau, 1997); i.e. temperature increases which have already been observed in the Arctic (Chapin *et al.*, 2005; Serreze & Barry, 2011) and are predicted in the near future (IPCC, 2013).

The ITEX project comprehensively demonstrates that when tundra is heated, even over short time scales (Arft *et al.*, 1999) the plant community becomes more productive (Walker *et al.*, 2006; Elmendorf *et al.*, 2012a). The longer the duration of the ITEX experiment and the higher the temperature increase, the more pronounced the changes to the plant community. More productive shrubs, graminoids, and forbs increased in cover and canopy height at the expense of mosses and lichens (Walker *et al.*, 2006; Elmendorf *et al.*, 2012a). Earlier bud burst was the most obvious effect, allowing photosynthesis to occur over a longer growing season (Arft *et al.*, 1999), consistent with observed changes across northern latitudes (Myneni *et al.*, 1997).

One of the key findings of ITEX is that woody biomass and canopy height increase in response to warming, especially in deciduous shrubs (Walker *et al.*, 2006; Elmendorf *et al.*, 2012a). This draws obvious parallels with observations of shrub expansion across the Arctic and adds strength to the argument that temperature increases are important in this process (Myers-Smith *et al.*, 2011a).

1.5.3. Positive feedbacks and shrub expansion

Ecosystem feedbacks to shrub expansion may play an important part in further facilitating their growth in a positive feedback mechanism (Sturm *et al.*, 2001a; Sturm *et al.*, 2005; Wookey *et al.*, 2009). Deep snowpacks of up to one meter high may build -up on the leeward side of a small stand of shrubs and thus protect the shoots of younger individuals (Sturm *et al.*, 2001a; Sturm *et al.*, 2005). In addition to this, soil under snow-packs are insulated from the air temperature in winter and dip no lower than -10°C compared with soil under a thin snow pack which closely follows ambient air temperature, which can dip to -40°C (Sturm *et al.* 2001b; Sturm

et al. 2005). Experimental manipulation of snow depth has shown that deep snow allows continued microbial activity and, as a result, nitrogen (N) mineralisation increases (Schimel *et al.*, 2004). This provides a positive feedback, whereby the snow beds provide more bioavailable N for further growth and expansion of productive shrubs (Sturm *et al.*, 2001a).

Equally important as an ecosystem feedback to shrub expansion is the quality of litter input. Higher quality (low C:N) of litter input from *Betula glandulosa* increased pools of N, and had higher N turnover rates in the organic layer, than adjacent birch hummock tundra (Buckeridge *et al.*, 2010). It is therefore likely that productive shrubs facilitate their own expansion through summer and winter processes which increase N availability and turnover rates and increase productivity in an otherwise N-limited system (McKane *et al.*, 2002).

1.5.4. Geographical extent of shrub expansion

Although there is a general consensus that across much of the Arctic shrubs are increasing their range and becoming more productive (Myers-Smith *et al.*, 2011a), there are important exceptions to this. It has been observed over the last three decades that, high arctic tundra has undergone only modest increases in productivity (2-6 %), whereas large increases have been observed in warmer, lower latitude areas (Epstein *et al.*, 2012). This has been supported by a number of field studies in Greenland (Daniëls & de Molenaar, 2011; Daniëls *et al.*, 2011), Svalbard (Prach *et al.*, 2010) and Disko island (Callaghan *et al.*, 2011), which have not reported significant vegetation change despite regional climate warming. A tundra warming experiment using OTCs on Ellesmere Island, Canada, observed no change in

community composition after three years, suggesting that these high arctic communities may be very resilient to change (Hudson & Henry, 2010). This supports Wookey and Robinson's (1997) prediction that the lack of substantial soil nutrient pools in polar desert and semi-desert ecosystems would constrain responses to warming in these regions in the absence of further nutrient inputs.

At a different vegetation assemblage on Ellesmere Island, a long term study (25 years) showed increases in aboveground (Hudson & Henry, 2009) and belowground biomass (Hill & Henry, 2011).This observation may, however, be exceptional for the High Arctic; described as a 'polar oasis' and contains substantial soil organic matter and nutrient stocks (Henry, 1998; Hill & Henry, 2011), it has relatively large nutrient and moisture influxes from an upslope glacier resulting in high vegetation cover. This is consistent with patterns observed at lower latitudes, where the large increases in shrub biomass have been observed in moister riparian zones (Tape *et al.*, 2006; Naito & Cairns, 2011). ITEX syntheses have also shown that shrub biomass increases most in warmer and wetter plots (Walker *et al.*, 2006; Elmendorf *et al.*, 2012a) which is supported by NDVI data showing that the warmer areas of the Arctic have already witnessed the highest productivity increase. This prompts the suggestion that the area most likely to experience shrub expansion is the 'Low Arctic' where the environment and climate are milder. More southerly areas of the Arctic nearer the treeline, are likely to undergo the largest changes in ecosystem processes and properties and this is where research into the impacts of shrub expansion on carbon cycling should be focussed.

1.6. Treeline change and forest expansion

The arctic treeline is defined as the position of the northern-most tree (over 2 m) of a tree species (Hustich, 1979), north of which is treeless tundra and south of which is the 'tree-tundra', a mosaic of stands of trees and tundra vegetation (Payette *et al.*, 2001). Further south, below the Timberline is the uninterrupted boreal forest or 'taiga', where higher temperatures allow trees to grow larger and taller (Payette *et al.*, 2001). The treeline is formed by different species at different points in its circum-polar distribution. These species are principally of the genera *Betula*, *Larix*, *Pinus* and *Picea*, with *Betula* and *Larix* most consistently at the northern-most limit of tree growth (Hustich, 1979; Callaghan *et al.*, 2002).

Tree survival, and therefore the position of the treeline, are is highly sensitive to extremes of temperature and is therefore predicted to advance in response to climate warming (Callaghan *et al.*, 2002; Grace *et al.*, 2002; Harsch *et al.*, 2009). Research has shown that altitudinal treelines grow up to a summer 'thermal limit' (Korner & Paulsen, 2004), and could be responsive to climate warming. However, in a meta-analysis of treeline studies, it was found that only half of those studied had moved in response to warming (Harsch *et al.*, 2009). The strongest response of treelines has been in areas experiencing strongest winter warming (Harsch *et al.*, 2009). Trees could be particularly responsive to winter climate change as their height ensures that shoots receive no protection from winter cold, therefore increases in winter temperature should equate to increases in survival (Grace *et al.*, 2002). The largest increases in temperature at high latitudes have been observed over the winter period (Serreze & Barry, 2011), therefore we should expect further advance of the polar treeline.

Diffuse treelines, where stands of trees are ever-increasingly interspersed with tundra until they can grow no further north/up-slope (Payette *et al.*, 2001) were found to be the most likely to respond positively to climate warming rather than abrupt treelines (Harsch *et al.*, 2009). This 'forest-tundra' is found further south in the Arctic and is where some of the highest increases in productivity have been recorded in past decades (Epstein *et al.*, 2012) and is predicted to expand rapidly into the tundra by 2050 (Pearson *et al.*, 2013). Some recent examples of this can be found in sub-arctic Sweden where stands of *Betula pubescens* were found both to have increased in size and to have colonised further up-slope (Rundqvist *et al.*, 2011) and to higher latitudes (Hofgaard *et al.*, 2013). In sub-arctic Quebec, only more southerly tundra within a diffuse treeline area were found to be undergoing recruitment of tree species (Gamache & Payette, 2005). In addition to this, it has been observed that land management via processes such as changes in reindeer husbandry can also result in the increases of forest cover via herbivory of lichens (Tommervik *et al.*, 2009). Conversely, herbivory by reindeer can stabilise the treeline through direct shoot mortality (Van Bogaert *et al.*, 2011), as can herbivory by other species such caterpillars of lepidopteran moths (Van Bogaert *et al.*, 2011; Bjerke *et al.*, 2014).

1.7. Potential interactions between shrubs and trees and soil carbon.

Earth system models have predicted that increases in productivity at northern high latitudes that are being driven by climate change will result in increased C sequestration in the soil (Cramer *et al.*, 2001; Qian *et al.*, 2010; Todd-Brown *et al.*, 2013). The proposed mechanism for this is through an increase in litter fall (Qian *et*

al., 2010). However, recent work has cast doubt on this prediction. It was suggested that extrapolations of current ESMs do not take into account more subtle, yet critical plant-soil interactions that exert potentially critical influence over the fate of soil C (Todd-Brown *et al.*, 2013). Indeed recent empirical data from treelines (Wilmking *et al.*, 2006; Hartley *et al.*, 2012) and boreal forests (Kane & Vogel, 2009) have shown that more productive vegetation stores less C in the soil than less productive vegetation. The plant-soil interactions which Todd-Brown *et al.* (2013) allude to are mechanisms such as 'positive priming', whereby labile carbon is delivered to the soil from plant roots. This stimulates the microbial community into mineralising nutrients from organic complexes, but, in so doing, causes decomposition of native organic matter in the soil (Kuzyakov, 2002). This was a mechanism proposed to explain low C stocks in a sub-arctic forest where it was observed that 'old' C was being respired at the peak of forest productivity (Hartley *et al.*, 2012). Meta-analyses of CO_2 enrichment experiments have shown that the excess C fixed by forests can be cancelled out by increases in priming of old soil C (van Groenigen *et al.*, 2014). Further, when priming effects are incorporated into ESMs, they can reduce the soil C storage predicted by CO_2 'fertilisation' by up to 50 % (Sulman *et al.*, 2014).

Productive shrub and forest expansion onto the carbon-rich tundra is therefore not a straight-forward case of increased productivity resulting directly in carbon sequestration. The simple consideration of priming effects (Sulman *et al.*, 2014) in more productive systems casts doubt on the additional storage potential of these plant functional types (PFTs). Furthermore, the examination of empirical evidence suggests that there could be losses of C from the Arctic, should shrubs expand their

ranges. Plant-soil interactions are of fundamental importance to C fluxes in the Arctic when considering vegetation change (Wookey *et al.*, 2009). Specific plant-soil interactions need to be considered to better understand the future fate of soil C in the Arctic.

1.7.1. Ectomycorrhizal fungi and soil carbon

Ectomycorrhizal (ECM) fungi live in symbiosis with the majority of trees and shrubs, exchanging nutrients, water and pathogen resistance for photosynthate-C from their plant hosts (Smith & Read, 2008) . They can be defined morphologically from any other mycorrhizal group by their complete coverage of root tips with a fungal mantel and hyphae that grow in between epidermal and cortical cells, called the 'Hartig net' (Smith & Read, 2008). ECM invest autotophic C in a network of extramatrical mycelia, which are heavily branched hyphae that have considerably greater surface area than the plant's roots (Smith & Read, 2008). The extent of the mycelia varies greatly by species, from smooth-mantled 'contact' species to extremely dense or long-ranging networks with large potential to explore the soil (Agerer, 2001; Agerer, 2006). ECMs make up a polyphyletic group that has evolved multiple times from free-living ancestors (Tedersoo *et al.*, 2010). For this reason, many species retain the ability to produce extra-cellular enzymes similar to those of free living fungi with a high potential to degrade soil organic carbon (Cullings *et al.*, 2008; Talbot *et al.*, 2008; Bödeker *et al.*, 2014; Phillips *et al.*, 2014; Brzostek *et al.*, 2015). It is doubtful that ECM species produce these enzymes as a method of acquiring C for their own growth (Lindahl & Tunlid, 2015), but to break down complex organic molecules in order to free N for their tree hosts (Bödeker *et al.*, 2014).

The major deciduous shrub groups known to be proliferating onto the tundra as a result of climate warming (*Betula, Alnus, Salix*) all have strong symbioses with ECM fungi (Michelsen *et al.*, 1996; Treu *et al.*, 1996; Cripps & Eddington, 2005). As the range of these species expands, more soils will be exposed to the action of ECMs and potentially lead to the stimulation of soil organic matter (SOM) decomposition through the ability to produce a suite of oxidative and hydrolytic enzymes (Cullings *et al.*, 2008; Talbot *et al.*, 2008; Bödeker *et al.*, 2014; Phillips *et al.*, 2014; Brzostek *et al.*, 2015). In particular, it was found that a genus of ECM fungi, *Cortinarius*, which commonly colonises deciduous shrubs (Deslippe *et al.*, 2011), retains the ability to use peroxidase enzymes, especially when inorganic N is at low concentrations (Bödeker *et al.*, 2014). This was seen as a mechanism to free N from organic complexes for uptake by the fungus, but can result in stimulated decomposition of organic matter, as previously 'secure' C is exposed for decomposition to the microbial community (Talbot *et al.*, 2008). This could be of particular significance in arctic ecosystems/ heath environments where inorganic N availability is typically very low (Hobbie *et al.*, 2002; Read & Perez-Moreno, 2003) but abundant in undecomposed organic forms (Hobbie *et al.*, 2000; Read & Perez-Moreno, 2003). Carbon stores in arctic soils could therefore be highly vulnerable if exposed to ECM networks. It is therefore of critical importance to understand how ECM communities relate to PFTs and how they respond to changes in C supply.

Ericoid mycorrhizal fungi (ERMs) are dominant in many heath ecosystems (Read & Perez-Moreno 2003; Tybirk 2000). They form strong symbioses with ericaceous dwarf shrub genera such as *Empetrum, Vaccinium* and *Calluna* (Read & Perez-

Moreno 2003). ERM species are evolved to degrade the complex C compounds that make up the litter of their hosts (Read & Perez Moreno 2003). As such, they are able to exude a larger number of C-degrading enzymes than many ECM species, with a higher specificity to 'recalcitrant' C compounds such as lignin and phenols (Bending & Read, 1997; Talbot *et al.,* 2008).

One of the most significant differences between ECM and ERM fungi is the degree to which they explore the soil in search for nutrients (Smith & Read 2008). ERM fungi do not typically explore many μm beyond their host roots where they form typical coils in infected root hairs (Smith & Read 2008). There is a lot of diversity in the growth forms or exploration types (ETs) of ECM species (Agerer 2001). Some, like ERM fungi do not explore away from the root (e.g. contact ETs such as *Russula* spp.), some forage the soil extensively, forming cords and/or dense mycelial mats across the soil (Agerer 2001). Therefore, depending on the assemblage of species associated with a tree or shrub, the amount of ECM growth could be very large, as could the amount of C degrading enzymes that are exuded into the soil (Phillips *et al.,* 2014). It has been found that in forests, ECM fungi could produce as much, if not more extracellular enzymes than free-living fungi (Phillips *et al.,* 2014). In this way, although ECM species produce a lower diversity of C degrading enzymes (Talbot *et al*. 2008) and may produce less per unit area than ERMs; the net production of enzymes from ECM fungi associated with a single root maybe far higher than in ericaceous systems.

1.7.2. Snow accumulation and winter processes

A significant proportion of biogeochemical cycling in forest and tundra ecosystems occurs over winter, with up to a third of ER estimated to take place over the winter months (Fahnestock *et al.*, 1999). This is because microbial communities remain active at sub-zero temperatures (Clein & Schimel, 1995), and active microbial growth has been recorded in tundra soils at -2 °C (McMahon *et al.*, 2009) with survival possible at -39 °C (Panikov *et al.*, 2006).

Winter snow cover is critical to maintaining microbial activity, as deep snow insulates the microbial community from air temperatures which regularly dip as low as - 40 °C in the Arctic and Sub-Arctic (Sturm *et al.*, 2001a; Sturm *et al.*, 2005; Grogan & Jonasson, 2006). Shrub vegetation can create snow-packs of 50-100 cm in depth, where it gathers on the lee-side of the shrub, insulating the soil to maintain relatively stable temperatures between 0 and -10 °C (Sturm *et al.*, 2001a). The changes in local snow depth associated with shrub expansion in the Arctic (increase of up one metre) are a far larger change than changes in general landscape-scale overall snow depth observed in recent decades (± 10 cm per decade, depending on location (Park *et al.*, 2013)). The formation of deeper snow packs formed by the presence of shrubs may therefore have an important effect on microbial winter processes and C cycling via snow drift creation that exceeds any direct effects of climate change on snow depth.

Although the direct effect of snow depth on carbon cycling processes is relatively well established and it is known that increases in snow depth scales with biogeochemical cycling (Schimel *et al.*, 2004; Sturm *et al.*, 2005), the effect on

carbon cycling year-round is less known. It has been shown in a number of different ecosystems that deep snow over winter can influence microbial cycling in the summer (Blankinship & Hart, 2012). Forest ecosystems foster a large, active community of fungi (Voriskova *et al.*, 2014). In contrast, tundra ecosystems harbour stress tolerant microbial communities that are adapted to colder soil temperatures in winter, when they show low rates of activity (Robinson, 2001). There are clear differences in snow accumulation between low stature tundra vegetation and more productive plants which are currently encroaching. The importance of this difference in abiotic environment needs to be addressed from a year-round perspective.

1.7.3. Litter decomposition

ESMs predict that increasing productivity will result in C sequestration via an increase in litter-fall (Qian *et al.*, 2010). Although it is clear that more productive groups of plants such as trees and shrubs will create more litter, the speed at which this litter decomposes is fundamental to whether it will be stored in the soil for any significant amount of time. The effect of new shrub species on litter and C already present in tundra systems is also of key importance. Novel decomposer communities and litter input may stimulate faster decomposition in the soil as a whole.

It was hypothesised that an expansion of shrubs into the Arctic would increase its sink capacity because shrub litter was found to decompose at slower rates than other arctic PFTs (Cornelissen *et al.*, 2007). However, this prediction contrasts with comparisons of soil C which show that some the most productive areas of arctic

landscapes have the lowest C storage (Wilmking *et al.*, 2006; Hartley *et al.*, 2012). This highlights that litter decomposition rates are highly species-specific and the identity of the original tundra species is all-important. For example, litter of deciduous shrubs decomposes at slower rates than litter of graminoid species, so, a shift from graminoid vegetation to deciduous shrubs would result in a slower rate of litter C turnover (Cornelissen *et al.*, 2007). However, the key vegetation substitution some sub-arctic ecotones (Hartley *et al.*, 2012) is deciduous shrubs and trees for evergreen, ericaceous dwarf shrubs. Litter of deciduous plants are known to decompose faster than litter of evergreen shrubs (Aerts *et al.*, 2006; Cornwell *et al.*, 2008). Additionally, high levels of N in the litter of deciduous shrubs are known to stimulate fast cycling of C in the soil (Buckeridge *et al.*, 2010; DeMarco *et al.*, 2014). Therefore, to understand the effect of vegetation change on carbon cycling via changes in litter input, the interactions between the species identity and decomposition environment needs to be considered.

1.8. Treeline at Abisko, sub-arctic Sweden

The Sub-Arctic (Fig. 1.3) is of interest and importance for a number of reasons. This transition between boreal forest and tundra has been observed to be changing rapidly in response to climate change. Productivity increases have been most pronounced at the southern limit of the tundra, where trees begin to encroach (Epstein *et al.*, 2012). It is these 'diffuse' treelines in the sub-Arctic (Payette *et al.*, 2001) which have shown the strongest expansion with recent warming (Harsch *et al.*, 2009). Therefore this dynamic area of the terrestrial biosphere could have a disproportionately large interaction with the global carbon cycling and future climate change.

The alpine treeline in the Nissunsnuohkki area, c. 4.5 km south of the Abisko Scientific Research Station, in sub-arctic Sweden represents an excellent area in which to investigate controls exerted on soil processes by key PFTs (Sjögersten & Wookey, 2009). At this location (68° 18' N, 18° 49' E (Fig. 1.3)) the diffuse treeline is formed by *Betula pubescens* ssp *czerepanovii* (mountain birch). The understorey is a mixture of *Empetrum nigrum, Vaccinium myrtillus, Vaccinium vitis-idaea*, *Vaccinium uliginosum* with some patches of *Juniperus communis*. The *Betula* genus forms the treeline in many arctic ecosystems (Hustich, 1979) and *B. pubescens* has been shown to be responsive to climate warming at its northern limit in Fennoscandia (Hofgaard *et al.*, 2013) and at an alpine treeline at Abisko (Rundqvist *et al.*, 2011). Mountain birch forests suffer defoliation by the geometrid moths *Epirrita autumnata* and *Operophtera brumata* which is recognised as an important control over productivity in the region (Bjerke *et al.*, 2014).

Figure 1.3: Map of the circumpolar Arctic and Sub-Arctic with the location of Abisko, Sweden noted. Taken from The Arctic Biodiversty Assessment 2013 (CAFF, 2013).

Vegetation above the treeline at Abisko is predominantly ericaceous heath, made up of dense mats of *Empetrum nigrum* with other ericaceous species such as *Vaccinium vitis-idaea*, *Vaccinium uliginosum* and patches of deciduous *Betula nana*. These are typically slow growing communities adapted to stressful tundra environments which contribute to dense mats of humic C (Tybirk *et al.*, 2000). The transition between forest and tundra heath moves through a transitional band of shrubs consisting of *Betula nana* and *Salix* spp. A typical ecotone in at this treeline is shown in Fig. 1.4. The plant-soil interactions of these three distinct and important vegetation

communities can therefore be compared within very short distances without the introduction of geographical biases resulting from changes in slope, aspect or altitude. At the same time, the effect of specific PFTs on soil C can be examined at large scales because of the mosaic nature of the treeline at this location.

Figure 1.4: Treeline ecotone at Abisko with *Betula pubescens* (back), *Betula nana* and *Salix* spp. (right) and *Empetrum nigrum* dominated heath (Left). Photo: Thomas C. Parker.

The transition from heath to forest via shrubs can be seen as a 'space for time' transition in vegetation; the heath represents present-day tundra communities, the deciduous shrub communities will be more prevalent over the next 10-50 years with further warming (Myers-Smith *et al.*, 2011a) and forest communities could colonise tundra within the next 100 years, as evident in the paleo-record (Lloyd, 2005). Using this gradual change in vegetation across the forest-tundra ecotone, we can understand how soil carbon storage and cycling in tundra soils will respond to the

increases in productivity predicted over the next 100 years (Todd-Brown *et al.*, 2013).

The treeline ecotones represent not only a transition in productivity but also a transition in plant-soil interactions in various different forms, which can be hypothesised to increase C cycling rates with increasing productivity. Firstly, the ecotone can be seen as a transition in the dominance of mycorrhizal fungi from slow-cycling ericoid mycorrhizal (ERM) fungi (Dickie *et al.*, 2013) to faster cycling ECM fungi, capable of stimulating decomposition via extensive hyphal exploration of the soil. Ericaceous dwarf shrubs engineer a slow-cycling soil system by producing litter of low C:N ratios which favours uptake organic N via their ERM fungi (Read & Perez-Moreno 2003). This results in strong accumulation of soil C as mineral N availability remains low other microbial communities are excluded, leaving the N cycle 'closed' and dominated by ericaceaous plants and ERM fungi. The transition to shrub and forest ecosystems on the ecotone represents an 'opening' of the N cycle where N may become more easily accessible (including organically bound N (Talbot et al. 2008)) and C cycling may be faster as a result.

The ecotone is a transition in snow depth, from deep snow packs in forests and shrubs to thin, wind-blown snow cover on the tundra heath. This could translate into a gradient of winter microbial activity with (relatively) fast-cycling in the deep snow areas and slow cycling in the shallow snow areas (Grogan & Jonasson, 2006). Thirdly, the transition from heath to forest could be seen as a transition from evergreen litter which is typically slow to decompose (Cornwell *et al.*, 2008), through to more carbohydrate- and nitrogen-rich litter, which stimulates

decomposition in the shrub and forest vegetation (Buckeridge *et al.*, 2010). When studying these transitions, it is clear that the shrub-dominated systems share some key traits with adjacent forest ecosystems: ECM symbionts, snow accumulation and deciduous litter may result in low carbon storage, as has been shown in forest ecosystems (Hartley *et al.*, 2012). An investigation into the effects of vegetation transitions in the Arctic therefore needs to address these different impacts separately, whilst including potential interactions between them.

The information above is summarised in Figure 1.5. 'Space for time' changes in vegetation across treeline ecotones also represent transitions in the amount of ECM growth and their influence over SOM dynamics, depth of snow over winter and rate of litter decomposition. The are all hypothesised to contribute to increased C cycling and reduced C storage in the soil as productivity increases (Fig. 1.5).

The direct effect of climate warming on the biogeochemical processes identified (mycorrhizal growth, winter temperature and snow accumulation and litter decomposition) should also be considered (Fig. 1.6). If current anthropogenic GHG emissions remain on course, the Arctic could warm by up to 11 °C over the next 100 years (IPCC 2013). This will cause huge changes in thermal regimes in tundra northern high latitude ecosystems, notably a shortening of the snow-covered season and a lengthening of the growing season (Euskirchen *et al.* 2006). Warmer temperatures can lead to increases in mycorrhizal growth as more explorative growth forms such as *Cortinarius* spp. are selected for as shrub productivity increases (Deslippe *et al.* 2011). This genus has high potential to produce peroxidase enzymes (Bödeker *et al.* 2014) which can stimulate decomposition,

therefore, increases in temperature can increase decomposition via higher ECM growth rates. The direct effect of atmospheric warming may increase winter temperatures as well as a reduction of the snow-covered season across all ecosystem types (Euskirchen *et al.* 2006). This could lead to increasing microbial decomposition rates and a further loss of C from the soil (Panikov *et al.* 2006). Temperature explains large amounts of variation in decomposition across forest-tundra ecotones in Fennoscandia (58 % (Sjögersten & Wookey 2009)), therefore increases in temperature in this region may result in increases in decomposition. Taken together, the direct effects of increases in air temperature on the ecology of the tree-line ecosystem may result in increases in C cycling rates and reductions of C storage as is predicted across the arctic with changes in thermal regimes (Euskirchen *et al.* 2006). In summary, without considering vegetation change, many of the processes described may increase in activity with warming in accordance with typical kinetic models which predict increasing biological activity with increasing energy input (temperature) (Davidson *et al.*, 2006). This is summarised in Figure 1.6.

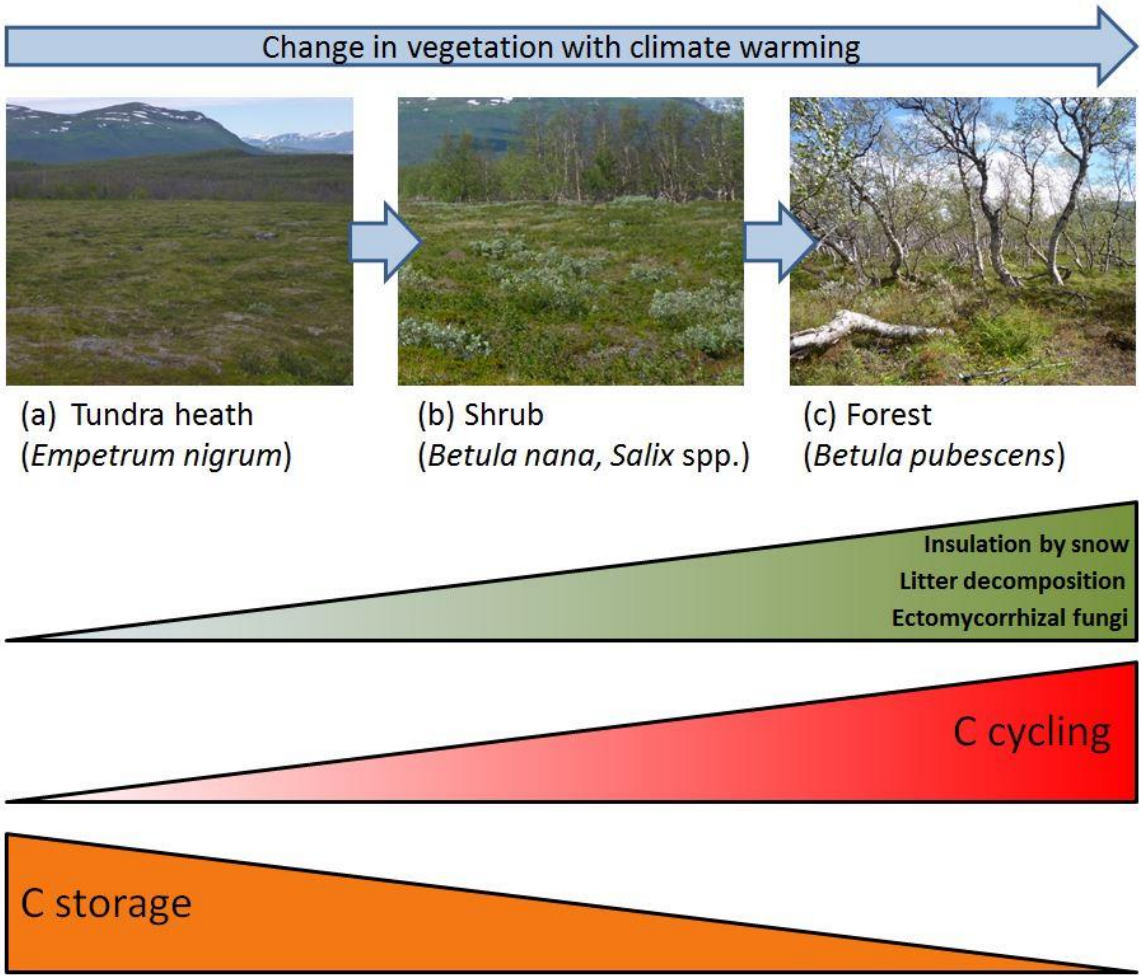

Figure 1.5: Conceptual diagram showing contrasting vegetation communities across ecotones: (a) Tundra heath, (b) Shrub, (c) Forest with shrubs and forests predicted to expand across tundra vegetation (Pearson *et al.*, 2013) this ecotone represents a 'space for time' transition. Hypothesised strength of ecological influences on C cycling and storage are represented below.

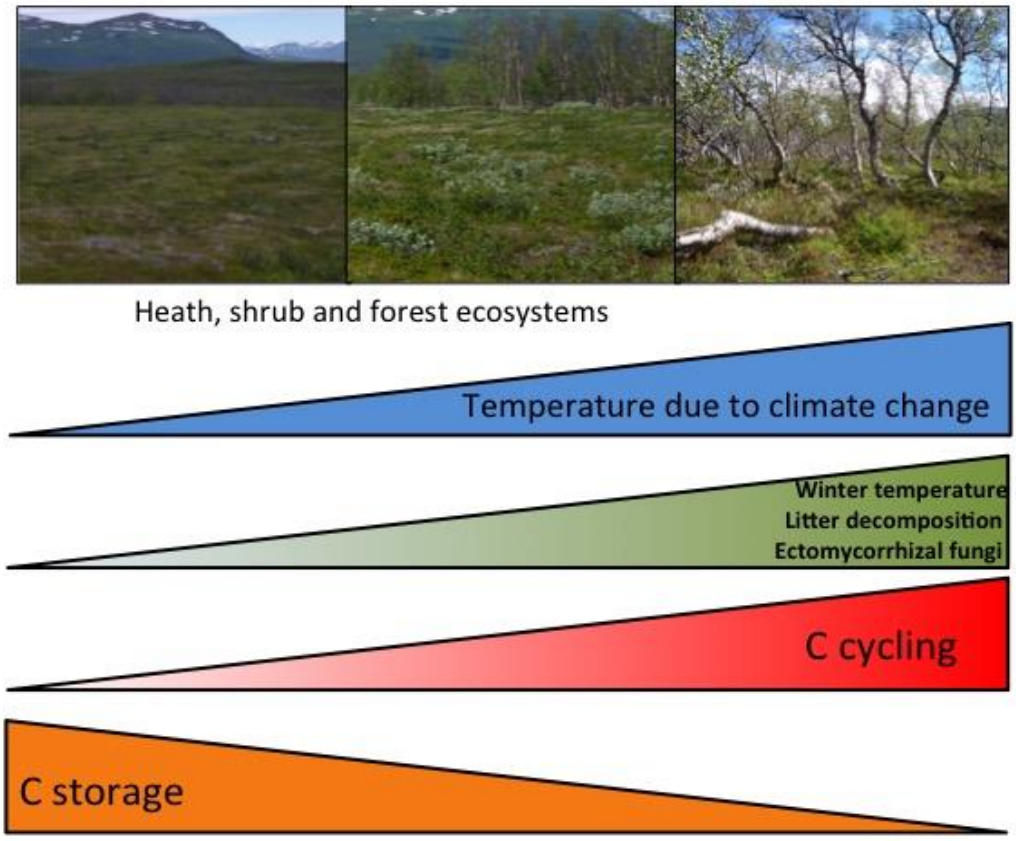

Figure 1.6: Conceptual diagram showing the direct effect of temperature increase on important processes in heath, shrub and forest ecosystems and their knock-on effects on carbon cycling and storage independent of change in vegetation dynamics outlined in Fig. 1.5.

1.9. Aims and objectives

This thesis will synthesise four studies conducted at the sub-arctic treeline in the Nissunsnuohkki area at Abisko, sub-arctic Sweden, in order to quantify and understand the interplay between plant and soil processes, and the potential consequences of global change and shifts in PFTs and community composition. Each study addresses plant-soil interactions which drive dynamics of soil C, but are linked by the theme of the relationship between productivity and soil C cycling. It will address hypotheses relating to ecological mechanisms which can be condensed into four over-arching hypotheses:

1. Shrub and forest systems store less C than heaths as a result of fast C cycling related to ECM activity;

2. Deep snow accumulations in forest systems protect soil microbial communities from the harshest of winter conditions and therefore increase both winter and summer microbial activity;

3. Defoliation of forest ecosystems results in reduction in autotrophic C supply to the soil, therefore slowing microbial decomposition of soil C;

4. Carbohydrate-rich litter input and summer decomposition environment interact to result in fast decomposition rates in the forest and shrub systems compared to the heath.

Each of these concepts was addressed individually and the methods used for each chapter are summarised here:

Chapter two: To understand carbon storage and cycling across the treeline ecotone we established multiple, spatially replicated transects from forest to heath. These ran through 'knee-high' shrub zones to characterise gradual changes in vegetation which could be representative of vegetation change across the Arctic. These transects were replicated over an area of approximately 2 km^2, therefore allowing results gained from the transitions to be realistically extrapolated to the landscape-scale. Along these transitions soil C stocks were measured, which involved detailed inventories of soil organic matter content, bulk density, horizon depth and elemental analysis of organic and mineral horizons. This was repeated in another landscape which has similar vegetation transitions but a wetter climate (Vassijaure). At Abisko, at each plot at along the transect, thaw-season respiration rates over two years and ECM hyphal production rates over the growing season of 2013 was then measured.

Chapter three: In order to understand the influence of snow depth and duration on soil microbial processes throughout the year, a spatially replicated soil temperature monitoring experiment was firstly set up in heath and forest soils. Concurrently, soil monoliths were transplanted between heath and forest, and *vice versa*, with control monoliths which were transplanted back into their 'home' environment. This experimental approach was used to expose each soil type to two contrasting winter environments and then to measure respiration rates and microbial biomass inthe

growing season to understand the importance of winter environment on summer C cycling rates.

Chapter four: A natural disturbance event occured in summer of 2013 when a moth outbreak caused wide-spread damage of the mountain birch forest canopy. In collaboration with the University of New Hampshire, the issue of whether reduced autotrophic C supply to the rhizosphere slowed microbial C and N cycling rates was addressed. Plots were set up on existing transects and on new plots with contrasting levels of defoliation to measure respiration rates, free inorganic N levels in summer and autumn, and ECM hyphal production rates. Collaborators collected ECM root tips from *Betula* hosts at defoliated and non-defoliated trees to understand how the ECM community changed and how extra-cellular enzyme production changes with defoliation. Together, these data could better explain the C and N flux data in this chapter and show how soil processes respond to canopy defoliation.

Chapter five: An extensive decomposition experiment at the ecotone transects was set-up to identify the most important factors determining decomposition rates across the vegetation types. Recently senesced litter from dominant heath, shrub and forest vegetation was measured for mass loss over 21 months with each species of litter transplanted between all three environments. Concurrently litter bags were placed under snow manipulations designed to recreate those under different vegetation types to understand the role of insulation by snow for decomposition rates. CP/MAS ^{13}C-NMR spectroscopy was used to characterise remaining C composition of decomposed litter of *Betula pubescens* and *Empetrum nigrum* in heath and forest environments. This was then compared with undecomposed 'controls' to identify

which compounds had been significantly depleted and the relative importance of species biochemistry and decomposition environment to loss of important C structural compounds.

Chapter 2: Rapid carbon turnover beneath shrub and tree vegetation is associated with low soil carbon stocks at a sub-arctic treeline

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2.1 Abstract

Climate warming at high northern latitudes has caused substantial increases in plant productivity of tundra vegetation and an expansion of the range of deciduous shrub species. However significant the increase in carbon (C) contained within above-ground shrub biomass, it is modest in comparison with the amount of C stored in the soil in tundra ecosystems. Here, a 'space-for-time' approach was used to test the hypothesis that a shift from lower-productivity tundra heath to higher-productivity deciduous shrub vegetation in the sub-Arctic may lead to a loss of soil C that out-weighs the increase in above-ground shrub biomass. It was hypothesised that a shift from ericoid to ectomycorrhizal systems coincident with this vegetation change provides a mechanism for the loss of soil C. Soil C stocks, soil surface CO_2 flux rates and fungal growth rates were sampled along replicated natural transitions from birch forest (*Betula pubescens*), through deciduous shrub tundra (*Betula nana*) to tundra heaths (*Empetrum nigrum*) near Abisko, Swedish Lapland. It is shown that organic horizon soil organic C (SOC_{org}) is significantly lower at shrub (2.98 ± 0.48 kg m^{-2}) and forest (2.04 ± 0.25 kg m^{-2}) plots than at heath plots (7.03 ± 0.79 kg m^{-2}). Shrub vegetation had the highest respiration rates, suggesting that despite higher rates of C assimilation, C turnover was also very high and less C is sequestered in the ecosystem. Growth rates of fungal hyphae

increased across the transition from heath to shrub, suggesting that the action of ectomycorrhizal symbionts in the scavenging of organically bound nutrients is an important pathway by which soil C is made available to microbial degradation. The expansion of deciduous shrubs onto potentially vulnerable arctic soils with large stores of C could therefore represent a significant positive feedback to the climate system.

2.2 Introduction:

Northern high latitudes, particularly north of 60° over land, and across the Arctic Ocean, have warmed by between 1-4°C since 1960, and at a rate substantially greater than the global mean (Serreze & Francis, 2006; Hansen *et al.*, 2010; Serreze & Barry, 2011). The 'Arctic Amplification' of global warming is also predicted to accelerate in the coming decades, further accentuating the contrasts with overall planetary warming (Serreze & Barry, 2011). In parallel with this strong warming trend, one important change in arctic and sub-arctic tundra ecosystems has been an increase in productivity (Guay *et al.*, 2014) where some areas have experienced increases of up to 10 g phytomass m^{-2} yr^{-1} in the last 30 years (Epstein *et al.*, 2012). Contributing towards productivity increase has been an expansion of the range of woody deciduous shrub species within the genera *Betula, Salix* and *Alnus* (Tape *et al., 2006)* .Shrub range expansion has now been documented to be occurring at many sites across the Arctic at ecosystem (Myers-Smith *et al.*, 2011) and plot scales (Elmendorf *et al.*, 2012b). This concurs with changes predicted by warming experiments (Elmendorf *et al.*, 2012a). Plant-soil interactions play a key role in global biogeochemical cycles, modulating the fate of carbon (C) fixed by plants, and the amount stored in the soil (Heimann & Reichstein, 2008; Metcalfe *et al.*, 2011). It is well documented that supply of C to, and respiration from, the soil and roots is broadly proportional to primary productivity in the system (Litton *et al.*, 2007; Chen *et al.*, 2011; Metcalfe *et al.*, 2011). However, although global scale analyses of the relationship between primary productivity and both plant and soil C stocks reveal general patterns (i.e. that the ratio of soil to vegetation C density increases with increasing latitude (Lal, 2005)), they mask important local and regional contrasts associated with specific plant functional types and, for example, their mycorrhizal symbionts. Despite their obvious importance, these patterns and

interactions are still not well understood (Arneth *et al.*, 2010; van Groenigen *et al.*, 2014).

In Northern terrestrial ecosystems, the expansion of woody species with more recalcitrant litter than the existing vegetation could lead to C sequestration in the soil and therefore a negative feedback to climate warming (Cornelissen *et al.*, 2007). A birch forest in northern Scandinavia, for example, was found to contain more recalcitrant carbon compounds than adjacent ericaceous heaths (Sjögersten *et al.*, 2003), which were suggested to be less prone to microbial decomposition. However, evidence is emerging that the supply of carbon via the rhizosphere of some woody species also stimulates decomposition of these recalcitrant (and potentially older) C stores (Hartley *et al.*, 2012) in a process known as 'positive priming' (Kuzyakov, 2002). This, therefore, may shift the balance between productivity and respiration, resulting in low soil C sequestration in spite of high net primary productivity.

Empirical data from field studies is providing growing evidence that specific relationships exist between the vegetation type and biomass in arctic and boreal ecosystems and the amount of C stored in the soil (Kuzyakov, 2002; Wilmking *et al.*, 2006; Kane & Vogel, 2009; Hartley *et al.*, 2012). These do not conform to the positive relationships between productivity and C storage predicted by global C cycle models (Cramer *et al.*, 2001; Qian *et al.*, 2010; Todd-Brown *et al.*, 2013). Arctic species' below-ground biomass does not increase with Leaf Area Index (LAI) above 1 $m^2 m^{-2}$ (Sloan *et al.*, 2013), and therefore may also defy predictions of carbon storage. At one site in northwest Alaska, Wilmking *et al.* (2006) revealed that recently advanced forest and shrub tundra had lower soil C densities in organic horizons than the adjacent

tundra. Furthermore, Hartley *et al.* (2012) demonstrated that soil C densities in a Swedish sub-arctic forest were significantly lower than a nearby tundra heath. Kane and Vogel (2009) also showed that less C is stored in Alaskan boreal ecosystems where there is greater above-ground biomass. Taken together, these studies indicate that existing patterns of above- and below-ground biomass and C stocks along *spatial* vegetation transitions may hold clues regarding the possible consequences of *temporal* shifts in vegetation communities in the future ('space-for-time substitution'). However, it is important to emphasise that C densities in many soils of the circumpolar north are often orders of magnitude higher than the phytomass in this region (Tarnocai *et al.*, 2009; Hugelius *et al.*, 2011; Epstein *et al.*, 2012) and have developed over decades to millennia; this raises the prospect of northern ecosystems increasingly being at 'dynamic disequilibrium' (Luo & Weng, 2011) with contemporary climate.

There are a number of phenomena that could lead to a net loss of C from tundra ecosystems when shrubs and forests encroach. Firstly, there is a concurrent increase in the abundance of ectomycorrhizal (ECM) fungi with increasing cover by trees and shrubs. These fungi are one of the primary recipients of autotrophic C (Hobbie, 2006) and are able to produce and exude a number of structural carbon-degrading compounds (Cullings *et al.*, 2008; Talbot *et al.*, 2008). Although it is uncertain the extent to which these compounds may interact with soil organic carbon (SOC) in the Arctic, it is clearly of pressing importance to find out. Secondly, the input of 'novel' litter into the system (i.e. from plant functional types not previously substantial components of the community) could lead to faster C cycling if the nutrients are in forms more accessible to the decomposer communities, physically or biochemically, than the litter of the plants they are replacing (e.g. ericaceous species) (Read & Perez-Moreno, 2003).

However a replacement of graminoids (grasses and sedges) may lead to the opposite effect (Cornelissen *et al.*, 2007). Thirdly, the accumulation of snow in drifts formed by taller vegetation and the resulting increased winter soil temperatures (Sturm *et al.*, 2005) may lead to faster C turnover in winter (Schimel *et al.*, 2004).

Other than the suggestion of 'positive priming' in sub-arctic birch forests, the ecological mechanisms by which C could be lost from the soil remain unresolved. Because the arctic tundra is undergoing increases in productivity (Epstein *et al.*, 2012; Guay *et al.*, 2014) on soils that contain a very substantial proportion of global soil C (Tarnocai *et al.*, 2009), there is a compelling need to understand the process implications for rates of soil organic matter (SOM) turnover and both C sequestration and release.

The increase of woody shrub cover in arctic systems occurs over a gradient from low densities to dominance over time (Myers-Smith *et al.*, 2011; Elmendorf *et al.*, 2012b) and it is important to understand the effect on C storage of this more subtle change as well as the larger-scale differences between forest and tundra. The ecotone between forest and tundra merits sampling over spatial scales sufficiently fine-grained to underpin an improved mechanistic understanding of the relationship between plant cover, C fluxes and soil C stocks. At fine (nominally defined here as 1 to 100 m lateral) scales, such transitions include subtle but important elements such as a transitional shrub community. In this case the 'space-for-time' substitution also potentially matches likely successional changes (vegetation shifts) associated with climate change, albeit with changes in soil C stocks likely trailing changes in vegetation (Sistla *et al.*, 2013).

This present study of SOC stocks and ecosystem respiration across the forest-tundra ecotone makes use of a dispersed 'mosaic-like' treeline near Abisko, Sweden. The following hypotheses were tested:

1. In spite of higher productivity (Shaver, 2010), deciduous shrub and forest plots have lower soil organic horizon and total SOC than heath sites, likely due to higher decomposition rates;
2. At small scales at tundra heath sites, deciduous shrub cover is correlated negatively with SOC densities;
3. Shrub and forest plots have high rates of C recycling (respiration), which would be a key indicator of C loss from the ecosystem;
4. ECM hyphal growth (a key link between plant productivity and soil C cycling) is comparable at shrub and forest sites, and both are higher than at heath sites.

2.3 *Material and methods*

Sites description

Twelve independent, short (<100 m) transects were selected within a permafrost-free landscape (c 2 km^2) spanning the sub-arctic/alpine treeline at Nissunsnuohkki (Abisko area, Sweden; ca. 68°18'N 18°49' E, 600 m asl, hereafter referred to as 'Abisko'). In this study, terminology of Walker (2000) and Kaplan *et al.* (2003), presented in ACIA (2005), is adopted to distinguish tundra plant growth forms and to place the study into circumpolar context. The treeline is formed by mountain birch (*Betula pubescens* Ehrh. ssp *czerepanovii* (Orlova) Hämet Ahti) with an ericaceous understorey and typically moves through a thick layer of shrub vegetation (*Betula nana* L. and grey willow (*Salix*) species (Specifically, *Salix glauca*, often accompanied by *Salix lanata*; other *Salix* spp., including *S. hastata* and *S. lapponum*, occur less frequently) - before becoming tundra heath, dominated by *Empetrum nigrum* L. ssp *hermaphroditum* (Hagerup) Böcher and *Vaccinium vitis-idaea* L. This transitional shrub-dominated vegetation is similar to the 'low- and high-shrub tundra' ('Continuous shrubland, 50 cm to 2 m tall, deciduous or evergreen, sometimes with tussock-forming graminoids and true mosses, bog mosses, and lichens') referred to in ACIA (2005), although generally not exceeding 1.5 m height and with the only one evergreen shrub species, *Juniperus communis* L., at low abundances. Tundra heath is here similar to the 'erect dwarf-shrub tundra' ('Continuous shrubland 2 to 50 cm tall, deciduous or evergreen, with graminoids, true mosses, and lichens') of ACIA (2005). Soils in the forest are micro-spodosols with a thin O horizon (< 5 cm) underlain by glacial till on a bed-rock typically of hard-shale (Sjögersten & Wookey, 2002). Soil pH in the organic horizon is 4.3 ± 0.1 at forest and 4.5 ± 0.1 at heath locations in the Abisko area (Table 1).

Transect lengths ranged from 52 to 97 m (Appendix 1) depending on the length-scale of the forest- heath ecotone. Care was taken to select vegetation transitions that were not present as a result of strong topographical influence; for example where water and snow accumulation due to dips and hollows dominate site conditions, and avoiding steep slopes (mean elevation change from heath to forest plots of -2.7 m (Appendix 1)). Transects were selected with a variety of contrasting compass bearings (Appendix 1) to ensure that there was no bias in the data due to shading or winter snow drifting. The 12 transects were grouped geographically into three blocks of four as shown in Figure 1.

Table 2.1: Vegetation characteristics along transects at Abisko across all blocks (means ± 1SE, n=12). "Canopy height" refers to the actual vegetation canopy for Heath, Shrub-Heath and Shrub communities, and the understorey for the Forest Edge and Forest (where mountain birch trees comprise the canopy)

	Plot on transect				
	Heath	Shrub-Heath	Shrub	Forest Edge	Forest
Distance from Heath (m)	n/a	14.6 ± 1.6	28.3 ± 2.9	44.9 ± 5.8	67.6 ± 5.9
Canopy height (cm)	14.7 ± 0.7	21.2 ± 1.2	32.0 ± 2.4	27.9 ± 3.0	19.0 ± 1.7
B. pubescens density (trees hectare $^{-1}$)				78.5 ± 11.4	78.5 ± 10.9
B. nana cover (%)	21.2 ± 2.7	36.9 ± 6.9	60.3 ± 4.8	32.2 ± 4.2	8.0 ± 2.2
E. nigrum cover (%)	65.4 ± 3.3	67.6 ± 3.4	66.9 ± 4.7	43.0 ± 6.5	45.4 ± 4.2
pH (organic horizon)	4.3 ± 0.1	4.6 ± 0.2	4.4 ±0 .1	4.5 ± 0.1	4.5 ± 0.1

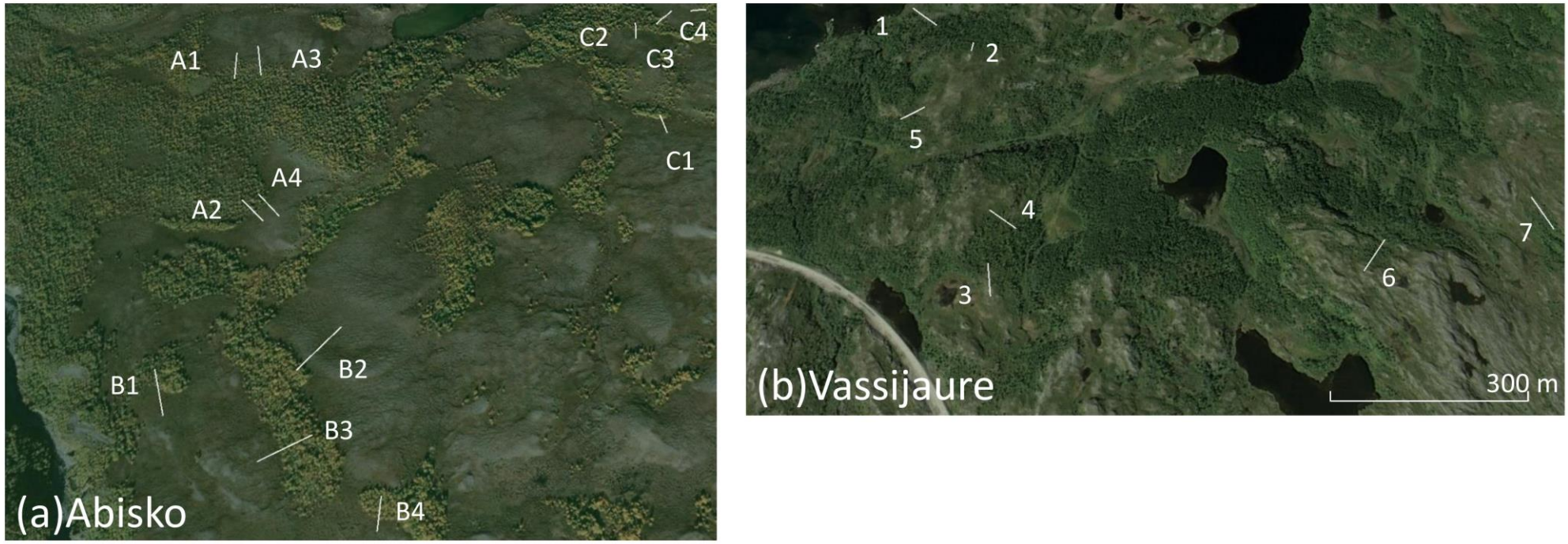

Figure 2.1: Google Earth images showing (a) Abisko transects and (b) Vassijaure transects across multiple treeline ecotones. At Abisko, A, B and C refer to different geographical blocks

Seven further transects (over approximately the same area as the Abisko transects) were sampled at Vassijaure (68° 26' N 18° 15' E, 517 m asl). This location has monthly temperatures similar to the Abisko area (both monthly means range from -11.9°C in January to 11°C in July) but a far higher mean annual precipitation (848 mm compared with 304 mm; for an overview of environmental conditions at the two sites, see Sjögersten & Wookey (2005)). Care was taken to distribute transects over an area similar in extent to Abisko, and to run transects over similar distances (c. 58 m). As with the Abisko sites, Vassijaure sites were selected to have little (on average) topographic change from H to F sites; this was, however, unavoidable for some sites (Appendix 1). Nonetheless, the most important apparent difference between sites was the vegetation community.

Five plots were established along each transect in order to represent best the transition in vegetation from heath to forest. These were; tundra heath (H), shrub heath (SH), shrub (S), forest edge (FE) and forest (F) (see Table 1 for further site details). H plots were chosen for an open heath environment with low *B. nana* cover and a low canopy height, and with vegetation dominated by *E. nigrum*. S plots were identified as areas dominated by *B. nana* with shrub height characteristically between 40 and 60 cm. SH plots were at locations intermediate between H and S plots, defined as having intermediate canopy height and *B. nana* cover, and generally located approximately equidistant to plots H and S. FE plots were located at the first *B. pubescens* tree along the transect from H to F and signified the forest margin. F plots were chosen to be in areas dominated by *B. pubescens,* approximately 10 to 15 m inside the forest edge.

Vegetation surveys

Percentage cover of selected species was estimated at each plot on transects. Five 0.25 m^2 quadrats were placed at each plot, one at the centre point and four more located 2.5 m from the centre point, every 90°, starting at a random bearing. In each quadrat, percentage cover of *B. nana* and *E. nigrum* was estimated by eye and the height of the tallest shoot was measured from ground level. Canopy height refers to actual canopy height at plots H, SH and S, and understorey canopy height at plots FE and F; at the latter two plot types *B. pubescens* forms the canopy (estimated to be 2 to 4 m vertically). Density of *B. pubescens* individuals > 50 cm high was measured within a 5 m radius of the centre points of sites FE and F.

Soil organic carbon (SOC) estimation

SOC was measured at every plot (H, SH, S, FE and F) on all transects at Abisko and the H, S and F plots of transects at Vassijaure. Five soil cores were taken at 2 m from the central point at headings of 0, 72, 144, 216 and 288°. A two cm diameter soil corer was pushed (using a sharp knife inserted around the margin to cut fibrous materials, including roots, and to avoid compression) into the soil to a depth at which the corer could not be inserted any deeper (assuming that parent materials or large clasts were reached), and depth of organic and mineral horizons recorded. Subsamples of mineral and organic soil were collected and pooled for the five coring locations on each plot. Samples were homogenised, dried (80°C for 48 hours) and sieved through a 2 mm

sieve. Soil organic matter (SOM) content for each pooled sample was determined by loss on ignition (LOI) in a furnace at 550°C for 5 hours (Ball, 1964).

Bulk density (BD) was sampled once at the organic horizon at the centre point of every plot by vertically inserting a 6.5 cm diameter, 10 cm deep PVC collar, measuring depth of organic horizon in the collar and calculating volume of soil present. BD samples were dried at 80°C for 48 hours (to 'constant weight') before determining soil dry mass. Five transects were selected to measure BD of mineral horizons. The procedure was the same as for the organic horizon except that this was removed in order to expose the mineral horizon. BD of mineral horizons across all sites and transects was found to be very consistent (1.20 ± 0.067 g cm^{-3}; mean ± one standard error (n = 21)) therefore the mean bulk density across sites was applied to all mineral horizons in the calculation of SOM.

SOM content (kg m^{-2}) in organic and mineral soil was calculated according to

$$SOM = f \times BD \times h$$

Where f is the fraction of organic matter, BD the bulk density (kg m^{-3}), and h the height of the respective horizons (m; averaged across the 5 cores).

Soil organic carbon (SOC) was measured from all soil samples taken from Vassijaure (organic and mineral; H, S, F). Triplicate subsamples from each sample were measured for C content after combustion in a Vario EL Cube elemental analyser (Elementar, Hanau, Germany) and a mean was taken for each plot. The relationship between measured SOM (g g^{-1}) and SOC (g g^{-1}) was determined. Based on these samples, SOC can be calculated with high confidence ($P < 0.001$, $R^2 = 0.997$) according to

$$SOC = SOM \times 0.5248$$

This equation was applied to estimations of SOM at every plot to estimate SOC.

Respiration measurement

At all plots of the 12 Abisko transects, PVC collars with a diameter of 15 cm and a height of 7 cm were placed on the soil surface and sealed to the soil using a non-setting putty (Plumber's Mait®, Bostik Ltd, Stafford, UK). Collars were not pushed into the soil in order to avoid disturbing the rhizosphere. Effectiveness of the seal was confirmed as all measurements of respiration showed a linear and regular increase in [CO_2] which was comparable to closed system in laboratory conditions.

A portable EGM-4 infrared gas analyser with a darkened CPY-2 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. At H plots, this measurement includes the entire vegetation canopy and therefore represents ecosystem respiration (ER); however, at all other sites the vegetation canopy is higher than the chamber, and the respiration measurement is therefore the sum of the understorey shoot and cryptogam respiration, total root and microbial respiration. CO_2 flux was measured from all collars in June and September 2012 and June, July and September 2013. Respiration rates were calculated as the product of a linear function of CO_2 concentration increase within the closed system, over a period of 90 seconds. Tests with longer regression periods showed no improvement of fit compared with regression results obtained over 90 seconds. All collars on every transect at Abisko (60 collars in total) were measured over periods of two days from 0900-1600 hours. Complete blocks were measured on the same days to

avoid bias from variations in temperature and moisture over the two day periods. The order in which blocks and transects within blocks were measured was randomised, as was the order of sampling within transects (i.e. H to F or F to H).

Hyphal in-growth

Thirty-seven µm nylon mesh bags (5 x 4 cm) were filled with 25 g sand from the shore of Lake Torneträsk (68°21'N, 18°49'E). These allowed ingrowth of hyphae, anticipated to be primarily of ECM fungi (analysis of community DNA shows c 80% (Wallander *et al.*, 2013)) but not roots. It was assumed that no ericoid mycorrhizal fungi grew into the sand as they not known to explore far from ericaceous root hairs (Smith & Read 2008). In addition to this No plants were present above-ground within 1 m of the sampling point. Sand was sieved to between 0.125 and 1 mm, rinsed under a flow of water for 1 minute then microwaved in a microwave (800 W) for 12 minutes, reaching a temperature of 98°C. This process was repeated and rinsed a final time before drying for 48 h at 80°C. Bags were inserted within 0.5 m of the PVC collar at the centre of the plots. The bags were left in the field for 92 days between 16th June and 16th September 2013. Sand was removed from the mesh bags and freeze-dried using a ModulyoD freeze drier (ThermoFisher Scientific, Waltham, MA, USA) for 72 hours within 6 hours of recovery.

One gram of sand from each bag was sonicated for 10 minutes in 30 ml of H_2O, a 4 ml aliquot of the solution was filtered onto a nitrate cellulose filter paper using a Millipore filtration kit, and fungal material was stained with trypan blue. Hyphal length was counted under 200x magnification (Primo Star, Zeiss, Oberkochen, Germany) using the

line intersect method (Brundrett *et al.*, 1994). This was repeated to make duplicates for each in-growth bag, a mean of which was taken as the final measurement.

Defoliation event

In 2012 and 2013 there was a significant joint outbreak of the geometrid moths *Operophtera brumata* and *Epirrita autumnata* across the Abisko and Vassijaure areas, causing large scale defoliation the *B. pubescens* canopy and damaging the understorey. In a separate study at these sites, complete defoliation was observed to reduce respiration rates but only at 50 cm from the base of a tree, there was no significant effect of defoliation on soil CO_2 flux further away from the tree (See Chapter 4). In the present study, all collars for respiration measurement are at least 2 m from the closest tree and therefore we do not consider defoliation to have affected respiration rates significantly. ECM in-growth into sand was reduced by *B. pubescens* defoliation (average F and FE plot defoliated by 50.5 %) by an average of 26.6 % (Chapter 4). Therefore, the results presented in the present study in F and FE plots will likely be an underestimation compared to a 'healthy' year. At our plots the outbreaks were confined to the forests and there was no evidence of defoliation of H, SH or S plots.

Statistical analysis

Differences in organic horizon SOC, mineral horizon SOC and total SOC between vegetation types, within sites (Abisko or Vassijaure), were analysed using one-way ANOVAs. If the raw data did not meet the assumptions of parametric analysis, they were transformed using a natural log. If vegetation type was statistically significantly related to SOC, differences between vegetation types were analysed using a Tukey's

Honestly Significant Differences (HSD) test. A generalised linear model, following Poisson distribution and a log-link function, was used to analyse the relationship between *B. nana* cover and organic horizon SOC. Repeated measures nested ANOVAs following a linear mixed effects model were used to analyse for differences in respiration rates between vegetation types. A nested ANOVA following a linear mixed effects model was used to analyse hyphal in-growth between vegetation types. The respiration and hyphal in-growth data were nested within transect then block, which were assigned as random factors. Respiration and hyphal growth data were square root transformed prior to analysis to meet the assumptions of the parametric model. Differences between vegetation types as analysed by nested ANOVAs were identified using one degree of freedom Wald tests. All analyses were carried out on R studio v0.97.551.

2.4 Results

Soil organic carbon across ecotones

At Abisko there are significant differences in organic horizon SOC (SOC_{org}) between vegetation types (Fig. 2.2, Table 2.2 for statistics). Both S (2.98 ± 0.48 kg m^{-2} (mean ± 1SE)) and F (2.04 ± 0.25 kg m^{-2}) plots have significantly lower SOC_{org} than the H plots (7.03 ± 0.79 kg m^{-2}) but are not significantly different from each other. Differences can be observed in SOC_{org} between H (7.03 ± 0.79 kg m^{-2}) and SH (4.55 ± 0.61 kg m^{-2}) where *B. nana* cover increases by an average of 15.7 % across an average lateral distance of 14.6 ± 1.6 m (Table 2.1). Furthermore, there is a significant ($P < 0.001$ Fig. 3) negative relationship between the % cover of *B. nana* and SOC_{org}.

At Vassijaure there is a significant relationship between vegetation type and SOC_{org} with a significant difference between H (5.51 ± 1 kg m^{-2}) and F plots (2.18 ± 0.29 kg m^{-2}) (Fig. 2.2, Table 2.2). The difference in SOC_{org} between H and S (3.01 ± 0.72 kg m^{-2}) was not as pronounced at Vassijaure as at Abisko and was not statistically different ($P = 0.066$). At both Abisko and Vassijaure there are no significant differences in mineral SOC (SOC_{min}) between vegetation types (Fig. 2.2, Table 2.2). Reflecting this, total SOC (SOC_{tot}) follows a similar pattern to SOC_{org} across the vegetation types and at both sites, with a decrease in SOC_{tot} from H to F. There is a significant relationship between vegetation type and SOC_{tot} at Abisko (Fig. 2.2, Table 2.2), with SOC_{tot} reducing from 9.01± 0.74 kg m^{-2} at H plots to 4.51 ± 0.51 kg m^{-2} at F plots. The first significant reduction in SOC_{tot} compared to H plots was at the FE plots (5.76 ± 0.84 kg m^{-2}). As with SOC_{org}, SOC_{tot} at Vassijaure follows a very similar pattern (Fig. 2.2). In this case

the differences in SOC_{tot} between H (9.98 ± 1.53 kg m^{-2}) and F (4.53 ± 0.49 kg m^{-2}) plots are statistically significant (P = 0.016).

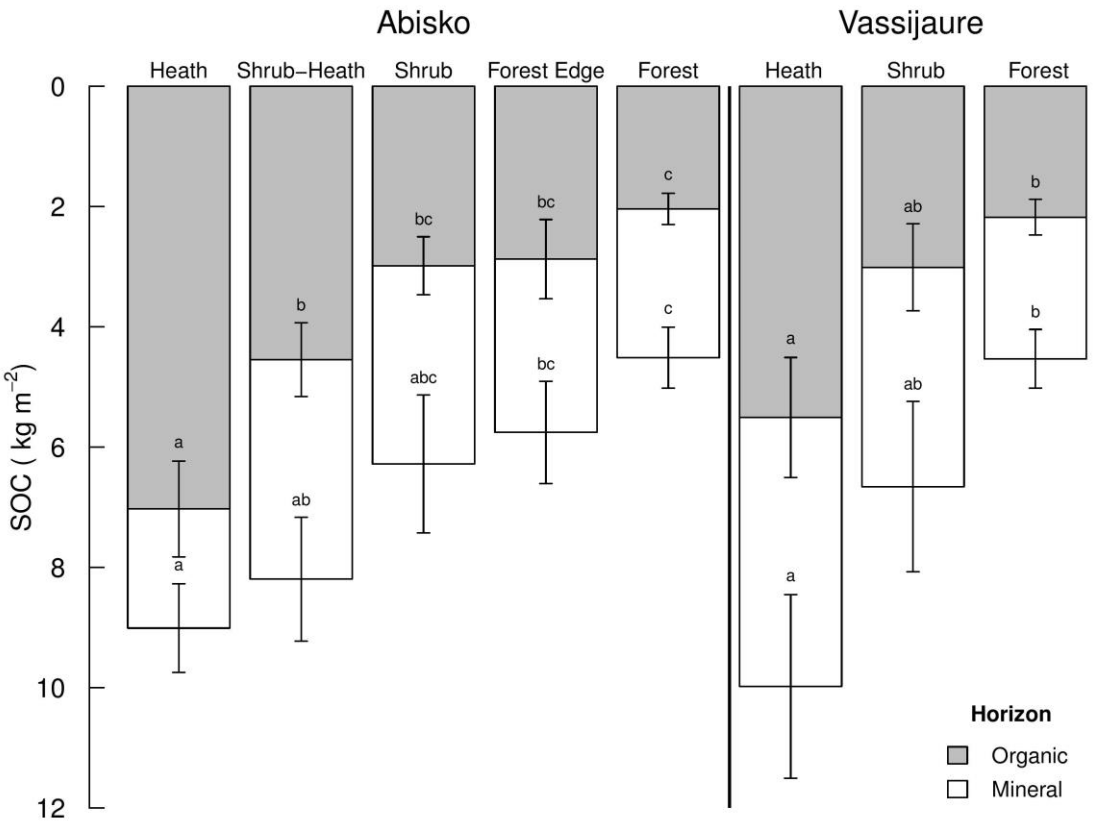

Figure 2.2: SOC at Abisko (dry/mesic, n = 12) and Vassijaure (mesic/wet, n = 7) across multiple heath-forest ecotones. The lower error bars (± 1SE mean) refer to total SOC (Organic + Mineral). The upper error bars (± 1SE mean) refer to organic horizon only SOC. Different letters show significant differences between means (P < 0.05) from Tukey HSD post-hoc tests (see Table 2 for test statistics). Letters refer to differences within site and horizon (Organic or Total).

Table 2.2: Test statistics for one way ANOVAs analysing differences in organic horizon SOC (SOC_{org}), mineral horizon SOC (SOC_{min}) and total SOC (SOC_{tot}) between vegetation types within sites (Abisko and Vassijaure). Data marked "*" have been natural log transformed for analysis.

Site	Abisko			Vassijaure		
	F value	d.f.	P value	F value	d.f.	P value
SOC_{org}	11.18	4,55	<0.001	5.60	2,18	0.01
SOC_{min}	0.66*	4,55	0.62	1.76	2,18	0.2
Total	6.38*	4,55	<0.001	4.94	2,18	0.02

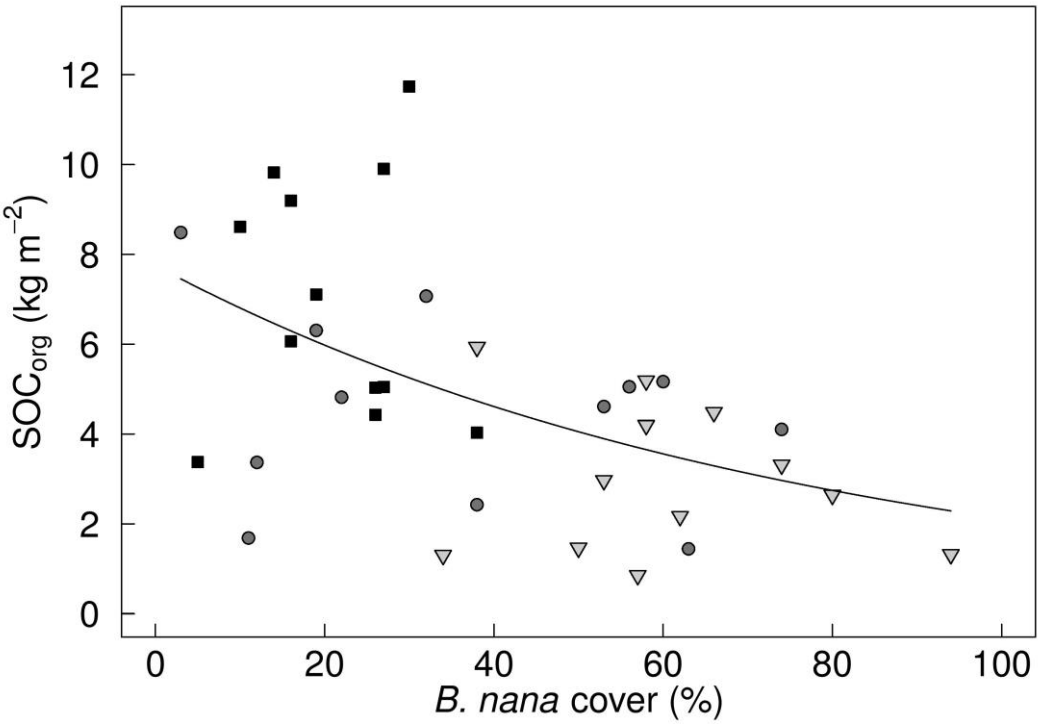

Figure 2.3: Relationship between % cover of *Betula nana* and SOC of the organic horizon at H (squares), SH (circles) and S (triangles) sites ($y = 10^{2.04 - 0.013x}$). Modelled line represents a significant relationship between the two variables (generalised linear model (Poisson distribution, $z = -3.722$, $P < 0.001$, d.f. = 35))

Respiration rates at Abisko ecotones

Respiration was significantly ($P = 0.008$) associated with vegetation type (Fig. 2.4). Mean respiration over all measurement points was highest at shrub plots (3.49 ± 0.21 μmol CO_2 m^{-2} s^{-1}), followed, in decreasing order, by SH, F, FE and H plots (3.23 ± 0.20, 3.03 ± 0.22, 2.93 ± 0.32 and 2.71 ± 0.13 μmol CO_2 m^{-2} s^{-1}, respectively); only the latter (H) was significantly different from S plots ($P < 0.001$). When respiration is expressed per kg SOC_{org}, however, it was significantly associated with vegetation type ($P < 0.001$, Fig. 2.5); S, FE and F plots respired at very similarly high rates (1.37 ± 0.29, 1.44 ± 0.22, 1.48 ± 0.19 μmol CO_2 (kg SOC_{org})$^{-1}$ s^{-1}, respectively), followed by SH and H plots (0.77 ± 0.15 and 0.48 ± 0.08 μmol CO_2 (kg SOC_{org})$^{-1}$ s^{-1}, respectively), which were significantly lower ($P < 0.001$).

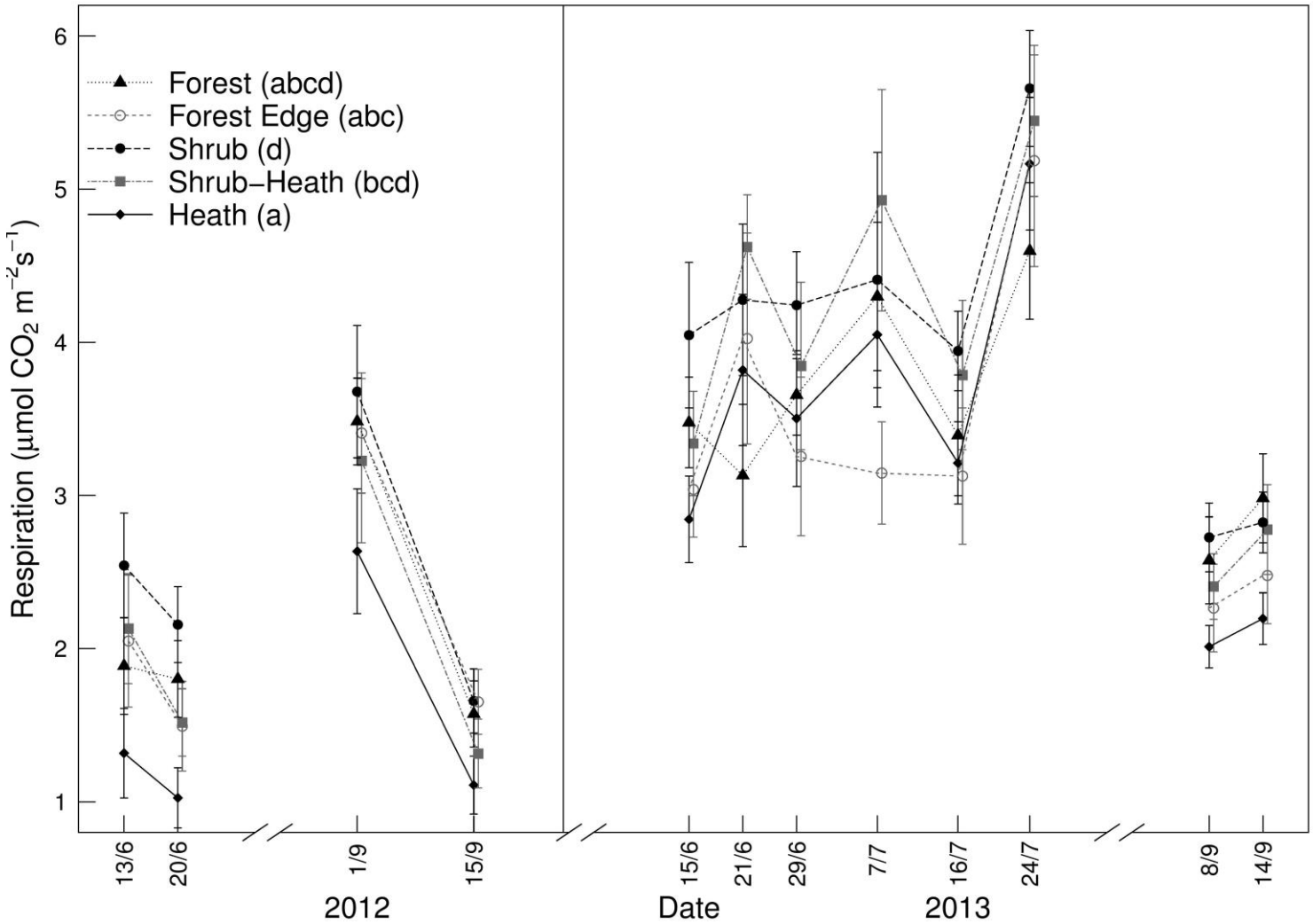

Figure 2.4: Dark respiration over two years of measurement across five vegetation types (n = 12). Repeated measures nested ANOVA: F = 3.92, P = 0.0083, response variable was square root transformed before analysis to meet assumptions of the linear model. Different letters in brackets at the figure legend represent significant differences ($P < 0.05$) between vegetation types within the statistical model using one degree of freedom Wald tests.

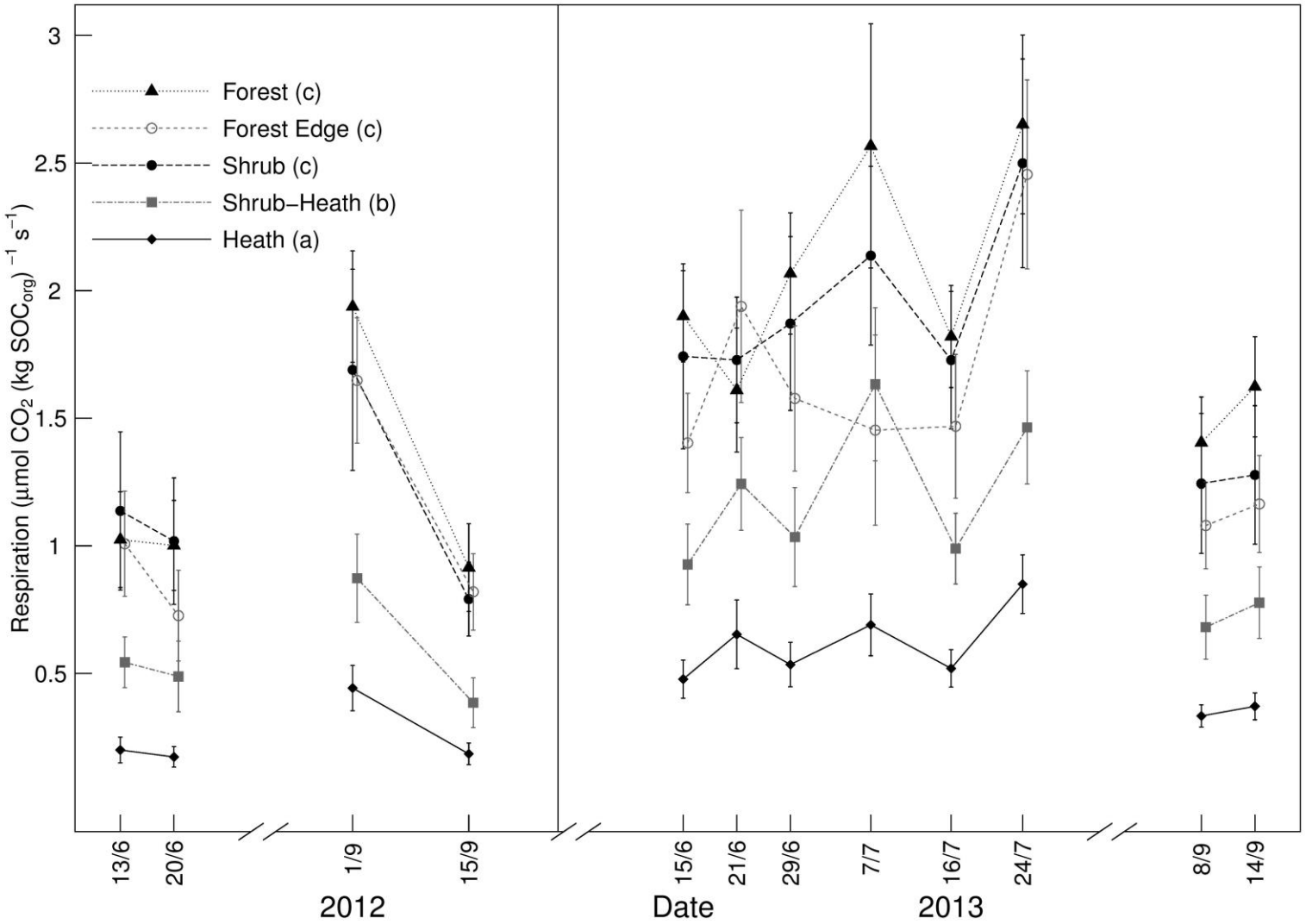

Figure 2.5: Dark respiration (expressed per kg SOC at each plot) measured over 2 years at five vegetation types (n = 12). Repeated measures nested ANOVA: $F = 12.90$, $P < 0.001$, response variable was square root transformed before analysis to meet assumptions of the linear model. Different letters in brackets at the figure legend represent significant differences ($P < 0.05$) between vegetation types within the statistical model using one degree of freedom Wald tests.

Hyphal in-growth at Abisko ecotones

Hyphal in-growth increased steadily along the transect from H (6.79 m hyphae (g sand)$^{-1}$) to FE plots (17.70 m hyphae (g sand)$^{-1}$) with more hyphal growth in S and FE plots than H plots (Fig. 2.6). There were lower growth rates at the F plots with a decrease to 10.67 m hyphae (g sand)$^{-1}$ from the FE plots. The overall pattern was not statistically significant as indicated by the nested ANOVA (P = 0.077).

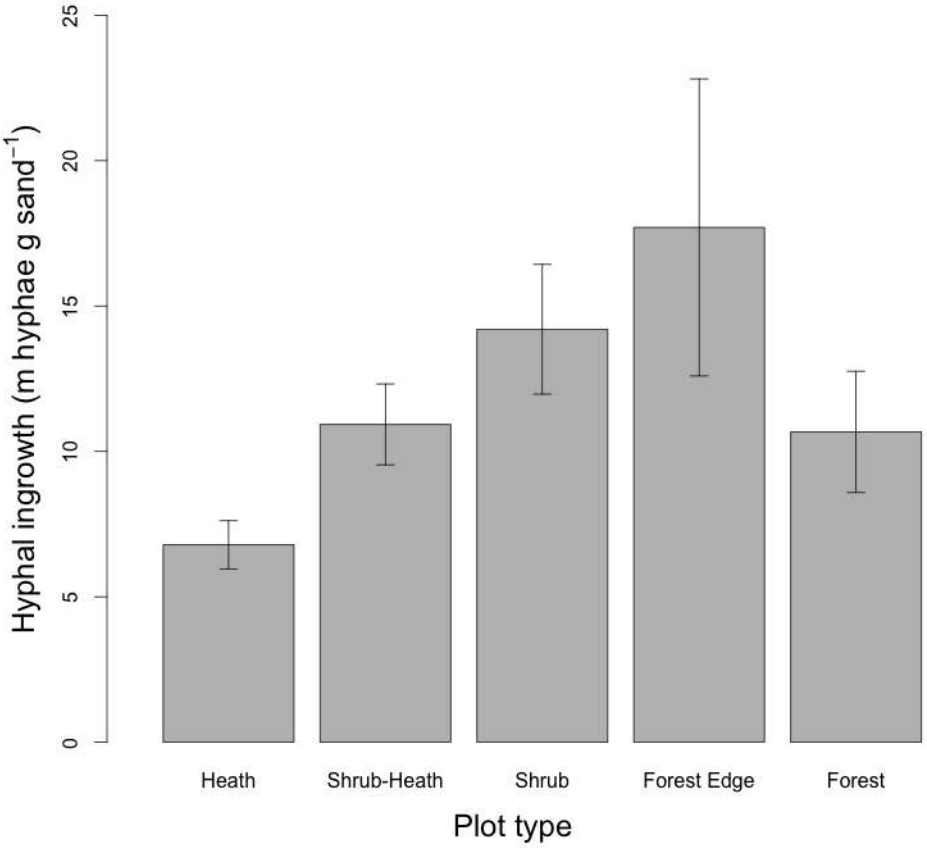

Figure 2.6: Hyphal in-growth of fungi over summer 2013 at Abisko transects. Nested ANOVA: F = 2.28, P = 0.077, response variable was square root transformed before analysis to meet assumptions of the linear model.

2.5 Discussion

This study provides strong evidence to support a number of hypotheses relating to vegetation cover and C storage in the soil. First, it demonstrates, using 17 independently replicated transects over two landscapes, that SOC stocks are similar in deciduous shrub-dominated systems and forest systems, but substantially lower than in adjacent, lower productivity, tundra heath systems (Hypothesis 1). These data show that this is true at multiple scales, from negative relationships between cover of *B. nana* and SOC (Hypothesis 2), to changes in SOC over ecotones. This emphasises a close link between the dominance of non-ericaceous woody species present in a community and the amount of C stored in the soil. It was shown that the changes in SOC over ecotones hold true at the landscape scale (both ca. 2 km^2 sampling areas), and also are similar in contrasting climatic contexts (sites with large differences in mean annual precipitation).

Until now, only Wilmking *et al.* (2006) had shown that SOC is depleted in shrub tundra compared to tussock tundra over permafrost in NW Alaska. Our sites are not underlain by permafrost, and they are relatively freely-draining; moisture and thermal status, alone, are therefore unlikely to explain contrasting rates of organic matter decomposition in shrub and forest communities compared with tundra heaths. Previous work at Abisko (Hartley *et al.*, 2012) showed that SOC densities in sub-arctic birch forests were lower than at tundra heaths. They did not, however, consider other woody vegetation (specifically, non-ericaceous shrub-dominated communities) in the same landscape; neither the ecological similarity between forest and shrub-dominated systems nor whether they exert the same controls over

SOC and how it is cycled. Furthermore, our study reveals a fine-scale negative relationship measured between *B. nana* cover and SOC (Hypothesis 2). This may be important in predicting how ecosystems will respond to gradual vegetation change as observed at plot scales (Elmendorf *et al.*, 2012b) and in warming experiments (Elmendorf *et al.*, 2012a).

Root biomass is an especially important component of C storage in arctic ecosystems which in most cases is larger than aboveground biomass (Iversen *et al.*, 2015). However, a full inventory of root biomass was beyond the scope of this study, but Hartley *et al.* (2012) provide data to indicate that it represents from ca. 8 to 18% of total below-ground C stocks in nearby heath and forest plots, respectively, in Abisko. Furthermore, at such sites, fine root C does not increase linearly with LAI above 1 m^2 m^{-2} (it tends to plateau at approximately 0.25 kg C m^{-2} (Sloan *et al.*, 2013)). This suggests that extra C sequestered in above-ground biomass may not be associated with a proportional increase in root biomass. The mechanism for this is high root turnover at high LAI meaning that high production of roots in more productive vegetation types does not result increased storage of C in root biomass (Sloan *et al.*, 2013). However, this has not been found for course roots in tundra (Campioli *et al.*, 2009) or forest systems (Bolte *et al.*, 2004).

The small C stocks under forest and deciduous shrub vegetation are being recycled (respired) substantially faster than adjacent, more SOC-rich, ericaceous heaths (Hypothesis 3). When the flux data are standardised and presented per unit SOC (i.e. potentially available substrate) present at each plot, it becomes clear that plots with high productivity (shrubs and trees) also return C rapidly to the atmosphere via

respiration compared to adjacent tundra heath communities (Fig. 2.5). Even without standardising the respiration data per kg SOC_{org}, respiration is highest in deciduous shrub vegetation (Fig. 2.4). Although photosynthesis was not measured, previous work shows that photosynthetic rates can be up to five times higher in deciduous shrub vegetation compared to tundra heath (Shaver, (2010); but note that Fletcher *et al.* (2012) also provide evidence of some depression in rates of GPP per unit leaf area in transition zones compared with adjacent 'main' vegetation types). So, whilst not all components of the C fluxes and stocks across our vegetation transitions could be quantified, it was shown that the larger amounts of C that are likely assimilated into deciduous shrub plots compared to heath plots are quickly metabolised and returned to the atmosphere through respiration.

Our findings suggest that the increased amount of C fixed by shrubs is cycled at a faster rate and therefore not sequestered in the soil to the same extent as predicted by some models (Qian *et al.*, 2010; Todd-Brown *et al.*, 2013). Our data are consistent with measurements at other shrub sites with relatively warm soils, which have been shown to be slight net sources of CO_2 (Cahoon *et al.*, 2012). These authors concur that a shift to shrub dominance in the Arctic will increase rates of C cycling and result in loss of C to the atmosphere if temperatures continue to increase. At another site in the low Arctic of Northwest Territories, Canada, a warming experiment with strong increases in shrub productivity yielded no extra standing above-ground litter compared to control (Zamin *et al.*, 2014), suggesting that the increase in productivity is concurrent with faster recycling and release of C from the ecosystem.

Here, vegetation transitions, thought to represent a plausible space-for-time scenario, were used to understand better the patterns that exist between vegetation and soil C and the possible future of soil C under vegetation change. The future flux of C is, however, highly dependent on a large number of interacting biotic and abiotic factors, several of which have not been investigated directly in this study. The vegetation of arctic tundra can be highly heterogeneous over small spatial scales (Walker *et al.*, 2005), and contrasting vegetation types can have significantly different fluxes of C. Moist sedge tundra, for example, is far more productive, with faster rates of C cycling, than adjacent dry heaths (Kade *et al.*, 2012). Increased shrub abundance may therefore have contrasting effects on sedge tundra than on ericaceous heaths. Additionally, where carbon cycling is slow due (topographically) to waterlogged conditions (Zona *et al.*, 2011), shrub vegetation may have a less pronounced effect on SOM decomposition due to the relatively greater importance of physico-chemical constraints (e.g. anoxia) on microbial activity. Shrubs have, in fact, been observed to increase in wet soils that have experienced climate warming (Elmendorf *et al.*, 2012b), but a key question is by how much they will influence rates of C cycling once established.

Observations of low SOC under more productive vegetation hold true in both areas of very high and low rainfall (Sjögersten & Wookey, 2005) in the sub-Arctic, i.e. at geographical scales for which forest and shrub expansion have been observed in the Fennoscandian sub-Arctic (Tommervik *et al.*, 2009; Rundqvist *et al.*, 2011). This adds confidence that the hypothesised 'vegetation effect' that was observed can be extrapolated over larger areas with contrasting climates. It is also hypothesised that expansions of shrubs and trees across the sub- and low Arctic tundra, the majority

of which is underlain by permafrost (Tarnocai *et al.*, 2009), may result in net losses of SOC from organic horizons which are supplemental to changes caused by climate drivers (e.g. soil warming and drying, and active layer deepening). The patterns that are observed in this study should be applicable in continuous permafrost regions where shrub expansions (Myers-Smith *et al.*, 2011) and productivity increases (Epstein *et al.*, 2012) are occurring. Indeed, Wilmking *et al.* (2006) observed similar decreases in stocks of C in a permafrost-underlain region. It is therefore likely that shrub expansion in tundra that is underlain by permafrost will result in loss of SOC.

Our results suggest that because there are similarly low SOC stocks in shrub and forest vegetation, there may be similar plant-soil interactions at work. One of the likely key differences between forest and shrub systems and tundra heaths at our study sites is the dominance of ECMs (Read & Perez-Moreno, 2003) in symbiosis with *B. nana* and *B. pubescens,* amongst others (Hypothesis 4). There is some evidence to support this, as it was found that there is a general increase in ECM growth along the transects from the heath to the edge of the forest. The decrease seen at F plots in Figure 6 is likely to be due to the partial defoliation of some F and FE plots which will have reduced C flow to the ECM community and reduced hyphal growth (Chapter 4) amongst other ECM community changes (Kuikka *et al.*, 2003). This would act to dampen the effect that was observed (Fig. 6) and it should be expected that FE and F plots would have higher ECM growth rates in non out-break years.

There is increasing evidence that the action of ECM fungi in scavenging for nutrients results in the breakdown of SOC (Talbot *et al.*, 2008). The exact mechanisms for this are attracting considerable interest, and the relative importance of ECMs' potential saprotrophic ability, their influence as an 'accidental decomposer', and as a direct recipient of plant C for positive priming, will be important to know (Talbot *et al.*, 2008). One could view the transition from heath to shrub to forest as an increase in dominance of ECM fungi from heath to shrub vegetation, and then a plateauing at the forest which would explain the loss of SOC along this transition if the 'decomposers in disguise' hypothesis is true (Talbot *et al.*, 2008).

Hartley *et al.* (2012) showed, by radiocarbon analysis of respired CO_2, that 'old' SOC was being decomposed at peak growing season in a sub-arctic birch forest. They attributed this to recently assimilated ('young') C by the trees causing a positive priming effect (Kuzyakov, 2002); here, it is proposed that the ECM community is central to this process. ECMs receive up to 20% of total C fixed by trees (Hobbie, 2006) and are therefore a key interface between labile C input and C sequestered in the soil. ECMs have substantial potential to produce extracellular enzymes to break down a range of structural organic compounds (Cullings *et al.*, 2008; Talbot *et al.*, 2008; Phillips *et al.*, 2014). One such genus (*Cortinarius*) has been found to excrete SOC-targeting peroxidases in response to low nitrogen (N) availability in the soil in the same region as the present study (Bödeker *et al.*, 2014). This finding may be of key importance in heath systems with relatively high soil organic C contents (e.g. H and SH plots), which typically also have low N availability (Read & Perez-Moreno, 2003), as the ECMs may degrade soil C in

order to mineralise N (Bödeker *et al.*, 2014). The observation in the current study that areas of high above-ground productivity and ECM growth (Fig. 6) (Hypothesis 4) have the highest rates of C cycling (Hypothesis 3) and lowest SOC (Hypothesis 1) lends support to the hypothesis that the ECM symbiosis is a mechanism by which C is lost from the soil. This could be important following an expansion of vegetation with ECM associations into heath soils where nutrients such as nitrogen are more likely to be bound in organic forms (Read & Perez-Moreno, 2003).

One other mechanism that could explain, or contribute towards, the patterns in SOC that were observed is the influence of winter processes. Over winter, an insulating layer of snow is trapped by shrubs and trees (Sturm *et al.*, 2005), which contrasts with heath sites where drifting elsewhere results in only thin or no snow cover. This insulating snow layer may maintain a more active microbial community (Schimel *et al.*, 2004) with higher winter respiration rates (Sullivan, 2010), which could also contribute to the loss of SOC from the system. As with the ECM example, the pattern in SOC across the transect will be mirrored by a similar pattern in abiotic constraints over biogeochemical processes such as snow accumulation.

Lastly, the transition in vegetation from heath to forest represents a transition in chemical composition of litter input; there is a reduction in chemical recalcitrance and decomposability of litter from heath (evergreen dominated) to forest (deciduous dominated). *Empetrum nigrum* leaf litter has high concentrations of phenolic compounds, which results in low decomposition and accumulation of SOC (Tybirk *et al.*, 2000). This contrasts with deciduous shrubs and trees (Cornelissen *et al.*, 2004; Cornwell *et al.*, 2008) and specifically *B. nana*, which decomposes faster than

E. nigrum (Aerts *et al.*, 2006). At our sites, there is a substantial cover of *E. nigrum* in the understory of the forest and shrub plots (Table 1), yet accumulation of SOC were not observed at these plots. It is therefore likely that the chemical composition of the litter input is not the most important determinate of SOC storage at these plots. Much like the decomposition of *B. pubescens* litter (Sjögersten & Wookey, 2004), it could be hypothesised that decomposition of *E. nigrum* litter (amongst other litter types) is enhanced in shrub and forests systems due in part to the presence of a strong decomposing fungal community (Lindahl *et al.*, 2007; Bödeker *et al.*, 2014) .

In conclusion, evidence is presented for a marked contrast in below-ground C cycling rates across the forest-tundra ecotone at a sub-arctic treeline. These results, based on a fully replicated design and covering contrasting landscape settings, not only confirm that mountain birch forests have relatively low soil C densities, but also that shrub vegetation has equally low SOC storage and faster C turnover. This relationship holds across different microclimatic conditions (contrasting precipitation at comparable mean temperatures), supporting the hypothesis that treeline vegetation type strongly controls SOC storage. These data emphasise the importance of plant-soil interactions and of the relative size, responsiveness and vulnerability of phytomass and SOC stocks to climate and vegetation change in the Arctic. Documented increases in productivity and above-ground phytomass may be modest compared to potentially vulnerable soil C that could be metabolised as a result of shrub expansion or other biotic and abiotic drivers of change in the circumpolar North. If shrub- and tree-dominated communities continue to expand northwards, then increases in productivity may accelerate C cycling (and release) to

a greater extent than any additional sequestration of C. Improved process understanding is required to underpin improvements in Earth System models.

Chapter 3: Snow accumulation over winter contributes to fast summer carbon cycling in a sub-arctic forest

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3.1 Abstract

Shrubs and trees are expanding onto tundra ecosystems in areas that are becoming more productive due to climate change. Snow accumulation around tall-stature vegetation can have important influences on carbon cycling during winter months by insulating the soil microbial community from the extreme cold and fluctuations of temperature. At a sub-arctic treeline in Sweden data loggers measured soil temperature across replicated transitions between mountain birch forests (*Betula pubescens* Ehrh. ssp *czerepanovii*) and tundra heath, to measure the influence of vegetation on soil thermal regimes. It was hypothesized that deep snow in forest plots would increase soil microbial activity over the growing season due to more insulation and better growing conditions over winter. To this end, a spatially replicated soil transplant experiment between forest (high snow) and tundra heath plots (low snow) was conducted. Respiration rates over the growing season of 2013 were measure, as were respiration and microbial biomass in June 2014. Respiration of forest soils, which were transplanted into heath, was significantly lower than paired control soils over 2013. Data showed that this depression in respiration rates was partly due to low plant cover in the transplanted cores but also the result of the transplant manipulation. In 2014, respiration of heath soils was higher in those that were transplanted into forests. Paired measurements of microbial biomass did not show

any significant differences between transplant and control cores. This work suggests that deep snow cover forests in may contribute towards the low storage of soil C in these systems. It then suggests that continued expansion of high-stature vegetation may stimulate the decomposition of C stored in the Arctic.

3.2 Introduction

Soils in northern high latitudes store up to half of the global soil carbon (C) stocks (Tarnocai *et al.*, 2009). In arctic ecosystems, winter is the longest season and of importance to year-round ecosystem processes. Snow cover exerts a profound influence on biological processes and winter respiration may contribute up to a third of annual ecosystem respiration (ER) (Fahnestock *et al.*, 1999). Environmental influences over winter on soil processes may therefore have important interactions with the large store of soil carbon in the Arctic (Tarnocai *et al.*, 2009).

There is a growing body of evidence demonstrating the importance of snow cover for microbial activity over winter (Fahnestock *et al.*, 1999; Schimel *et al.*, 2004; Grogan & Jonasson, 2006; Nobrega & Grogan, 2007; Sullivan *et al.*, 2008; Sullivan, 2010; Grogan, 2012; Oechel *et al.*, 2014). Tundra soil microbial communities can actively grow at -2 °C (McMahon *et al.*, 2009) and can continue metabolism at -5 °C (Clein & Schimel, 1995), with an exponential decrease in rates down to -39 °C where respiration is still measurable (Panikova *et al.*, 2006). There is a similar ratio of anabolism and catabolism at temperatures above and below 0 °C but with lower rates at lower temperatures, thus suggesting 'normal' function of the microbial community (Drotz *et al.*, 2010); although as temperatures fall towards 0 °C soil microorganisms initiate a range of physiological responses to survive freezing; (see (Schimel *et al.*, 2007) for a review). It is not surprising, therefore, that measurements of CO_2 emissions from soils year-round are revealing that winter fluxes are an important component of the C balance in the Arctic, potentially determining the sign of the net ecosystem C balance (Oechel *et al.*, 2014). Indeed, for soils at a high arctic site on Svalbard (Norway)

(Elberling, 2007) determined that between 14 and 30% of annual CO_2 production rates occurred during the six winter months (October to March) with continuous sub-zero air temperatures.

Annual duration of snow cover is very sensitive to climate change in the pan-Arctic region (Jones *et al.*, 2012) and has been observed to decline over the past 40 years (Brown & Robinson, 2011). Snow depth in high latitude systems has also been observed to be decreasing by up to 10 cm in the last 30 years, with the exception of localised areas in Siberia (Park *et al.*, 2013) This could therefore interact with soil processes over winter as insulation decreases. Therefore climate change could slow C cycling over winter through reduced insulation of the microbial community.

Another change in arctic ecosystems that is driven by climate warming is the expansion of shrub genera such as *Betula, Salix* and *Alnus* (Tape *et al.*, 2006; Myers-Smith *et al.*, 2011) as part of an overall 'greening' trend (Epstein *et al.*, 2012). These shrub species increase the canopy height of the tundra by up to one meter and cause snow accumulations in the windward side of the vegetation (Sturm *et al.*, 2001). The accumulation of snow also represents a potential positive feedback mechanism where the deeper snow enhances microbial activity, therefore increasing nutrient availability and encouraging further growth of shrubs (Sturm *et al.*, 2005; Elberling, 2007).

The local increases in snow associated with shrub expansion (e.g. over 100 cm (Sturm *et al.*, 2005)) are far higher than the observed pan-arctic decreases in snow cover directly due to climatic influences (up to 10 cm (Park *et al.*, 2013)). Considering the close coupling between snow depth and soil temperature, and thus winter respiration,

the increase in snow associated with shrub expansion could represent a stronger positive feedback to CO_2 flux than the possible negative feedback associated with the subtler, yet larger scale, reduction in snow depth (Park *et al.*, 2013). Furthermore, Grogan & Jonasson (2006) found that winter ER was significantly increased by snow accumulation in a Swedish sub-arctic birch forests only in areas where winter precipitation was low; there was no corresponding difference in ER between low and high stature vegetation in areas where snowfall was high (Grogan & Jonasson, 2006). This suggests that shrub expansion and associated snow accumulation will be particularly important to ER in areas with low snowfall, or where high wind speeds cause substantial redistribution of snow in the landscape.

Winter snow cover also has important 'memory effects' on subsequent summer C cycling rates, with the general trend observed that reduced winter snow cover results in reduced growing season CO_2 efflux from the soil (Blankinship & Hart, 2012). A number of mechanisms could be driving this: A reduction in root respiration due to frost damage (Oquist & Laudon, 2008; Haei *et al.*, 2013), and reduced soil moisture at depth (Chimner & Welker, 2005); but, conversely, in some cases snow can cause waterlogging and reduce ER due to anoxic conditions (Natali *et al.*, 2011). In permafrost soils increases in snow can increase CO_2 efflux due to a deepening of the active layer, making more SOM available for microbial degradation (Nowinski *et al.*, 2010; Rogers *et al.*, 2011).

Following a decline in winter snow-depth, springtime soil respiration may also be increased due to the release of dissolved organic carbon (DOC) resulting from physical damage by freeze-thaw cycles (Oquist & Laudon, 2008; Haei *et al.*, 2010; Haei *et al.*,

2013) and a subsequent shift to a fungal decomposer community (Haei *et al.*, 2011). In the same boreal study system, decreases in snow leads to reduced annual decomposition of organic matter, but the relative importance of each season under this treatment is not known (Kreyling *et al.*, 2013).

Observations in sub-arctic Sweden have revealed that productive forest and shrub ecosystems store less carbon in the soil than adjacent, lower productivity, heaths (Hartley *et al.*, 2012). The high rates of C cycling observed in Chapter 2 in the growing season within the forest and shrub communities, compared to nearby heaths, could partly be due to the protection (insulation) afforded to the microbial community by the deeper winter snow cover (Grogan & Jonasson, 2006).

Here, a spatially replicated soil transplant experiment was conducted between forest and heath ecosystem types at a sub-arctic treeline, with contrasting winter environments, to test the importance of winter snow cover for subsequent summer carbon cycling rates. The design of this experiment, and its physical location, enabled us to eliminate a number of environmental co-variables (e.g. waterlogging and permafrost thaw) which could confound the interpretation of the results. The following hypotheses were tested:
H1: Deep snow in forests insulates the soil, making it warmer and less variable than in heath soils;

H2: The winter-insulated community has higher metabolism over the subsequent summer;

H3: Winter-insulated soils have a larger microbial biomass which can explain higher respiration rates.

3.3 Materials and Methods

Sites description

12 paired heath (H) and forest (F) plots were selected from 12 replicated ecotones at a permafrost-free area spanning the subarctic/alpine treeline at Nissunsnuohkki (Abisko area, Sweden; ca. 68° 18'N 18° 49' E, 600 m asl). The treeline is formed by stands of mountain birch (*Betula pubescens* Ehrh. ssp *czerepanovii* (Orlova) Hämet Ahti) with an ericaceous understorey interspersed with tundra heath. F plots were chosen to be in areas dominated by *B. pubescens,* approximately 10 to 15 m from the edge of the forest stand. H plots were chosen for an open heath environment with low *B. nana* cover and a low canopy height, and with vegetation dominated by *Empetrum nigrum* ssp *hermaphroditum*. Tundra heath is here similar to the 'erect dwarf-shrub tundra' ('Continuous shrubland 2 to 50 cm tall, deciduous or evergreen, with graminoids, true mosses, and lichens) of ACIA (2005). Soils in the forest are micro-spodosols with a thin O horizon (< 5 cm) underlain by glacial till on a bed-rock typically of hard-shale. Soil pH at the organic horizon is 4.3 ± 0.1 in the forest and 4.5 ± 0.1 in the heath (Table 3.1).

Distance between F and H plots ranged from 52 to 97 m with a mean length of 67.6 m. Care was taken to select vegetation transitions that were not present as a result of strong topographical influence - for example where water and snow accumulation due to dips and hollows dominate site conditions - and avoiding steep slopes (mean elevation change of -2.7 m). Transects were selected with a variety of contrasting compass bearings to ensure that there was no bias in the data due to shading or winter snow

drifting. For details, see Appendix 1. The 12 transects were grouped geographically into three blocks of four as shown in Figure 3.1.

Table 3.1**:** Vegetation characteristics along transects at Abisko across all blocks (means ± 1SE, n=12). "Canopy height" refers to the actual vegetation canopy for Heath (H), and the understorey for Forest (F), where mountain birch trees form the (open) canopy.

	Plot on transect	
	Heath	Forest
Distance from Heath (m)	n/a	67.6 ± 5.9
Understorey Canopy height (cm)	14.7 ± 0.7	19.0 ± 1.7
B. pubescens density (trees ha^{-2})		785 ± 109
B. nana cover (%)	21.2 ± 2.7	8.0 ± 2.2
E. nigrum cover (%)	65.4 ± 3.3	45.4 ± 4.2
pH (organic horizon)	4.3 ± 0.1	4.5 ± 0.1

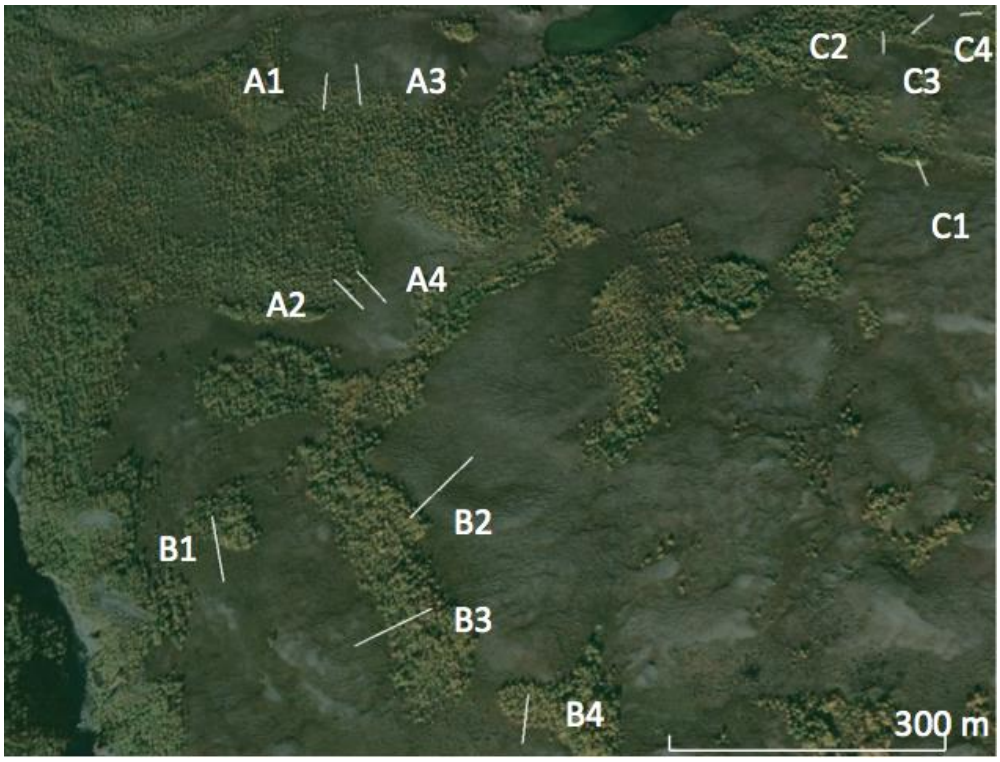

Figure 3.1: Google Earth images showing Abisko Ecotones. A, B and C refer to different geographical blocks

Data loggers

A Tinytag Plus Two logger with PB-5001 thermistor probe (Gemini Data Loggers, Chichester, UK) was set up at six paired H and F plots (A1, A2, B2, B4, C1, C4, i.e. two from each block) to measure soil temperature at 5 cm depth. Loggers recorded, hourly, from June 2012 until June 2014. Hourly mean and standard error data for vegetation type were generated from the six replicate H and F plots for the duration of the measurement period. Measurement seasons (summer and winter) were allocated based on the date at which soil temperature at 5 cm depth at all heath plots deviated and remained below 0 °C (Winter), and when they deviated and remained above 0 °C (Summer) at all heath plots. According to this definition based on the temperature of the soil, Winter 2012 lasted from 16/10/2012- 14/5/2013 and Winter 2013 from 20/10/2013- 18/5/2014. At transect B2 at the F plot, a HOBO U23-002 thermistor shielded from direct sunlight using a well-ventilated screen, connected to a HOBO microstation data logger (Onset, Bourne, MA, USA), was used to measure air temperature from July 2012 to August 2013. Surveys of soil moisture were carried out at all H and F plots. Using a HH2 ThetaProbe soil moisture meter (Delta-T Devices, Cambridge, UK), a mean of three soil moisture measurements all within 20 cm of a central point, at 5 cm depth was taken. This was done five times through June and July 2013.

Soil transplants

Intact soil monoliths (herein referred to as 'core') were transplanted between H and F plots at each transect, with appropriate controls. At each plot, two 15 cm diameter circles were cut in the soil approximately 2 m apart at each plot. At each circle, a 16 cm deep, 15 cm diameter PVC tube was driven into the soil until the entire collar (top of

the PVC tube) was level with the understorey vegetation. The core was then carefully extracted to avoid excess disturbance of the soil. Of the two extracted cores, one was randomly selected to be transplanted to one of the holes created in the contrasting vegetation community (e.g. H to F and F to H) and one was selected as control and inserted back into the hole it came out of. Good stability of cores and close contact with surrounding soil was ensured by carefully replacing soil removed during extraction of PVC tubes. In all F soil cores, the base of the core comprised mineral soil, therefore confirming that the entire organic horizon had been transplanted; this was not the case with H soil cores, where the organic horizon often exceeded 16 cm. All transplants were completed in five days by 6 June 2012, this was therefore counted as the first day of the experiment. On 12 June and 21 July 2013, each soil core was carefully rotated 90° to disrupt any connection (such as roots and fungal hyphae 'importing' autotrophic C into cores from surrounding vegetation) between the soil in cores and the native soil/parent material (which was often comprised of large clasts). One soil moisture measurement at 5 cm depth was taken at every transplant core on 15/6/2013 but this was not repeated to ensure minimal disturbance and minimal increased aeration of the cores.

Respiration measurement

A portable EGM-4 infrared gas analyser with a darkened CPY-2 chamber (PP Systems International, Amesbury, MA, USA) was used to measure respiration of all soil cores. Respiration in this study is defined as the sum of microbial, root and shoot (including cryptogam) respiration within the chamber. Respiration was measured from all collars in June and September 2012, July and September 2013, and in June 2014. Respiration rates were calculated based on the linear increase of CO_2 concentration within the

closed system over a period of 90 seconds. Tests with longer regression periods showed no improvement of fit compared with regression results obtained over 90 seconds (see also Chapter 2). On each sampling day, all 48 collars were measured over a period of two days from 0900-1600 hours. Complete blocks were measured on the same days to avoid bias from variations in temperature and moisture over the 2-day periods. The order in which blocks and transects within blocks were measured was randomised, as was the order of sampling within transects (i.e. H then F, or F then H). On 26th July 2013, a vegetation survey was conducted of the species present within the PVC tube of each transplant collar and percentage cover of ericaceous species (E_{cov}) and graminoid and forb species (G_{cov}) recorded, the sum of the two representing total vascular plant cover (Tot_{cov}).

Table 3.2: Percentage vegetation cover (± 1SEM to one decimal point) of key species and groups (July 2013) present in transplant collars and forest and heath plots

Soil origin	Treatment	*E. nigrum*	*V. myrtillus*	*V. vitis-idaea*	*V. uliginosum*	Grass	Forb	Feather moss	Tot_{cov}
Forest	Transplant	1.5 ± 0.9	0.0	6.2 ± 1.5	0.4 ± 0.3	1.4 ± 1.2	0.5 ± 0.4	0.4 ± 0.4	10.0 ± 2.2
	Control	6.3 ± 3.9	1.1 ± 0.6	6.7 ± 1.4	1.7 ± 1.7	16.7 ± 8.9	9.2 ± 7.9	5.0 ± 4.2	41.5 ± 12.2
Heath	Transplant	24.6 ± 5.4	0.0	5.4 ± 2.5	2.1 ± 0.6	0.2 ± 0.2	0.0	3.8 ± 2.5	32.3 ± 4.7
	Control	25.2 ± 6.5	0.0	4.5 ± 1.2	4.1 ± 1.2	0.0	0.0	0.0	33.8 ± 6.9

Microbial biomass estimation

The organic horizon from four 1 cm diameter, 5 cm deep soil cores was extracted from each soil collar (transplants and control) on 19 June 2014, immediately after completion of respiration measurement at each collar. The cores were homogenised and large roots removed by hand on the day of sampling. Two 2 ± 0.1 g subsamples of soil (fresh weight (FW)) from each core were weighed into glass vials, lid put on to maintain field moisture, and incubated at 19 °C for three days. Following the chloroform fumigation method (Vance *et al.*, 1987), one of each of the paired subsamples was then fumigated in an evacuated desiccator with 25 ml of $CHCL_3$. After boiling the $CHCL_3$ for 3 minutes, samples were left to fumigate for 24 hours. Fumigated and non-fumigated samples were extracted in 20 ml H_2O and shaken at 150 rpm for 30 minutes. Samples were then immediately filtered through a Whatman number 42 filter and acidified with 1 ml 1 % H_2SO_4 to prevent microbial activity prior to analysis. Samples were diluted to 1:3 and then analysed for total organic carbon (TOC) in solution using a Sievers 5310c TOC Analyser (GE Analytical Instruments, Boulder, CO, USA). Microbial biomass of the soil taken from collars was calculated as the difference in TOC between fumigated and non-fumigated extractions.

Data analysis

In all linear mixed effects analysis of the transplant soil cores, the soil type (H or F) was defined as 'Origin' and the placement of the soil core (in either H or F plots) was defined at 'Site' and inputted into linear mixed effects models as fixed effects. Respiration measured from transplants in the forest was found to be significantly higher than at heath sites in the growing season of 2012, i.e. before the impact of winter

conditions (linear mixed effects model; P = 0.002; Table 3.4)). The reason for this is unknown, but in order to understand the effect of winter temperature on summer respiration rates in 2013, the mean respiration rate for each collar over 2012 was calculated and the rates measured at every collar in 2013 were divided by this average (one 2012 average flux per collar). The standardised data from 2013 were then analysed using a repeated measures nested ANOVA following a linear mixed effects model with measurements nested within block, transect and repeated measures. This was done after log transformation of the response variable in order to meet the assumptions of the linear model. Reduction of snow cover is known to reduce vascular plant cover (Kreyling *et al.*, 2012), therefore Tot_{cov} (see table 3.2 for vegetation cover)was included in the analysis of respiration as a fixed effect covariate, after it was found to best refine the linear mixed effects model (compared to combinations of E_{cov} and G_{cov}). Microbial biomass and respiration rates in response to transplant treatment were also analysed using a nested ANOVA following a linear mixed effects model. In all analyses, if the data violated the assumptions of the linear models, they were natural log transformed, with no further transformation required. All analyses were carried out using R studio V0.98.501.

3.4 Results

Soil temperature and temperature

Temperature was lower at H plots than F plots in both winter seasons: (Table 3.3, Fig. 3.2). This coincides with deep snow at the F plots and shallow snow depth at the H plots in both winters (Table 3.3). Winter mean temperatures contrast with only small differences in mean summer soil temperatures between vegetation types (Table 3.3). Soil temperatures over winter 2012-13 in H plots were also more variable than those at F plots as soil temperature was more strongly coupled to air temperature (F: coefficient = 0.06, H: coefficient = 0.24 (Fig. 3.3)).

Forest soil (not in the transplants) had consistently lower moisture than soil at the heath plots remaining on average at 24.9 % compared to 35.2 % (Fig. 3.4). The difference was less pronounced in the soil cores where all transplants and controls were ~ 30 % (Table 3.4).

Table 3.3: Mean temperature and snow depth measured at H and F plots. All temperature measurements are an average of hourly means over the whole season. All error signified is ± 1SEM to 1 decimal point.

Plot	Summer 2012 (°C)	Winter 2012-2013 (°C)	Summer 2013 (°C)	Winter 2013-2014 (°C)	Winter 2013 Snow (cm)	Winter 2014 Snow (cm)
Forest	5.5 ± 0.1	-1.2 ± 0.0	4.8 ± 0.1	-0.5 ± 0.0	46.8 ± 3.4	73.9 ± 7.0
Heath	5.0 ± 0.1	-4.2 ± 0.1	4.1 ± 0.1	-3.5 ± 0.1	13.1 ± 1.8	14.0 ± 2.5

Table 3.4: Soil moisture and DOC measured at H and F plots and within transplant collars. Moisture content in collars measured on 15/6/2013, DOC sampled on 19/6/2014. All error signified is ± 1SEM to 1 decimal point.

Soil	Transplant treatment	Moisture (%)	DOC (µg g soil $^{-1}$)
Forest	Transplant (to heath)	30.1 ± 2.6	20.6 ± 3.1
	Control	29.6 ± 1.5	17.5 ± 2.0
Heath	Transplant (to forest)	31.2 ± 2.0	16.6 ± 3.7
	Control	32.7 ± 1.3	16.0 ± 2.2

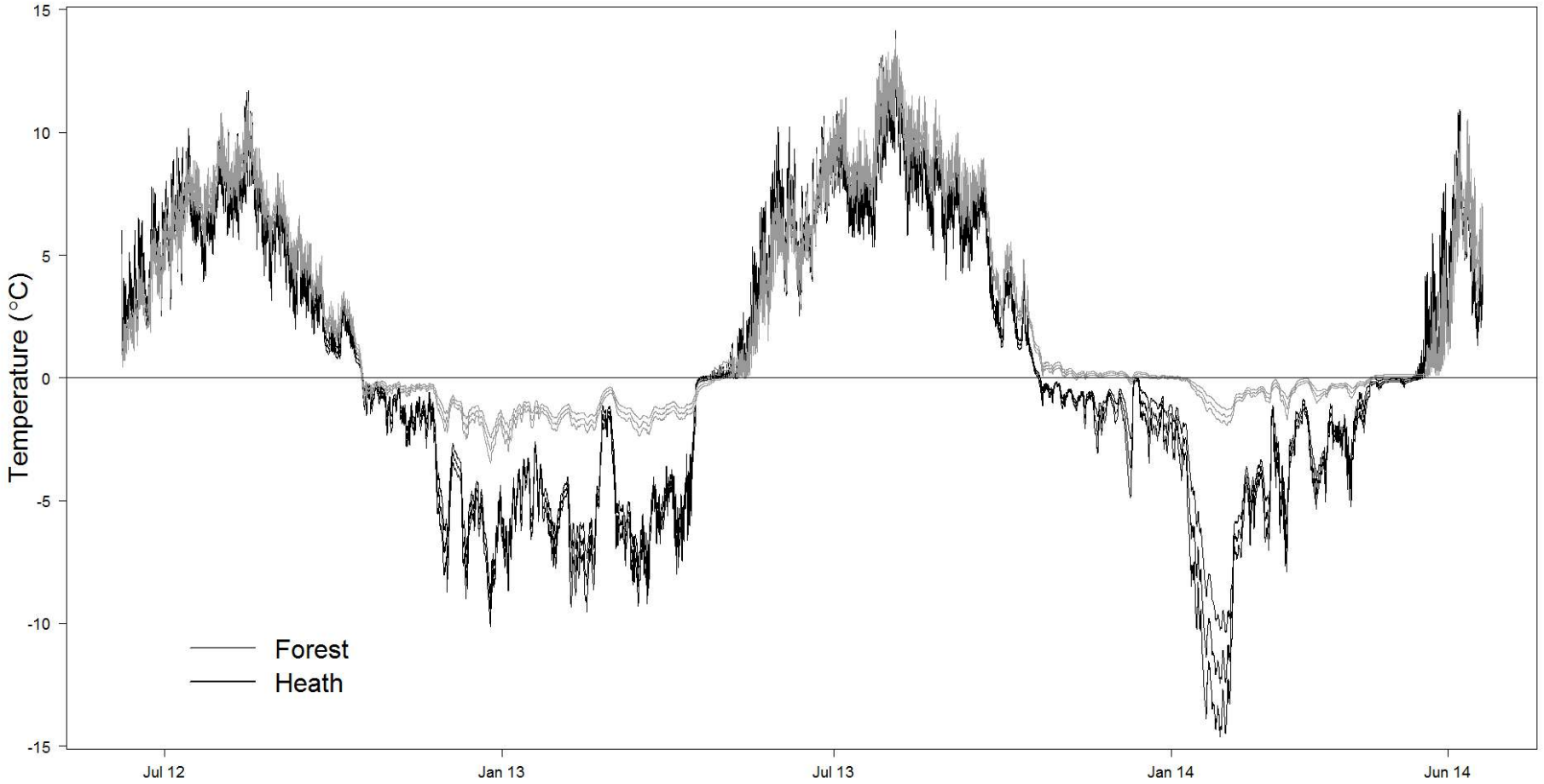

Fig. 3.2 Hourly soil temperature at replicated H (black) and F (grey) plots. Middle line of three represents the running hourly mean of all plots, outer two lines represent a running ± 1 standard error of the hourly mean (n = 6 per plots type).

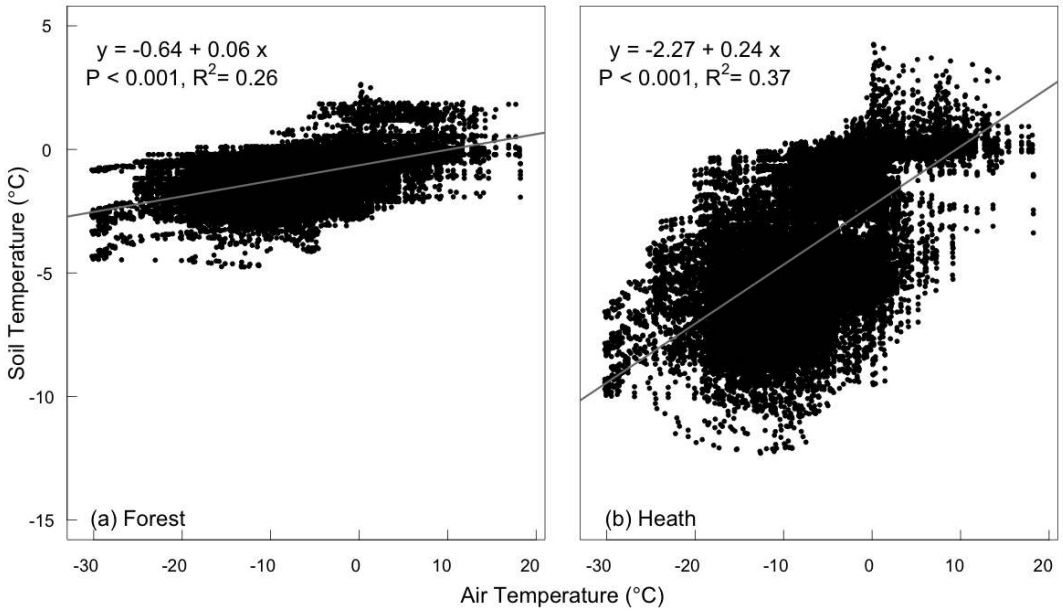

Figure 3.3: The relationship between air temperature and soil temperature at 5 cm depth in (a) Forest plots and (b) Heath plots over the winter of 2012-13.

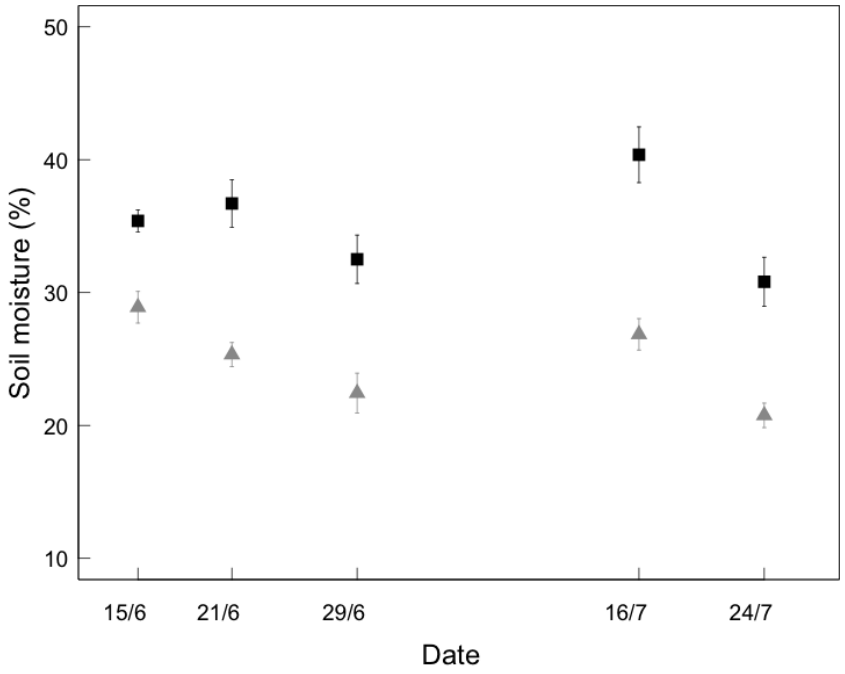

Figure 3.4: Soil moisture at 5 cm depth measured at Heath (Black Squares) and Forest (Grey triangles) plots over summer 2013. Error bars represent ± 1 SEM

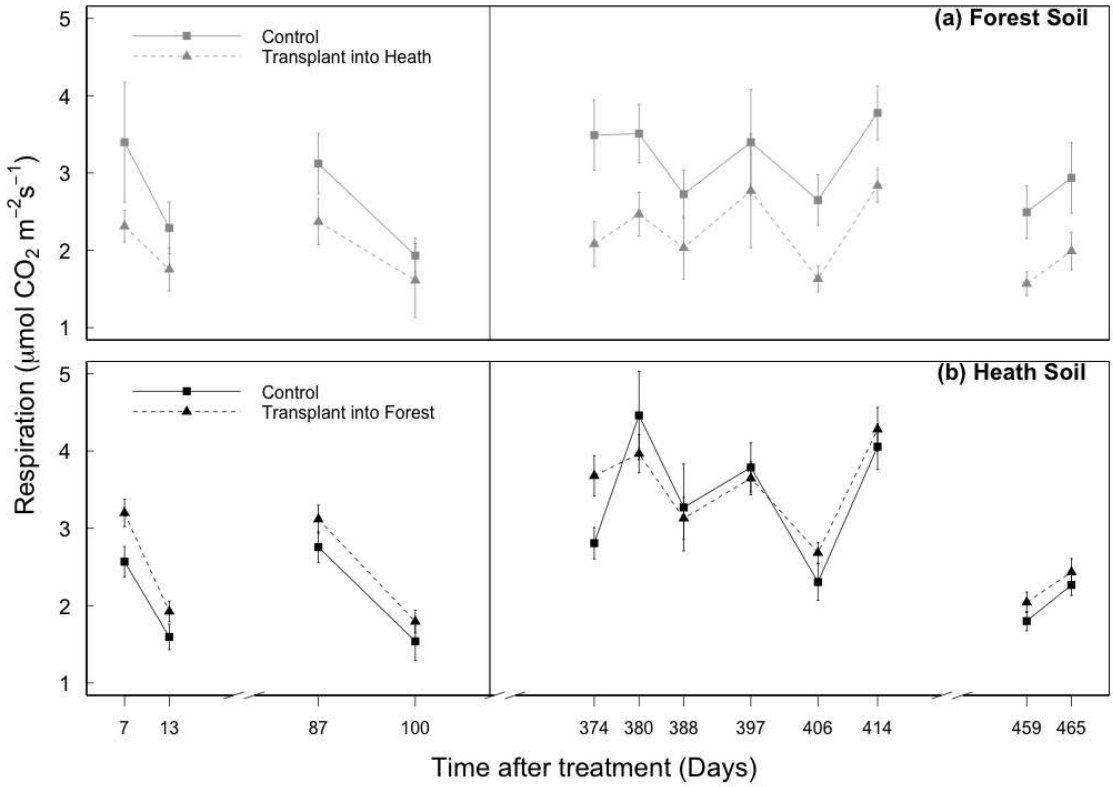

Figure 3.5 Dark respiration over 2012 (left) and 2013 (right) (with time after treatment indicated on the x axis) from (a) forest soils and (b) heath soils, transplanted into the contrasting environment (dashed lines) or remaining in 'home' environment as a control (solid line). Error bars represent ± 1SEM, n = 12).

Respiration rates from soil transplants

There was a significant effect of site on respiration rates of the soil cores in 2012 (before winter; P = 0.002, Table 3.5, Fig. 3.5), with rates in the forest (F control: 2.67 ± 0.23 µmol m^{-2} s^{-1}, H transplant: 2.5 ± 0.13 µmol m^{-2} s^{-1}) higher than cores in the heath (H control: 2.11 ± 0.13 µmol m^{-2} s^{-1}, F transplant: 2.01 ± 0.17 µmol m^{-2} s^{-1}). There was no effect of soil origin on respiration rates in 2012.

In a similar way to 2012, there was also a significant effect of site on respiration rates (P = 0.001, Table 3.5, Fig. 3.5), with cores in the forest (F control: 3.10 ± 0.14 µmol m^{-2} s^{-1}, H transplant: 3.23 ± 0.11 µmol m^{-2} s^{-1}) having higher rates than those in the heath (H control: 3.06 ± 0.15 µmol m^{-2} s^{-1}, F transplant: 2.15 ± 0.12 µmol m^{-2} s^{-1}). This difference was primarily driven by a reduction of respiration compared to control of the F soil transplanted in the heath (Fig. 3.5a). In 2013, there was however an important effect of soil origin (P = 0.002). The most important factor in this analysis was the total cover of vascular plants, Tot_{cov} (P < 0.001), which was positively associated with respiration rates (Table 3.5).

When fluxes in 2013 were standardised by dividing by the mean flux of each core in 2012 ($Respiration_{Rel}$, Fig. 3.6, Table 3.5), there was a near significant (P = 0.08) effect of site on fluxes, with higher respiration rates in the forest. Again, there was a significant effect (P = 0.005) of Tot_{cov} which was positively associated with respiration rates.

Table 3.5: Test statistics from nested ANOVAs testing the effect of soil origin (H or F), site (H or F) and total vascular plant cover (T_{cov}) on respiration rates from soil cores. Significant ($P < 0.05$) factors are highlighted in bold.

Data	y transformation	Factor	d.f.	F	P
2012 Respiration	Log_e	Origin	1,32	0.70	0.41
		Site	**1,32**	**10.53**	**0.002**
		Origin*Site	1,32	0.38	0.54
2013 Respiration	Log_e	**Origin**	**1,32**	**11.19**	**0.002**
		Site	**1,32**	**12.81**	**0.001**
		Origin*Site	1,32	0.23	0.64
		Tot_{cov}	**1,32**	**24.69**	**< 0.001**
2013 $Respiration_{Rel}$	Log_e	Origin	1,32	0.001	0.98
		Site	1,32	3.16	0.08
		Origin*Site	1,32	0.02	0.89
		Tot_{cov}	**1,32**	**6.19**	**0.02**

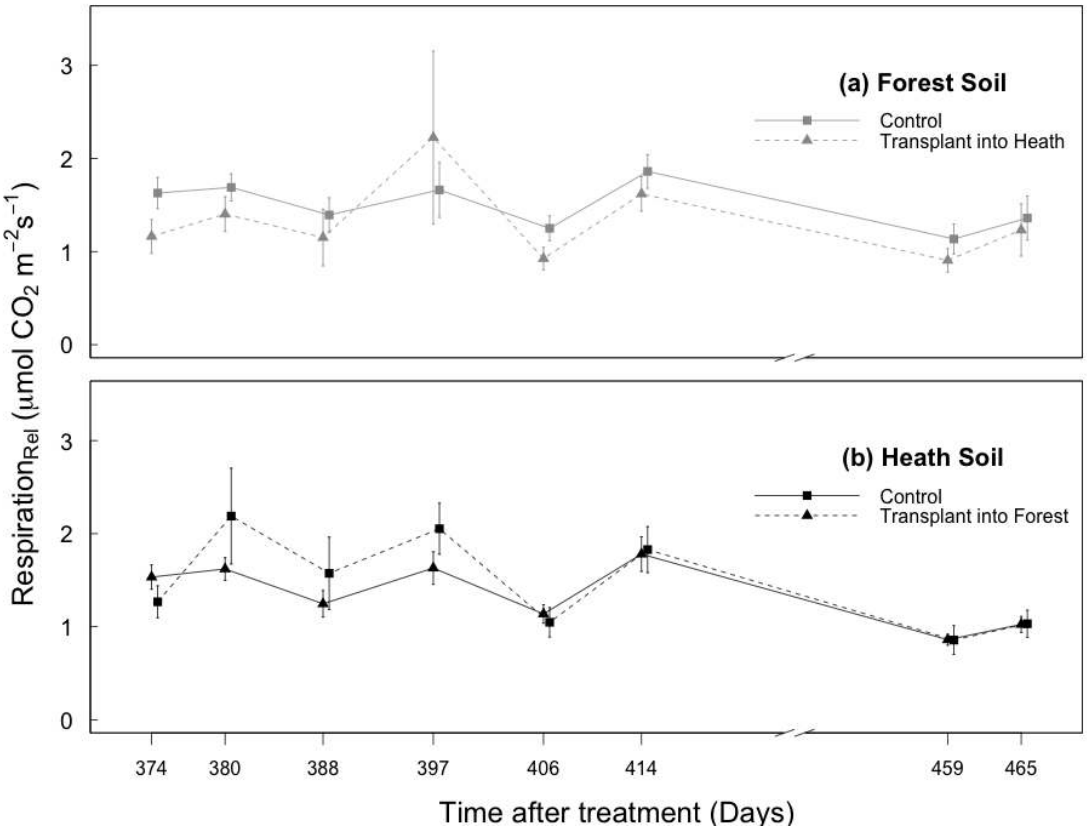

Figure 3.6: Dark respiration in 2013 standardised by dividing measured rates by rates recorded in 2012 ($Respiration_{Rel}$) from (a) forest soils and (b) heath soils. Error bars represent ± 1SEM, n = 12).

Final respiration and microbial biomass measurements

There was a significant effect of site on respiration rates in June 2014, with significantly higher respiration in F than in H plots (P = 0.009, Fig. 3.7a, Table 3.6). Unlike respiration rates recorded during 2013, where F control cores had the highest rates (Fig. 3.5), this was primarily driven by high rates recorded from H transplanted into F plots (2.35 ± 0.26 µmol m^{-2} s^{-1}(Fig. 3.7a)). In contrast, H control cores had the lowest mean rate (1.67 ± 0.27 µmol m^{-2} s^{-1}), and this resulted in a significant interaction between site and soil origin within the linear mixed effects model (Table 3.6). This was also reflected in respiration rates standardised by mean rates observed in 2012, before the two winter periods, where there was a close to significant effect of site on $Respiration_{Rel}$ (P = 0.054, Fig. 3.7b). Microbial biomass was highest in F control soil cores but there was no effect of origin, or site, or an interaction between the two on microbial biomass measured from the soil cores (Table 3.6, Fig. 3.7c). There was also no relationship present between microbial biomass C and respiration from these paired measurements (P = 0.36, Fig. 3.7d).

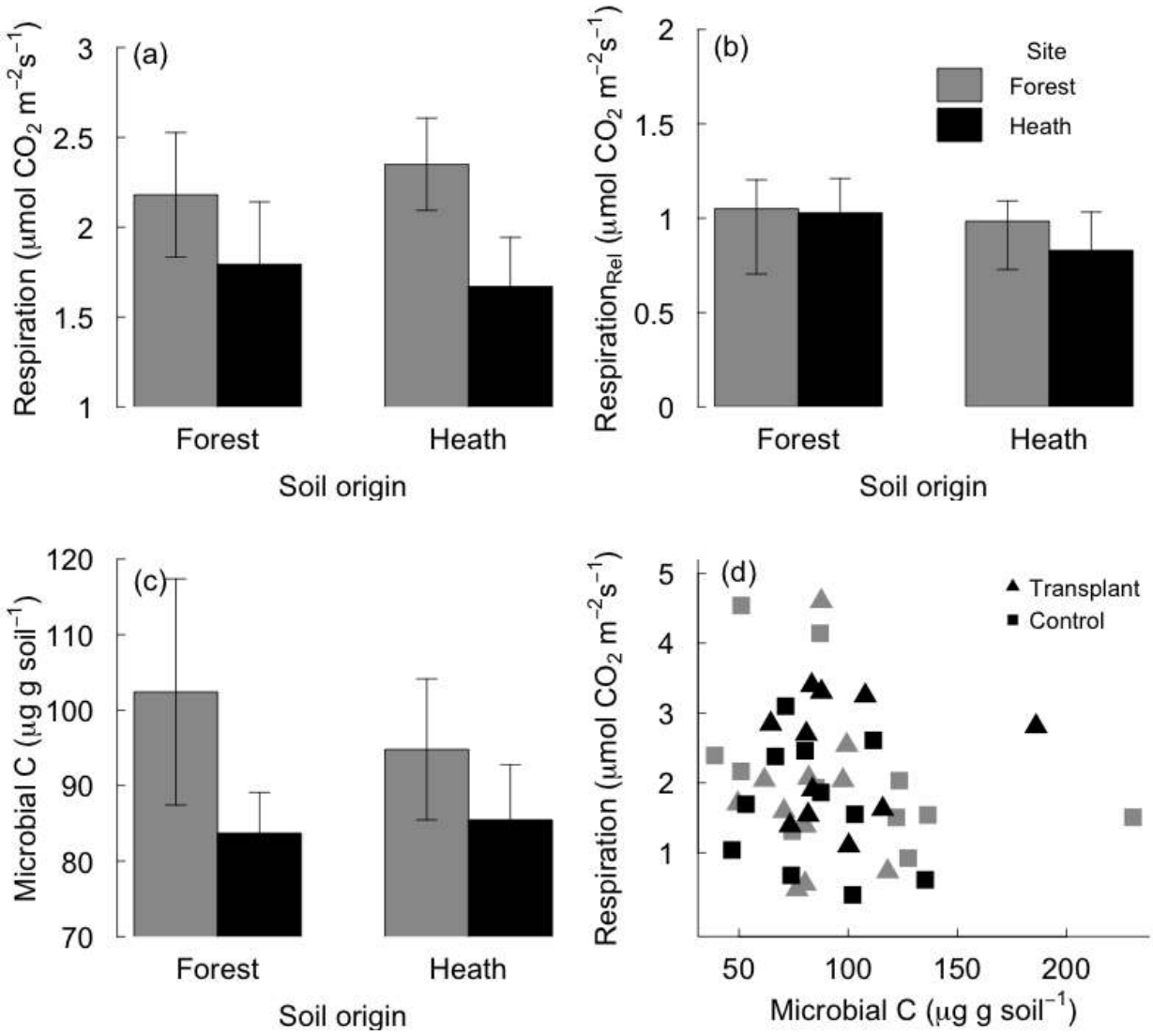

Figure 3.7: Dark respiration and microbial biomass measurements taken June 2014 from heath and forest soils transplanted in heath environment (Black bars) or forest environment (grey bars). (a) Dark respiration (b), respiration relative to 2012 (see Fig. 5 for more information), (c) microbial biomass C with soil origin indicated (n=12 per bar, bars represent ± 1SEM), and (d) microbial biomass plotted against respiration, showing no relationship between the two (P = 0.36).

Table 3.6: Test statistics from nested ANOVAs testing the effect of soil origin (H or F), site (H or F) and total vascular plant cover (T_{cov}) on respiration rates and microbial biomass in June 2014 from soil cores. Significant ($P < 0.05$) factors are highlighted in bold

Data	y transformation	Factor	d.f.	F	p
2014 Respiration		Origin	1,20	0.005	0.94
		Site	**1,19**	**8.47**	**0.009**
		Origin*Site	**1,19**	**3.21**	**0.021**
		Tot_{cov}	1,19	6.29	0.089
2014 $Respiration_{Rel}$		Origin	1,20	0.68	0.42
		Site	1,19	0.39	0.054
		Origin*Site	1,19	1.64	0.22
		Tot_{cov}	**1,19**	**6.05**	**0.02**
2014 Microbial biomass	Log_e	Origin	1,22	0.00	0.99
		Site	1,22	1.53	0.23
		Origin*Site	1.22	0.002	0.97

3.5 Discussion

This experiment demonstrated, over a replicated ecosystem scale, the importance of the interaction between vegetation and snow cover on summer carbon dynamics at an important treeline ecosystem. It showed that forest systems create a warmer, more stable environment for the maintenance of soil microbial processes (H1). Secondly, using a novel experimental approach, it suggested the importance of the winter snow pack created by forests, for maintaining high summer respiration rates (H2). In addition to this, evidence suggests that the respiration rates of heath soils can be stimulated by only two winters of increased snow pack, therefore suggesting a mechanism by which C could be lost if forests encroach onto heath. It was hypothesised (H3) that microbial biomass would be increased by being in a forest environment (with ameliorated winter temperatures related to deeper winter snow; Table 3.3, Figs. 3.2 and 3.3), but this was not supported by the data.

Our results are consistent with a significant body of evidence that tall stature vegetation, such as trees and shrubs, substantially changes the soil environment over winter (Sturm *et al.*, 2001; Sturm *et al.*, 2005; Grogan & Jonasson, 2006). This work has used a replicated design across a subarctic ecosystem to demonstrate without doubt that winters are warmer in forest soils (Fig. 3.2), due to snow accumulation, because they insulate the soil from extreme air temperature and fluctuations (Fig. 3.3) (H1). For example, when air temperature dropped below -10 °C, mean soil temperature in the heath could drop as low as - 12 °C whereas in the forest it did not drop below was -5 °C (Fig. 3.3), but mostly remained stable at ~ -1-2 °C regardless of the air temperature. In other words forest soils were protected

from serious temperature fluctuations and maintained at a temperature at which microbial population growth can continue (McMahon *et al.*, 2009). The depth of the snowpack created in the forests also allowed for a more stable temperature in the soil, which would make it less susceptible to freeze-thaw cycles (Haei *et al.*, 2010) and possibly even extreme winter warming events which can cause large scale ecosystem damage at multiple trophic levels (Bokhorst *et al.*, 2012).

The relationship between deep snow cover, soil temperature and microbial metabolism over winter is well known (Schimel *et al.*, 2004; Sturm *et al.*, 2005; Grogan & Jonasson, 2006; Monson *et al.*, 2006; Nobrega & Grogan, 2007). In the same landscape as the present study, Grogan and Jonasson (2006) showed that winter respiration was significantly higher at forest plots than heath plots. Furthermore, in similar plots to the present study, suggesting decomposition of 'old' SOC was being stimulated in the forest environment (Hartley *et al.*, 2013) . Therefore, although ER was not measured over winter, it is reasonable to suggest that fluxes of C were higher in the forested plots compared to the heath plots.

There were significantly higher respiration rates in the soil cores placed in the forest compared to those placed in the heath in 2012. This was not an intended treatment but it is nonetheless interesting. The transplant treatment was intended to change soil temperature over winter but it is possible that the small difference in summer temperature (0.5 °C) may have stimulated increase in respiration rates over the measurement period in 2012. Respiration rates of birch forest and ericaceous heath are known to be responsive to summer soil temperature increase in the summer over typical ranges of 4-8 °C (Sjögersten & Wookey 2002). Mean summer temperature

measured over 2012 was 5.5 °C in the forest and 5.0 °C in the heath, and the difference was 0.7 °C in 2013. A warming of tundra soil in sub-arctic Sweden by approximately 1 °C was found to increase heterotrophic respiration but the effect of summer warming and winter snow addition on ecosystem respiration was larger than summer warming alone (Dorrepaal et al. 2009). One question is whether small differences in summer temperature between vegetation types significantly effects respiration. The second is which season's temperature differences contributes the most to the respiration differences observed here: the indirect effect of the large temperature change over winter or the direct effect of small change in the summer. The data presented here not able to compare the relative importance of these drivers.

This work is the first to address the question of winter environment in influencing growing season C cycling rates in a sub-arctic context, and also the first to use the approach of soil transplants to simulate a future increase in snow due to treeline change on heath soils. Summer respiration rates were decreased in forest soils when transplanted to the heath (H1). This could be due to two mechanisms: Firstly, transplanting forest soil into the heath winter environment may have reduced microbial activity over winter due to less insulation which, in summer resulted in reduced activity which was measured as respiration. Secondly, a difference in plant cover between forest soils transplanted into the heath and controls due to, presumably, frost damage caused a reduction in respiration rates observed in the 2013 growing season.

Reduction of plant cover and associated respiration with reduction of snow cover is consistent with an experiment in boreal Sweden (Kreyling *et al.*, 2012). Other than this, a number of different factors were controlled for in order to address the response of soil microbial respiration rates to snow cover change. It was conducted in a permafrost-free and well-drained ecosystem which therefore eliminated the confounding effects of active layer (Rogers *et al.*, 2011) or soil moisture changes through saturation (Natali *et al.*, 2011), or drying (Chimner & Welker, 2005) associated with snow depth manipulations. Mean soil moisture was found to vary by only three per cent between all the treatments (Table 3.4). This, however, is only one measurement of soil moisture which varies enormously with time. A more detailed survey of the surrounding soil showed that forest soil was found to be consistently drier. Soil moisture may still be an important influence contributing to C cycling differences between forest and heath that was not sufficiently measured in this study. DOC did not vary significantly between collars as has been reported in other studies (Oquist & Laudon, 2008; Haei *et al.*, 2013). Again, this was only measured once so the conclusions that can be drawn are limited. The microbial communities in forest and heath may have been altered by the different snow depths and in turn had different summer respiration rates. The other factor identified as important in the statistical models was the vascular plant cover but when this is accounted for, the affect of winter environment still remained significant.

In contrast to the change in vegetation cover observed with forests soils when transplanted into a colder winter environment, there was no (measured) change in plant communities associated with heath soils (Table 3.2) transplanted into forest. Respiration of heath soils took two years to increase in response to transplantation

compared to the control treatment (effect detected in 2014: Fig. 3.7b). This is of particular interest because, in contrast to removing the 'normal' snow cover and seeing a reduction in respiration rates, it has been shown that in a soil, which normally experiences very cold temperatures in winter, respiration can be stimulated in the growing season by increasing winter snow for two years.

Winter snow-summer C cycling interactions may represent an important feedback between shrub expansion and soil carbon cycling when considering the large geographical scale of the pan-Arctic shrub expansion (Tape *et al.*, 2006; Myers-Smith *et al.*, 2011). Snow accumulation by shrubs (Sturm *et al.*, 2001) increases snow depth locally (by up to one meter) far more than the observed loss of snow depth due to climate change across the Arctic (Park *et al.*, 2013). If snow accumulation and associated warmer soils is integrated into earth system models, carbon storage is shown to be reduced due to continued winter decomposition (Gouttevin *et al.*, 2012). Therefore the large increases in snow associated with shrub expansion may have important effects on the carbon balance of the arctic as respiration is stimulated in winter (Grogan & Jonasson, 2006) and in the summer, as shown by this work. In the context of shrub expansion, C stored in the soil (Tarnocai *et al.*, 2009) far outweighs recent increases in sequestration of C into biomass as a result of increases in productivity (Epstein *et al.*, 2012). Recent work has suggested that C stored in tundras may be vulnerable to decomposition if colonised by shrubs (Chapter 2) or trees (Hartley *et al.*, 2012). The experiment here highlights another mechanism by which these soils could lose C in the future which is supplemental to effects mediated by shifts in soil-plant mycorrhizal status and/or rhizosphere priming.

The final hypothesis that the soil 'incubated' over winter in the forest would have a larger microbial biomass compared with soils in the tundra heath (H3) must be rejected; there were no differences in microbial biomass between sites or soil origins, and there was no significant relationship between microbial biomass and the respiration measured shortly beforehand (Fig. 3.7c,d, Table 3.6) as was expected (Wang *et al.*, 2003; Lipson *et al.*, 2005). This is consistent with studies which only show small increases in microbial biomass in sub-arctic soils in response to warming (Clemmensen *et al.*, 2006; Rinnan *et al.*, 2007) which need a concurrent increase in nitrogen availability to be significantly stimulated. It is therefore hypothesised that deep winter snow cover in sub-arctic forests influences microbial respiration in summer by changing the microbial community composition as opposed to total mass of microbes.

The soil transplants, with their PVC collars to depth and rotation to severe any connections at depth, were removed from the influence of the birch forest rhizosphere, therefore significant plant-soil interactions, for example priming (Hartley *et al.*, 2012) or ectomycorrhizal in-growth (Chapter 2) , were considered unlikely in this case (except for the effect of the small cover of understorey plants). A change in free-living fungi and bacteria are therefore likely to be influencing biogeochemical cycling in the transplanted soil cores. Previous work has found that soil fungal biomass (especially free-living fungi) is very high over winter (Schadt *et al.*, 2003; Zhang *et al.*, 2014). This is particularly the case under deep snow-packs which maintain temperatures no lower than -1 °C (Kuhnert *et al.*, 2012) where fungi are found to have highest enzyme production over the year (Voriskova *et al.*, 2014).

Significant fungal cell damage occurs below -10 °C, and fungi in soils which are annually exposed to temperatures this low have specific adaptations such as slow growth and low temperature growth optima whilst investing in antifreeze production (Robinson, 2001; Robinson, 2002). It is possible that the microbial population adapted to a tundra environment is not able to metabolise as rapidly in the summer as a population that has been 'incubated' at a warmer winter temperature and do not have a need for such adaptations.

Fungal populations have turnover times of up to hundreds of days, orders of magnitude higher than that of bacteria (Rousk & Baath, 2011). It is therefore likely that the fungal community in the forest which has been 'incubated' in a more stable and warmer environment than the heath will still be present into the growing season once the snow has gone and both communities are subject to similar thermal regimes. Differences were not observed in biomass between the two microbial communities, therefore the differences in respiration rates that were measure may be driven in part by a difference in the composition of the communities shaped by winter temperature patterns. Equally, bacterial communities differ in the winter compared to the summer (Lipson & Schmidt, 2004) and are therefore likely to vary depending on snow cover. Due to a shorter turnover time (Rousk & Baath, 2011) the effect of winter on summer process via bacterial communities maybe more limited but should not be ignored. This considered, it could be hypothesised that the increased snow cover in the forest fosters conditions which favour a faster metabolising, more fungi-dominated microbial community, which, in turn metabolises C more rapidly in summer.

In conclusion, this manipulation of winter snow cover, replicated over a landscape scale, shows that forest soils rely on deep snow and associated insulation for fast growing-season C cycling rates. This deep snow could stimulate summer decomposition rates in adjacent tundra heaths (i) if shrub and forest dominated systems expand in the future due to warming local climates, and (ii) if there is sufficient regional snowfall to produce an increase in snow-depth in these systems due to drifting. There were no differences detected in microbial biomass between transplant and control soil cores therefore it could be possible that snow accumulation in these forests fosters a fungal community that maintains fast rates of carbon cycling into the growing season. Continuing work on this system should therefore focus on quantifying the microbial communities, and their composition, created by snowpack accumulation in this treeline ecosystem.

Chapter 4: Slowed biogeochemical cycling in subarctic birch forest linked to mycorrhizal community change after a defoliation event.

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Author notes

This work came about as a collaboration of TCP, J-AS and PAW with HD, JS and SF from the University of New Hampshire. The chapter was written by TCP with contributions to the text by JA-S, PAW, SF and JS. TCP established the study plots, conducted respiration, made soil N measurments and ECM ingrowth measurements. HD sampled on plots set up by TCP. It should be clear that all mycorrhizal community composition and enzyme assays were sampled in the field by HD, processed in the lab by HD and JS, and analysed by JS with supervision from SF. Therefore, Figures 4.5,4.6,4.7,4.8 and Table 4.2 in the chapter and Table S2 and Figure S1 in Appendix 2 are contributed only with permission from HD, JS and SF.

4.1 Abstract

Sub-arctic birch forests (*Betula pubescens* Ehrh. ssp. *Czerepanovii*) periodically suffer large-scale defoliation events caused by the caterpillars of the geometrid moths *Epirrita autumnata* and *Operophtera brumata*. Despite their clear importance to forest ecosystem functioning through a large decrease in productivity, little is known about how the reduction in belowground C allocation affects soil processes. The response of soil processes following a natural defoliation event in sub-arctic Sweden was quantified by measuring respiration, nitrogen availability,

ectomycorrhizal (ECM) hyphal production, ECM root tip community composition and enzyme activity.

There was a reduction in respiration and an accumulation of soil inorganic N in defoliated plots, symptomatic of a slow-down of soil processes. This coincided with a reduction of ECM hyphal production and a shift in the ECM community to lower C-demanding lineages (e.g. /russula-lactarus) which produce more C-degrading enzymes. Microbial and nutrient cycling processes shift to a slower, less C-demanding state in response to canopy defoliation. These events are becoming more frequent with climate warming and therefore their impacts merit improved understanding.

4.2 Introduction

Mountain birch forests (*Betula pubescens* Ehrh. ssp. *czerepanovii* (Orlova) Hämet Ahti) are the dominant treeline forests in most of northern Fennoscandia (Tommervik *et al.*, 2009; Hofgaard *et al.*, 2013). This forest is responsive to climate change, amongst other important drivers such as changes in reindeer management (Tommervik *et al.*, 2009; Van Bogaert *et al.*, 2011). The birch treeline has been observed to have expanded both in latitude in the last century (Hofgaard *et al.*, 2013) and in altitude in the last 34 years (Rundqvist *et al.*, 2011).

Pathogenic insect outbreaks are important controls over productivity in temperate, boreal (Hicke *et al.*, 2012) and sub-arctic ecosystems (Bjerke *et al.*, 2014). Cyclical outbreaks of the defoliating Autumnal Moth (*Epirrita autumnata*) and the Winter Moth (*Operophtera brumata*) are common and widespread across the mountain birch forests of Northern Scandinavia (Jepsen *et al.*, 2008). These outbreaks occur in waves across the Scandes mountains, with an approximate 10 year frequency (Tenow *et al.*, 2007), causing considerable damage to the canopy of *Betula pubescens* forests (Jepsen *et al.*, 2013) and contributing towards large decreases in forest productivity (Bjerke *et al.*, 2014). It is not clear whether the frequency of these outbreaks is increasing with climate change but the area of forest affected by these outbreaks has increased by ~ 5 ° east in longitude into the colder continent and ~ 2 ° north in latitude (Jepsen *et al.*, 2008). This is due to a warming of winter climate, allowing over-winter survival of eggs in areas that were previously too cold (Jepsen *et al.*, 2008). With the distribution and severity of defoliator insect outbreaks expected to expand further with climate change (Bale *et al.*, 2002; Jepsen

et al., 2008; Jepsen *et al.*, 2011), there is a pressing need to understand how the mountain birch forest ecosystem responds to such disturbance.

Sub-arctic forests are known influence on soil carbon (C) cycling by allocating recently assimilated C belowground, stimulating the decomposition of soil organic C and the release of nutrients (Hartley *et al.*, 2012). Below-ground transfer of labile C from trees to the rhizosphere drives microbial activity, respiration (Högberg *et al.*, 2001), and N immobilisation (Kaiser *et al.*, 2011). Since defoliation reduces the ability of sub-arctic birch forests to fix C (Heliasz *et al.*, 2011), it is expected that defoliation events strongly reduce C inputs to the rhizosphere and slow biogeochemical cycles.

In addition to altering C allocation patterns, defoliation events in sub-arctic ecosystems accelerate nitrogen (N) inputs into the soil via direct frass addition (excreted waste from insects), thereby potentially altering N cycling (Kaukonen *et al.*, 2013). N immobilisation by microbial communities has been in the soil is known to be driven by autotrophic C inputs (Kaiser *et al.*, 2011) and belowground C allocation is positively correlated with forest productivity (Litton *et al.*, 2007). In productive temperate ecosystems the N cycle responds quickly to N additions from frass through redistribution of N into microbial communities (Lovett & Ruesink, 1995), or reabsorption by the affected trees (Russell *et al.*, 2004; Frost & Hunter, 2007). In a less productive ecosystem such as the sub-arctic, reduced C supply to the rhizosphere alongside frass addition may result in an accumulation of N in the soil (as has previously been observed (Kaukonen *et al.*, 2013)), akin to a saturated ecosystem (Aber, 1992).

Ectomycorrhizal (ECM) fungi are a major recipient of autotrophic C in forest ecosystems, with up to 21% of plant C allocated below-ground transferred to the ECM community (Högberg *et al.*, 2001; Hobbie, 2006). This C supply allows ECM species to maintain dominance in the organic horizon of boreal forest soils over free-living fungi (Lindahl *et al.*, 2007), but this dominance can be disrupted by cutting the autotrophic C supply (Lindahl *et al.*, 2010). Therefore any reduction in C inputs below-ground may translate directly to a reduction in C supply to the mycorrhizosphere (Gehring *et al.*, 1997; Högberg *et al.*, 2001). When trees are defoliated, ECM fungi with lower C demand from their autotrophic host may hold a competitive advantage over species with that require a larger C investment (Saikkonen *et al.*, 1999; Markkola *et al.*, 2004). Widespread defoliation should therefore drive a change in ECM community composition, selecting for less C-demanding ECM taxa.

Having evolved from free-living saprotrophic fungi, some ECM taxa retain the ability to degrade organic matter to access N and other nutrients and so play a role in nutrient cycles (Talbot *et al.*, 2008; Bödeker *et al.*, 2014; Meier *et al.*, 2014; Phillips *et al.*, 2014; Brzostek *et al.*, 2015). There is a large amount of variation between taxa in the ability to produce C degrading enzymes (Lindahl & Tunlid, 2015), therefore a change in community composition with defoliation may lead to changes in overall community enzyme production.

A mountain birch forest (*Betula pubescens* ssp. *czerepanovii*) in sub-arctic Sweden was defoliated by a joint outbreak of the winter and autumnal moths (*Operophtera*

brumata and *Epirrita autumnata*) in early summer 2013 after an outbreak the previous year. This gave us the opportunity to measure the belowground response of this ecosystem to the associated reduction in autotrophic C supply. In particular addressed the following research aims are addressed: 1. Measure belowground C and N cycling in response to defoliation of the *Betula* canopy. 2. Compare hyphal growth, root tip community composition and enzyme activity of the ectomycorrhizal community at defoliated and non-defoliated plots. 3. Better understand how changes (if any) in soil C and N cycling link to ectomycorrhizal community structure and function in a subarctic forest ecosystem.

4.3Materials and Methods

Study site

Study sites were established in the treeline birch forest near Abisko, Sweden (~68°18' N, 18°49'E). The forest is made up of mountain birch (*Betula pubescens* Ehrh. ssp. *czerepanovii* (Orlova) Hämet Ahti) with a dominantly ericaceous understorey of *Empetrum hermaphroditum, Vaccinium myrtillus, Vaccinium vitis-idaea, Vaccinium uliginosum* and some shrubs including *Betula nana, Salix* spp. and *Juniperus communis.* The soil is a thin spodosol developed over glacial till and bedrock typically of hard-shale, with a thin (<5 cm) O horizon. Soil pH of the organic horizon is 4.5 ± 0.1 SE (standard error) (Chapter 2). Further details on soil properties can be found in Sjögersten & Wookey (2002) and Hartley *et al.* (2010). The forest defoliation event by *Operophtera brumata* and *Epirrita autumnata* began in May 2013 as budburst occurred across the forest, although the exact timing was highly dependent on local microclimates. The trees were at their maximum extent of defoliation and caterpillars were no longer present in the trees by June 19th, 2013 (Fig. 4.1). There was also widespread defoliation due to an outbreak by the same species the previous summer, which is unusual, but it was not documented in detail in this study area.

Study design

Defoliation was estimated by eye at every *B. pubescens* individual for percentage of leaves remaining, which was then converted to percentage defoliated (assuming a

full canopy prior to defoliation). Defoliation values for each tree are means of independent estimates by two different observers. Based on the visual extent of defoliation, replicate trees across one forest stand were selected as either defoliated (max. 5% of leaves remaining) or non-defoliated (at least 95% of leaves remaining), with n = 5 per group (Fig. 4.1). In addition to these plots, other forest stands were sampled to assess defoliation impacts at the landscape scale. Plots (24 in total, consisting of all trees within a 5 m radius of a central point) were distributed across multiple stands of mountain birch over a 2 km^2 area (hereafter referred to as Landscape Scale (*LS*) plots). All trees in each plot were estimated for defoliation using the method above

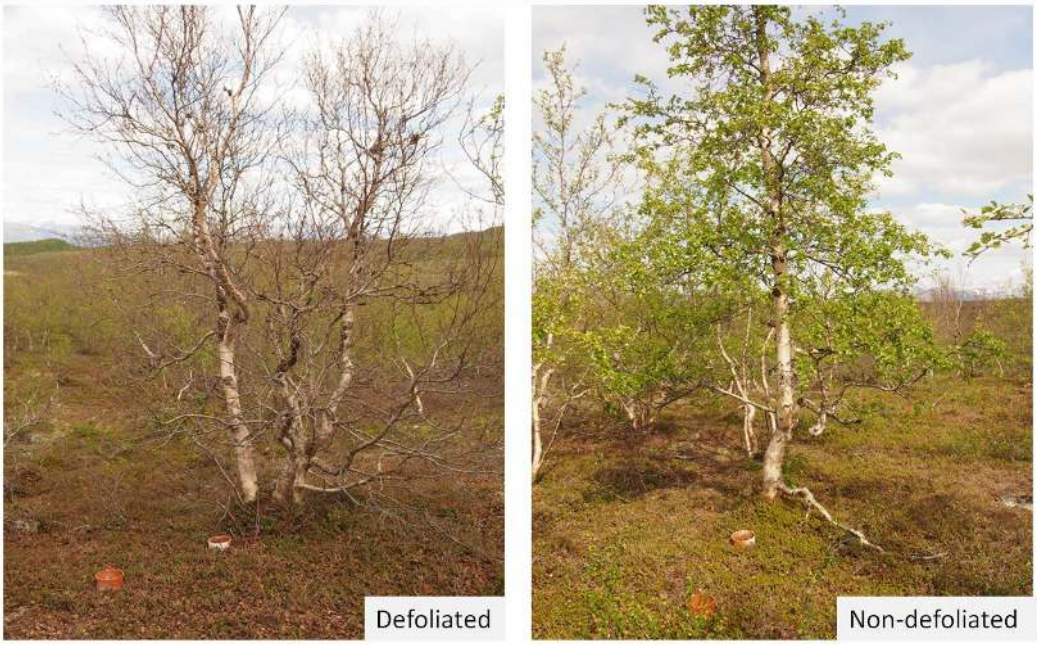

Figure 4.1: Two *Betula pubescens* study trees assigned to "defoliated" and "non-defoliated" categories based on the extent of defoliation by *Operophtera brumata* and/or *Epirrita autumnata* on 19th June 2013. Collars to measure soil respiration can be seen at 50 cm and 150 cm from the base of each tree.

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Respiration

At defoliated and non-defoliated plots, two PVC collars (15 cm diameter x 7 cm

high) were placed on the soil surface at 50 and 150 cm from the base of each tree (Fig. 4.1). To avoid disturbance to the rhizosphere, collars were not pushed into the soil, but were sealed to the soil using non-setting putty (Plumber's Mait®, Bostik Ltd, Stafford, UK). A good seal with the ground was confirmed as all respiration measurements showed a linear increase in CO_2 concentration over time (over 90 seconds).

Respiration measurements (which included both microbial and plant components) were made with a portable EGM-4 infra-red gas analyser with a darkened CPY-2 chamber (PP Systems International, Amesbury, MA, USA). CO_2 flux was measured five times at each collar through June and July 2013, after the defoliation event, and then twice in September 2013. Follow-up respiration measurements were made in June and July 2014 (one measurement each month). In 2014 'defoliated' trees did not re-grow their canopy, instead, investing in new shoots at their base. Respiration rates were calculated as the product of a linear function of [CO_2] increase over a period of 90 seconds within the closed system. Tests with longer measurement periods showed no improvement of fit. All collars were measured within a two-hour period between the hours of 0900-1600.

Soil inorganic nitrogen

Cation exchange membranes (2.5 x 5 cm; Resintech, West Berlin, NJ, USA) were used to measure soil inorganic N availability in summer (10^{th} – 24^{th} July 2013) and autumn (6^{th} – 20^{th} September 2013). Membranes were regenerated in 0.5 M HCl for one hour before being neutralised in 0.5 M $NaCO_3$ for five hours, replacing the

$NaCO_3$ every hour. The membranes were inserted vertically into the soil surface (0-5 cm) at the centre of all landscape scale (*LS*) plots. Care was taken to select soils with no moss species (e.g. *Pleurozium schreberi*) associated with N-fixing cyanobacteria (DeLuca *et al.*, 2002) to avoid measuring leached N from this potential source. A knife was used to create a vertical slit in the soil into which the membrane was inserted. The soil was then pushed together to ensure good contact and membranes were left *in situ* for 14 days. After collection, adhering soil particles were gently brushed away, after which the membranes were rinsed with deionised water. Membranes were stored at 3°C for 18 days before extraction (100 rpm for 60 minutes in 35 ml 2 M KCl (Qian & Schoenau, 2002)). Extractable NH_4^+ was quantified using flow injection analysis (FIAflow2, Burkard Scientific, Uxbridge, UK). Control strips (n = 10 per season) were taken into the field on the day of strip insertion but not placed in the field. They were taken back to the lab and stored at 3°C until field samples were analyzed, at which point they were processed in the same way. The mean amount of NH_4^+ adsorbed to control strips in each season was subtracted from field samples as an analytical blank.

Ectomycorrhizal hyphal production

Nylon mesh bags (5 x 4 cm; 37 µm mesh size), which allowed hyphal ingrowth, primarily of ECM fungi (analysis of community DNA in boreal and temperate forests shows c 80% ECM (Wallander *et al.*, 2013)) but not roots (Wallander *et al.*, 2001; Wallander *et al.*, 2013), were filled with 25 g of sand from the shore of Lake Torneträsk (68°21N, 18°49E). No plants were present aboveground within 1 m of the sand collection point. Sand was sieved to between 0.125 and 1 mm, rinsed under

a flow of water for 1 minute then microwaved (800 W) for 12 minutes, reaching a temperature of 98°C. This process was repeated and the sand was rinsed a final time before drying for 48 h at 80°C. The sand-filled bags were inserted at the landscape scale plots within 0.5 m of the ion exchange membranes at the centre of the plots. The bags were left in the field for 92 days between 16th June and 16th September 2013. At collection, the sand was removed from the bags and freeze dried using a Modulyo® freeze drier (ThermoFisher Scientific, Waltham, MA, USA) for 72 hours within six hours of recovery.

Sand from each bag (1 g) was sonicated for 10 minutes in 30 ml deionised water to disassociate the fungal hyphae from the sand particles. A 4 ml aliquot of the water-hyphae suspension was filtered onto a nitrate cellulose filter paper (0.45 µm pore size) and fungal material was stained with trypan blue (following Quirk *et al.* (2012). Hyphal length was estimated under 200x magnification (Primo Star, Zeiss, Oberkochen Germany) using the line intersect method (Brundrett *et al.*, 1994). This was repeated on duplicate samples for each mesh bag, a mean of which was taken as the final measurement.

Extraction, amplification and sequencing of fungal DNA from ectomycorrhizal roots

Root tips of non-defoliated and defoliated trees were collected and analysed to identify the ECM taxa colonizing the roots, along with their activity as measured by extracellular enzyme analysis. Samples for root tip analysis were taken on 7th July 2013. Samples were taken from five plots, three from the *LS* plots and two from two

additional mountain birch stands. At each of these plots, paired trees located within 5-10 m of each other were designated 'defoliated' or 'non-defoliated' based upon the percentage of leaves remaining (c. 0 – 15 % designated 'defoliated', c. 85 – 100 % designated 'non-defoliated'). Organic-horizon soil was sampled to a depth of 4 cm using a 5.7 cm diameter soil corer. Root samples were rinsed of adhering soil particles under a stream of tap water on a 1 mm-mesh sieve. Individual ECM root tips were then excised from larger root fragments under a stereomicroscope and stored and stored intact at 4˚C for up to 14 days until analysis (Pritsch *et al.*, 2011).

Single root tip DNA were extracted with the Extract-N-Amp kit (Sigma, USA) according to Avis *et al.* (2003). Fungal DNA was amplified using polymerase chain reaction (PCR) with the ITS1F-ITS4 primer set (Gardes & Bruns, 1993, White *et al.*, 1990) at 0.35-µM concentration in GoTaq G2 Master Mix (Promega, USA). The PCR consisted of a 3-min hot start at 95°C, 35 cycles of 30 s at 95°C, 45 s at 60°C, and 90 s at 72°C, and a final cycle of 5 min at 72°C. Negative controls (diethylpyrocarbonate-treated water) were included in each PCR run. PCR products were ran in 0.05% ethidium bromide 1.5% agarose (w/v) gels and photographed under UV light to confirm single PCR amplicons. After primers and unincorporated nucleotides were removed using ExoSAP (Affymetrix, Cleveland, OH, USA), as described by Kennedy & Hill (2010), amplicons were sequenced with the ITS4 primer on a 3730XL Applied Biosystems sequencer by Macrogen Corp. (Rockville, MD, USA). Sequence chromatograms were edited in FinchTV 1.4.0 (Geospiza, Seattle, WA, USA) or 4peaks 1.7 (http://nucleobytes.com/index.php/4peaks) to eliminate spurious base calls on the flanking ends of sequences.

Classification of fungal sequences

Fungal sequences were assigned to one of ~80 ECM lineages or monophyletic groups (Tedersoo & Smith, 2013). Lineages are designated by a '/' (slash) followed by the dominant genus, genera, or representative sequence, e.g., /cenococcum, /tomentella-thelephora, or atheliales3. Richness, evenness, and diversity (Shannon and Simpson indices) of ECM lineages were computed according to McCune & Grace (2002).

Fungal sequences were placed into ectomycorrhizal (ECM) lineages (Tedersoo and Smith 2013) based on maximum bit score matches to reference sequences in the UNITE 6.0 database (unite.ut.ee) accessed via PlutoF (Abarenkov et al. 2010; http://plutof.ut.ee/) with the International Nucleotide Sequence Database (INSD) option chosen. Mycorrhizal exploration type was assigned (ET; Agerer 2001) at the ECM lineage level following Tedersoo and Smith (2013) and of SH groups based on mantle structure and on previously published ECM morphology of taxa with 97% or higher match with sequences in this study.

Potential mycorrhizal N uptake

Potential mycorrhizal N uptake was estimated based by first grouping the ECM taxa associated with collected root tips into 'exploration types' (contact, short-, medium-, or long-distance) according to Tedersoo and Smith (2013) and additional references (Appendix 2, Table S2). NH_4^+-N potential depletion by ECM hyphae was calculated as the weighted average of the relative abundance of each exploration type in the

ECM community multiplied by NH_4^+-N depletion values specific to each exploration type.

Ectomycorrhizal (ECM) enzyme production

Hydrolytic and phenol oxidase enzyme activities were assessed according to (Pritsch *et al.*, 2011). Peroxidase activity, which is important in the breakdown of lignin in sub-arctic ecosystems (Bödeker *et al.* 2014), was measured as conversion of the substrate 3,3′,5,5′-tetramethylbenzidine (TMB; (Johnsen & Jacobsen, 2008)) in a 120-µl reaction volume after 15-min incubation. Following completion of each assay, reaction products read for fluorescence or absorbance on a Synergy HT (Biotek, USA). Substrate conversion was calculated as pmol mm^{-2} min^{-1} using standard curves for hydrolase substrates and the Beer-Lambert law for 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; $\varepsilon_{420} = 3.6 \times M^{-1}$ cm^{-1} and TMB ($\varepsilon_{450} = 5.9 \times 10^4$ M^{-1} cm^{-1}; (Josephy *et al.*, 1982), the latter after addition of 30 µL 1 M H_2SO_4 (Johnsen & Jacobsen, 2008).

Statistical analyses

Respiration data in 2013 and 2014 were analysed separately using a repeated measures two-way ANOVA following a linear mixed effects model with distance from tree and defoliation status (defoliated or non-defoliated) as main effects. Data were square root transformed to meet the assumptions of the parametric analyses. A linear model was used to analyse the relationship between NH_4^+ adsorbed to cation exchange membranes and the defoliation extent of the *B. pubescens* on the

landscape scale plots once the response variable was natural log-transformed. The relationship between defoliation extent and ECM ingrowth was analysed using a linear model. Once again, a natural log transformation of the response variable made the data appropriate for parametric analysis. One outlying point was removed from the ECM ingrowth analysis because it had a disproportionate effect on the statistical model, violating the underlying assumptions (Cook's distance > 0.5). A conservative Bonferroni test on its residual was used to confirm that this was indeed a statistical outlier ($P = 0.0058$) (Kutner *et al.*, 2005) .

Richness, evenness, and diversity (both Shannon's and Simpson's indices) of ECM lineages were compared between defoliated and non-defoliated trees with paired t-tests. To test a null hypothesis that ECM communities were not affected by defoliation, the non-parametric blocked multi-response permutation procedure in PC-ORD was used (McCune & Mefford 2011). The raw data matrix included counts of ECM fungal lineages and non-ECM fungi in defoliated and non-defoliated plots (n=5). Plots were considered as random blocks, performed within-block median averaging, and used distance function commensuration to give equal weighting to variables in the calculated Euclidian distance matrix (McCune & Grace, 2002).

The effect of defoliation effects on ECM enzyme activity at the community scale was evaluated by ANOVA in the generalized linear mixed-model procedure of SAS 9.4 (SAS Institute, Cary, NC, USA). Defoliation status was specified as a categorical fixed effect and plot as a random effect. Depending on enzyme class, enzyme activity among individual ECM roots varied by three or more orders of

magnitude. Transformation of raw data to a natural logarithmic scale proved satisfactory to dampen the influence of extreme values on plot means and to satisfy the ANOVA assumption of normality (studentized residuals, Shapiro-Wilk test, Type I error of $\alpha = 0.10$). Either equal or unequal fixed-effect variances were fitted by comparing the Bayesian Information Criterion value of competing models (Littell *et al.*, 2006). To determine if defoliation and ECM fungal lineages interacted to affect ECM-root enzyme activities a two-way ANOVA was used and a pre-planned comparison of defoliated and non-defoliated least-squares means for ECM lineages that had replicate observations among the sampled transects. Additionally, the effect of defoliation on the relative abundance of mycorrhizal exploration types was assessed using a two-way ANOVA with exploration type and defoliation defined as categorical variables and relative abundance as the response.

4.4 Results

Defoliated plots had a significantly lower respiration rates (2.63 μmol CO_2 m^{-2} s^{-1}) than non-defoliated plots (3.96 μmol CO_2 m^{-2} s^{-1}) in 2013 when measured 50 cm from the tree (P = 0.015; Table 4.1, Fig.4.2). However, the effect of defoliation overall was not significant (P = 0.068) due to a non-significant response to defoliation at 150 cm from the tree. Overall, there was a significant (P = 0.009) effect of distance on respiration rates and no significant interaction (P = 0.24) between distance and defoliation. The same pattern with lower respiration at 50 cm from defoliated trees continued into 2014.

Table 4.1: Analysis of variance of defoliation, distance from the base of the tree, and their interaction effects on respiration. Statistical results correspond to data shown in Figure 4.2. Significant (P < 0.05) factors are highlighted in bold.

Data	y transformation	Factor	d.f.	F	P
2013 Respiration	Square root	**Distance from tree**	**1,8**	**11.68**	**0.009**
		Defoliation	1,8	4.46	0.068
		Distance*Defoliation	1,8	1.62	0.24
2014 Respiration	Square root	**Distance from tree**	**1,8**	**8.83**	**0.017**
		Defoliation	1,8	3.25	0.11
		Distance*Defoliation	1,8	1.65	0.24

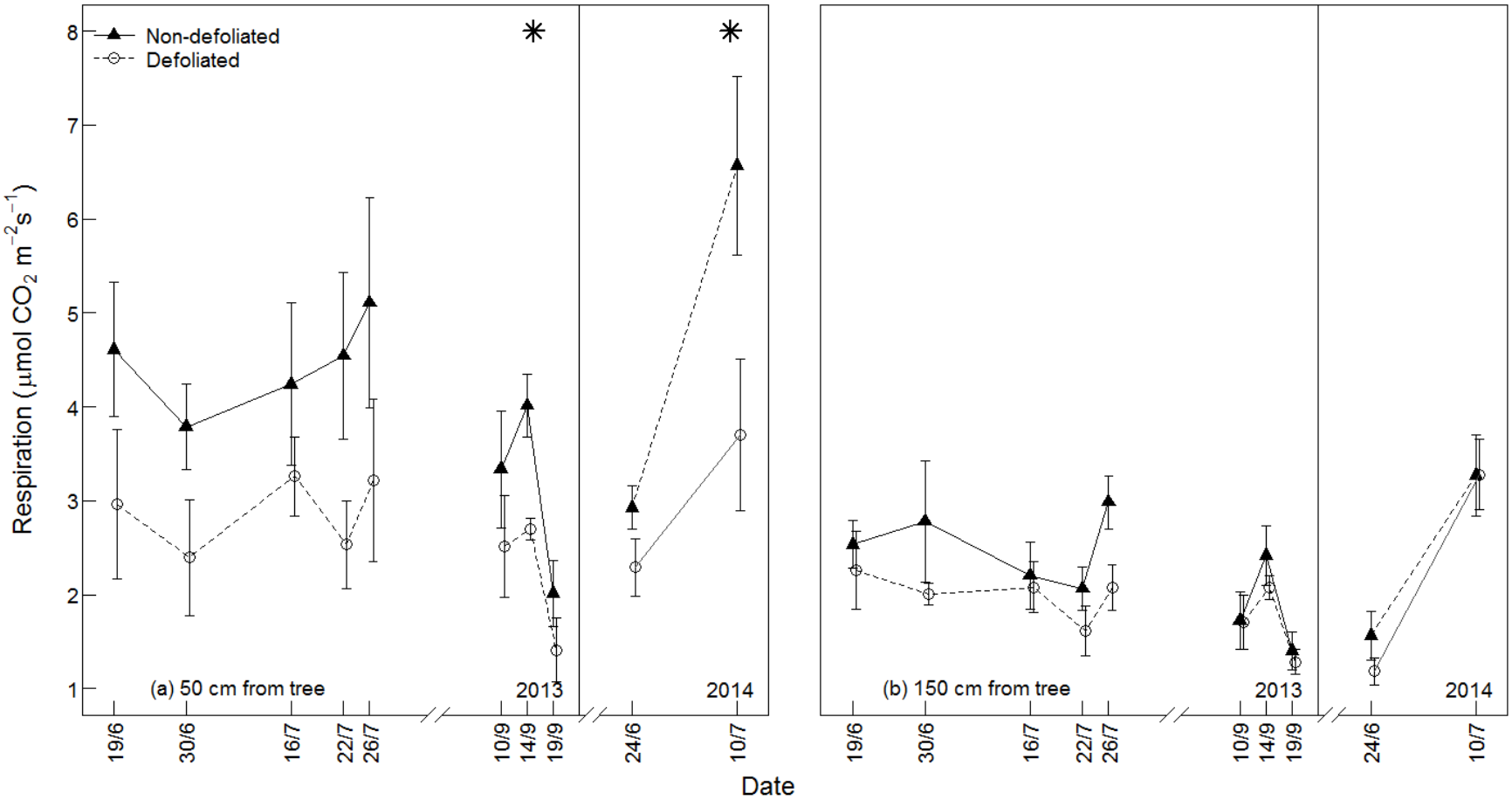

Figure 4.2: Soil respiration measured over the growing season of 2013 and 2014 at non-defoliated (closed triangles) and defoliated (open circles) plots at (a) 50 cm and (b) 150 cm from study trees. Error bars represent ±1 SEM (n = 5). * signifies a significant ($P < 0.05$) effect of defoliation within the statistical model in that year and distance from the tree, according to one degree of freedom Wald tests.

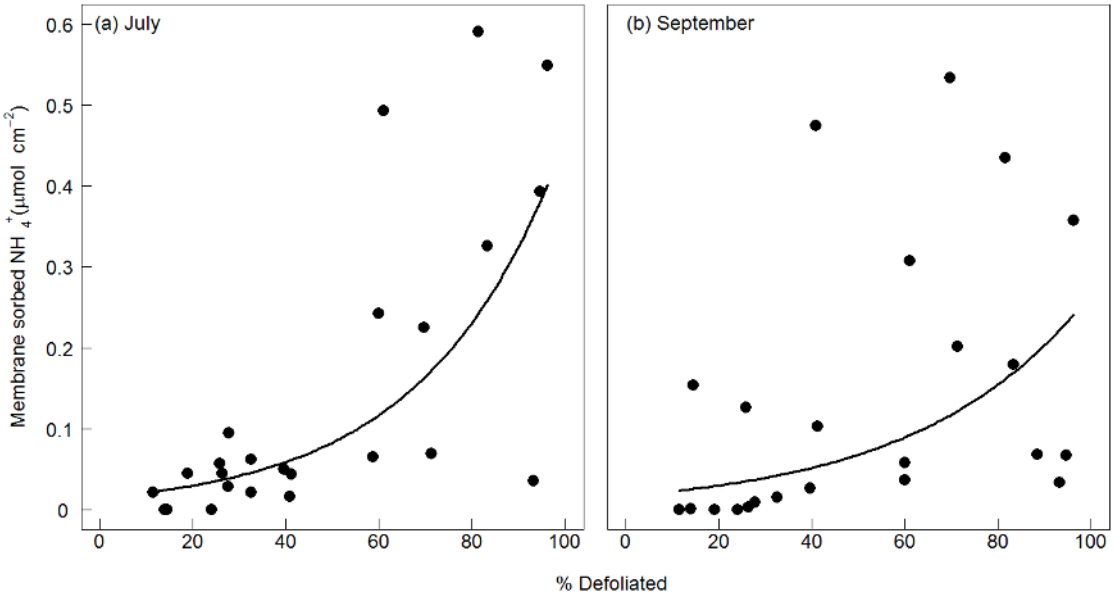

Figure 4.3**:** Resin membrane-sorbed ammonium (µmol NH_4^+ cm^{-2} membrane) at *LS* plots in (a) July and (b) September in relation to defoliation extent of *B. pubescens* (% defoliated). July: $y = e^{0.034x - 4.19}$, $R^2 = 0.56$. Non-linear regression: d.f. = 1,22, t = 5.49, P < 0.001. September: $y = e^{0.027x - 4.05}$ $R^2 = 0.31$. Non-linear regression: d.f. = 1,21, t = 2.43, P = 0.0036).

There was a significant relationship between defoliation extent (% defoliated) and the amount of NH_4^+ sorbed to resin membranes at both sampling points (July and September, 2013). The relationship was more significant in July (P < 0.001 Fig. 4.3a), however it was still present in September (P = 0.004 Fig. 4.3b). There was also a significant negative relationship between defoliation extent and ECM hyphal production over the months of June - September (P = 0.005, Fig. 4.4).

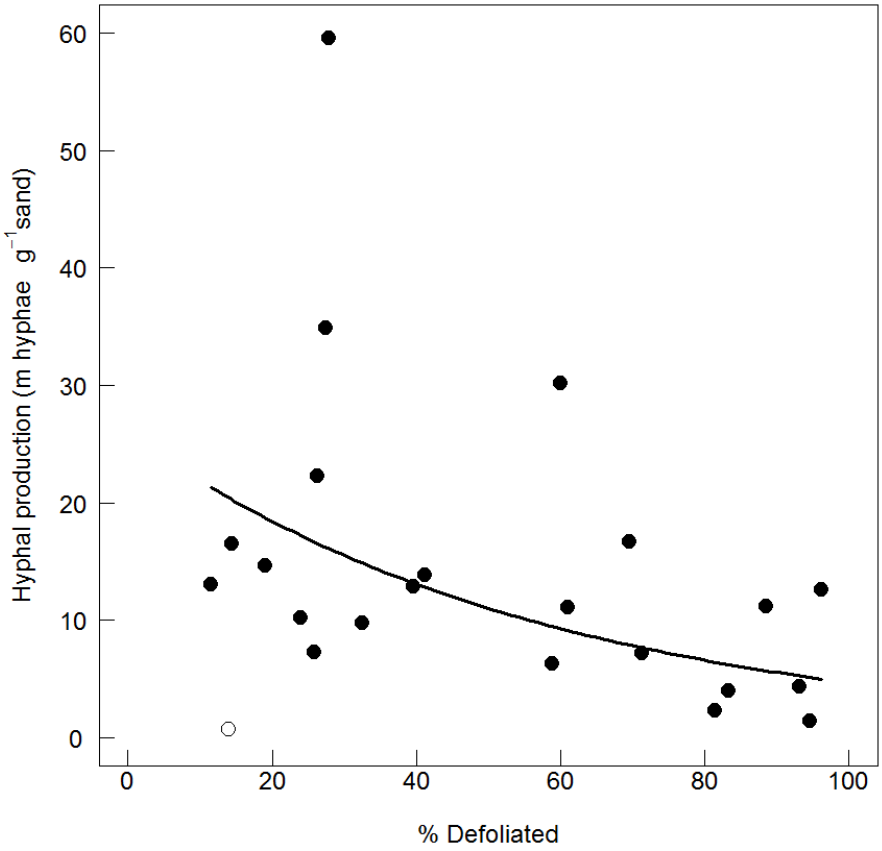

Figure 4.4: Hyphal in-growth in relation to defoliation of *B. pubescens* (% defoliated) at the *LS* plots. Line represents $y = e^{-0.017x + 3.26}$ $R^2 = 0.30$. Non-linear regression: d.f. = 1,20, t = 3.17, P = 0.0048. Statistical outlier is identified as an open circle, no other points were statistical outliers.

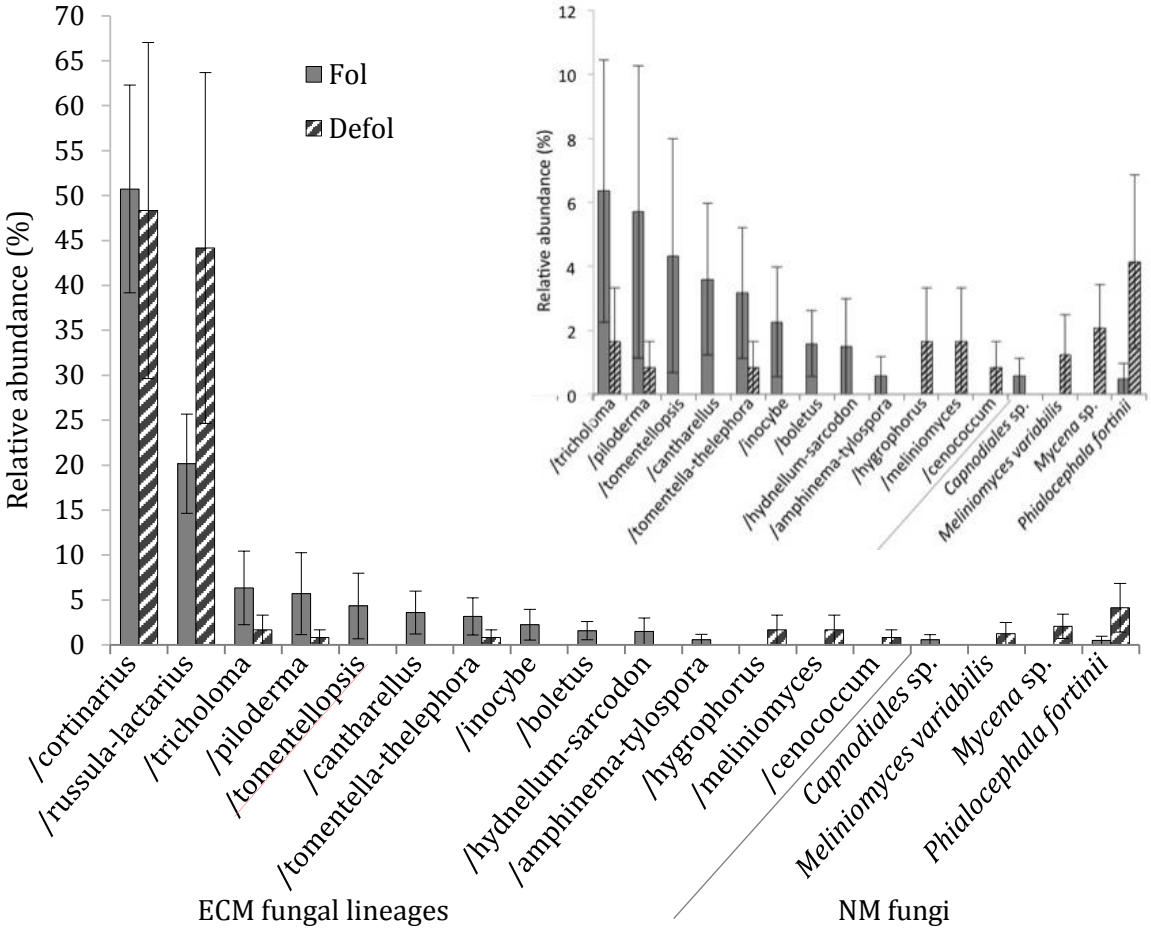

Figure 4.5: Main: Relative abundance of ectomycorrhizal (ECM) fungal lineages and non-mycorrhizal (NM) fungi sequenced from ectomycorrhizal root tips of control 'Fol' and defoliated 'Defol' *Betula*. Error bars are standard error of the mean (n = 5) and undefined for unreplicated fungal lineages . Absence in replicates was treated as zero for mean relative abundance calculation. Relative abundance of non-ectomycorrhizal fungi is shown but excluded from analysis. Inset: relative abundance of all but /cortinarius and /russula-lactarius lineages expressed on 0-12 % scale.

Table 4.2**:** Effect of defoliation on ectomycorrhizal fungal community richness (S), evenness (E), Shannon diversity (*H*'), Simpson diversity (*D*'), and composition. Values are means ± standard error (n=5).

	S	E	*H*'	*D*'	Composition
Non-defoliated	4.8±1.4	0.83±0.07	1.2±0.31	0.60±0.12	
Defoliated	2.6±0.9	0.43±0.19	0.5±0.24	0.28±0.13	
P > |t|	0.04[a]	0.05[a]	0.04[a]	0.05[a]	0.04[b]

[a]paired t-test
[b]blocked multi-response permutation procedure *T* statistic

The structure and function of the ECM fungal community associated with *Betula pubescens* roots was significantly affected by defoliation. ECM fungal richness, evenness, and diversity (both Shannon and Simpson diversity indices) declined in response to defoliation (Table 4.2). Fungal community composition was also altered, with some ECM taxa increasing and others decreasing with defoliation. The /cortinarius lineage was the most abundant ECM taxon of both non-defoliated and defoliated trees, representing 48-51% of relative ECM abundance (Fig. 4.5). Relative abundance of /cortinarius was not impacted by defoliation. In contrast, defoliation significantly affected the relative abundance of the /russula-lactarius lineage which made up 20% of the ECM community in non-defoliated trees, but increased to 44% following defoliation. Additionally, the ECM lineages /tomentella-thelephora, /tomentellopsis, /piloderma, /cantharellus, /inocybe, /hydnellum-sarcodon, /amphinema-tylospora, and /boletus, which together accounted for about 30% of ECM taxa associated with non-defoliated *Betula*, collectively declined to 3% of the ECM community with defoliation. The lineages /hygrophorus, /meliniomyces, and /cenococcum were detected on roots from single defoliated plots and not detected from non-defoliated plots. Non-mycorrhizal Fungi most closely related to root endophytes, such as *Phialocephala fortinii*, *P. sphaeroides*, and *Meliniomyces variabilis*, and free-living saprotrophs (*Mycena* spp.) were primarily recovered from ECM roots of defoliated *Betula* (Fig. 4.5). The changes in relative ECM community abundance of different exploration types with defoliation are summarised in Fig. 4.6.

Among ECM lineages, those with high extraradical mycelium biomass (medium- to long-distance soil exploration strategy (Hobbie & Agerer, 2010)) declined from 76% in non-defoliated trees to 43% in defoliated trees. This corresponded to an increase in contact exploration types (consisting of /russula-lactarius) and non-mycorrhizal fungi (Fig. 4.5). Insofar as exploration strategy is conserved in ECM lineages and corresponds to zones of hyphal nutrient depletion in soil (Agerer *et al*., 2012), alteration of ECM community composition by defoliation lessened the potential of ECM fungi to immobilize N in soil (Fig. 4.7). It should be noted that in this study potential ECM N immobilization was estimated from literature values and that direct measures of N uptake are needed for validation.

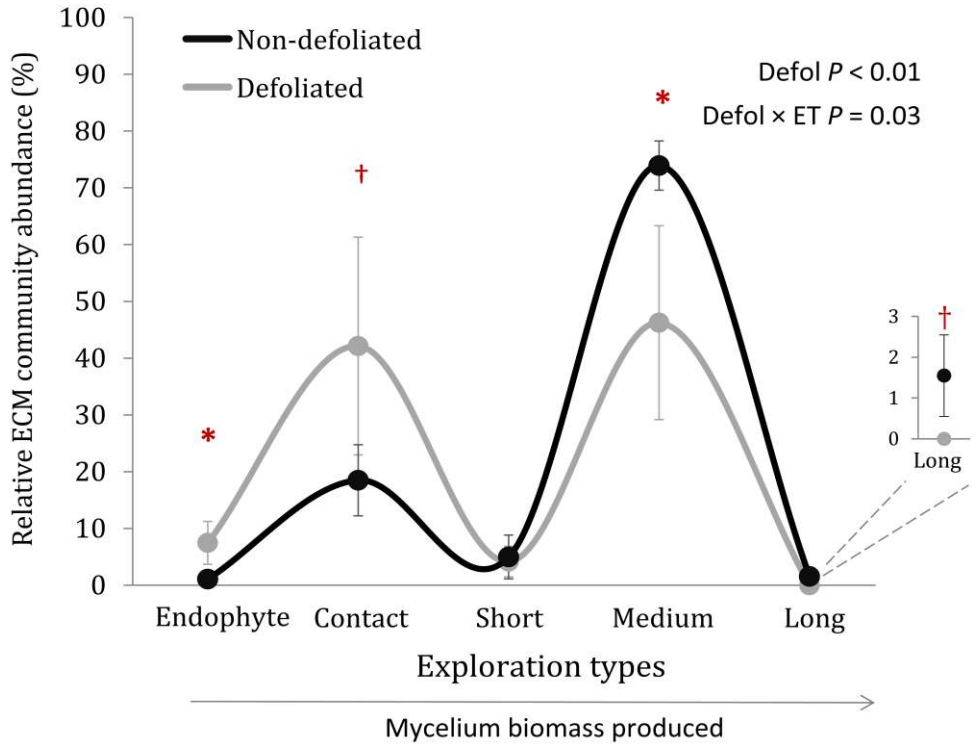

Figure 4.6: Effect of defoliation on relative abundance of non-mycorrhizal fungi and exploration types (ETs) of ectomycorrhizal fungi. Absence within replicates was counted as zero for mean relative abundance calculation. Bars are standard error of the mean. Symbols indicate defoliation affected relative abundance of an ET (Holm-Tukey test) at $P < 0.05$ (asterisk) and $P = 0.1$ (dagger).

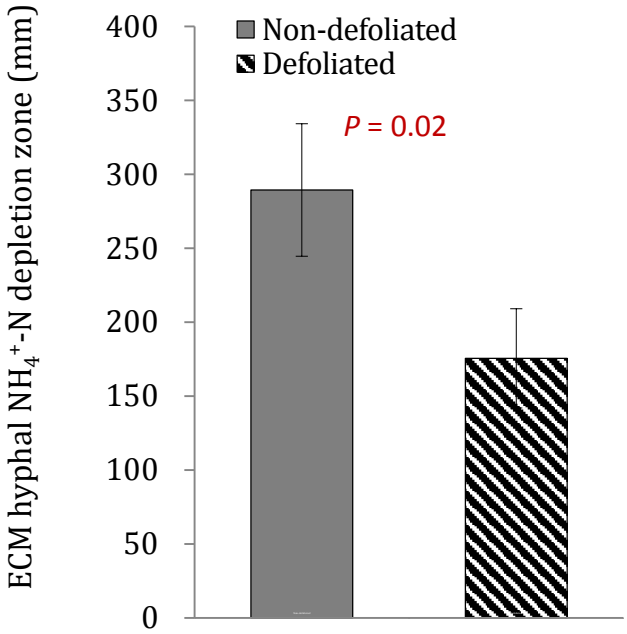

Figure 4.7. Effect of defoliation on potential N immobilization by ectomycorrhizal hyphae due to defoliation effects on mycorrhizal exploration type relative abundance. Least-squares means and standard errors are shown for replicate *LS* plots (n = 5).

Defoliation increased C-degrading (cellobiohydrolase, phenol oxidase, and peroxidase) enzyme pools on ECM-colonized root tips, but caused no change in the activities of N- and P-cycle enzymes (Fig. 4.8). As for the effects on individual ECM fungal taxa, defoliation did not alter enzyme activity of root tips colonized by /cortinarius, but increased cellulolytic enzyme activity of root tips colonized by /russula-lactarius (Fig. 4.9). Irrespective of defoliation, /russula-lactarius roots had higher phenol oxidase and peroxidase enzyme activities than /cortinarius roots. ECM lineages with low frequency (i.e. detected in two or fewer replicate plots) were not compared for activity of individual enzyme classes due to lack of replication.

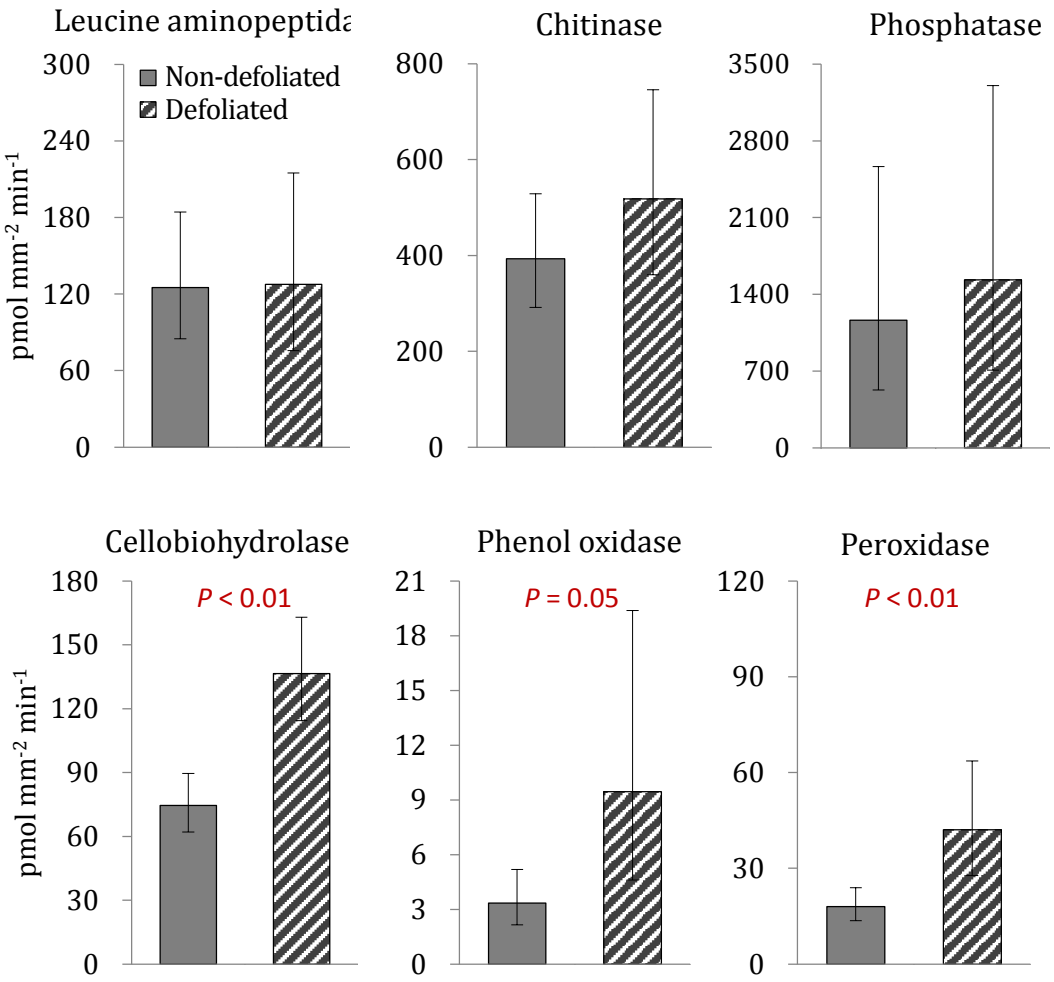

Figure 4.8: Effect of defoliation on enzymes on extracellular enzyme activity of ectomycorrhizal root tips. Data were analyzed on a natural logarithmic scale in order to meet the ANOVA assumption of normality; least-squares means and 90% confidence intervals, back-transformed to the measured scale, are shown. Sample size is n = 5 for all enzyme classes except phosphatase, n = 3 for control and n = 4 for defoliated.

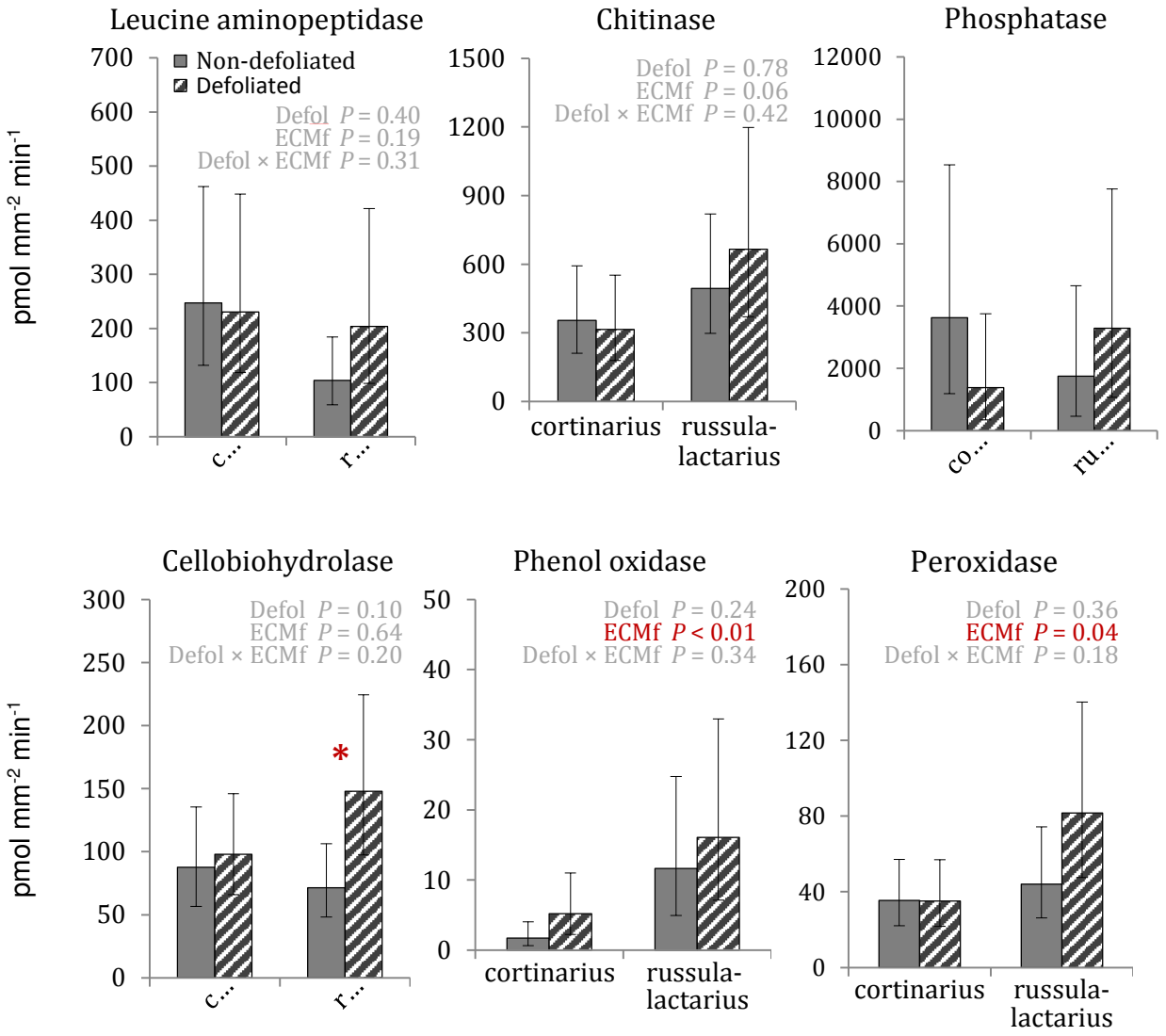

Figure 4.9: Effect of defoliation (Fol, non-defoliated; Defol, defoliated) and fungal lineage (ECMf; /cortinarius and /russula-lactarius) on extracellular enzyme activity of ectomycorrhizal root tips. Data were analyzed on a natural logarithmic scale to meet the ANOVA assumption of normality; least-squares means and 90% confidence intervals were back-transformed to the measured scale. Asterisk denotes increased (Holm-Tukey P < 0.05) / cellobiohydrolase activity of defoliated /russula-lactarius ectomycorrhizas. Sample size for enzyme classes except phosphatase: cortinarius, non-defoliated n = 5, defoliated n = 4; russula-lactarius, non-defoliated n = 5, defoliated n = 3; phosphatase: cortinarius, non-defoliated n = 3, defoliated n = 3; russula-lactarius, non-defoliated n = 3, defoliated n = 2, therefore, no statistics for phosphatase.

4.5 Discussion

This study has shown that defoliation of a *Betula pubescens* forest canopy slows biogeochemical cycling in the soil via a reduction of photosynthate supply. Amongst other potential factors, it is demonstrated that a shift in ectomycorrhizal community composition to a more conservative state could be an important factor contributing to the slow-down of C and N cycling in the soil.

It was shown that, when defoliated, biogeochemical cycling in a mountain birch forest slows. Previous work in this system showed that large defoliation events drastically reduce the strength of the ecosystem C sink (Heliasz *et al.*, 2011), with a reduction in photosynthesis. The reduction in C assimilation also slowed the loss of C from the soil. This was only statistically significant closer to the tree base (at 50 cm), presumably where the tree has a greater influence on soil carbon cycling rates. These data demonstrate that the respiration rates of roots, fungi and bacteria, and other soil organisms are sensitive to a reduction in above-ground C assimilation, as has been shown by experimental girdling and trenching experiments (Högberg *et al.*, 2001; Brzostek *et al.*, 2015). Although there was a slow-down in respiration close to trees, the effect of the defoliation on C cycling across the rest of the forest (> 50 cm from tree base) may be negligible, as there was no reduction in respiration further away from study trees. There will therefore be large areas of forest soil where soil CO_2 efflux is not affected by the defoliation of the canopy. These results should therefore be regarded as evidence that rhizosphere processes are slowed by aboveground defoliation, but it is unclear how this effect extends to the C budget of the forest as a whole.

This study in forest plots which have undergone almost complete defoliation in a relatively unproductive ecosystem (Myneni *et al.*, 2001; Karlsen *et al.*, 2008) showed the opposite response to more productive ecosystems which experienced a smaller reduction in belowground C allocation. Further south, in a temperate deciduous system, belowground respiration rates increased in response to defoliation (8% less foliage than control (Frost & Hunter, 2004)). This was similar to patterns of increased C allocation belowground by plants in response to partial herbivory (Bardgett & Wardle, 2003; Orians *et al.*, 2011). The authors explained the increase in C flux as being due to a combination of increased root growth, turnover, activity and labile C input, all as mechanisms to recover N that was lost from leaf biomass (Frost & Hunter, 2004). All of these processes depend on a high flux of autotrophic C to maintain roots and associated mycorrhizal symbionts (Litton *et al.*, 2007; Brzostek *et al.*, 2015), something that was not possible in the present study as the defoliated trees suffered almost complete defoliation. In a subalpine forest suffering a bark beetle outbreak, as productivity decreased with tree death, ecosystem respiration decreased, signifying an overall slowdown of the forest C cycle (Moore *et al.*, 2013). This is analogous to what was observed in the present study. Soil carbon cycling in sub-arctic forests relies on autotrophic C supply to mobilise microbial communities and continue the decomposition of soil organic matter (Hartley *et al.*, 2012). This study shows that when this C supply is cut, cycling in the soil slows, further supporting the hypothesis that it is recently fixed carbon which drives this cycle (Hartley *et al.*, 2012).

A decline in ECM hyphal production with increasing defoliation led to a shift in the ECM fungi colonizing *B. pubescens* roots from medium and long- distance exploration types (ETs) in non-defoliated plots to smooth-mantled contact species such as /russula-lactarius in the defoliated plots (Agerer *et al.*, 2012). The shift from longer to shorter ETs coincides with a loss of ECM diversity on *Betula* root tips. The species lost tend to be from the longer ETs. This is consistent with the hypothesis that when a host tree is defoliated, ECM species with a low C requirement hold a competitive advantage over those that invest in more extensive soil exploration (Saikkonen *et al.*, 1999; Markkola *et al.*, 2004). A competitive shift driven by defoliation therefore has negative effects on ECM biomass and diversity as longer ETs lose lose fitness and root tips shift to more of a *Russula* and *Cortinarius*- dominated system.

A similar shift to Russuloid ECM fungi on root tips and reduction in ECM biomass on roots was observed in another mountain birch forest (Saravesi *et al.*, 2015). The opposite occurred in a warming experiment in the Alaskan tundra where biomass of *Betula nana* increased; there was a shift to the more explorative *Cortinarius* spp. from the lower investment *Russula* spp, presumably as a result of increased C supply (Deslippe *et al.*, 2011). This change in the composition of the ECM community that was observed likely contributed to the reduction of ECM hyphal biomass observed in the in-growth bags in highly defoliated plots. A shift to higher allocation to C-scavenging extracellular enzymes by ECM fungi of defoliated trees also suggests that host C supply is an important control on ECM physiology in our study system, It is clear that the mycorrhizosphere is altered in both biomass and composition in response to reduced C supply, as has been observed in previous

defoliation studies (Gehring & Whitham, 1991; Delvecchio *et al.*, 1993; Gehring *et al.*, 1997; Kuikka *et al.*, 2003; Pestana & Santolamazza-Carbone, 2011).

The data from this study show that ECM hyphal production was reduced in defoliated plots as were respiration rates. This would agree with previous work showing that respiration by ECM hyphal networks contribute significantly to total heterotrophic soil respiration (Heinemeyer *et al.*, 2007; Vallack *et al.*, 2012). The effect of ECM community change on C flux is in this study, however, complicated by the co-occurring change in extracellular enzyme activity. Community-wide activity of C-degrading enzymes associated with ECM root tips were increased in defoliated plots compared to non-defoliated plots. This was driven primarily by the shift to increased relative abundance of the /russula-lactarius lineage; predominantly a contact exploration type (Agerer, 2006) which had higher extra-cellular oxidative enzyme activities compared to /cortinarius, the co-dominant ECM lineage in our study site. This could potentially lead to an increase in C degradation and soil microbial community-wide respiration in the vicinity of the ECM root tips.

The change in enzyme production at the root tips is likely to be minor relative to the observed decrease in hyphal production and associated reduction in soil enzyme activity. Our data along with others (Tedersoo *et al.*, 2012; Bödeker *et al.*, 2014) show that other lineages with potential to produce an extensive mycelium also has a significant capacity to produce C-degrading extracellular enzymes. Therefore, reduction in hyphal production may have reduced enzyme production away from the roots. Although /russula-lactarius lineages produced more enzymes at the root tip, the net effect of the observed ECM community response to defoliation may

have been a reduction in enzyme production through the soil and reduction of degradation of soil organic matter. It was shown that when trees are girdled (causing a similar effect as observed here), microbial community-wide enzyme production was reduced by ~ 40% (Brzostek *et al.*, 2015), causing a slow-down in respiration rates. The reduction in respiration rates observed that was observed is likely to be linked to change in the ECM community away from more explorative lineages.

Defoliation increased cellobiohydrolase activity in /russula-lactarius lineages. This could be seen as C acquisition by /russula-lactarius which could be a compensatory response to reduced photosynthate supply following defoliation. i.e., the 'Plan B Hypothesis' proposed by Talbot *et al.* (2008). Although some lineages of ECM fungi do not degrade polymeric C and rely strictly on ECM hosts for C (Veneault-Fourrey & Martin, 2013), evidence suggests that the /russula-lactarius lineage arose from wood-decomposing ancestors (Larsson & Larsson, 2003; Tedersoo *et al.*, 2010) and retained some capacity to access C via decomposition. For example, *Russula* sp. isolates cultivated on culture media and in the presence of *Fagus* leaf litter secreted similar levels of oxidative enzymes to litter-decomposing fungi (Burke *et al.*, 2014). Additionally, in decomposing leaf litter and forest soil, *Russula* spp. accounted for a large proportion of fungal cellobiohydrolase genes and gene transcripts, implicating a role of *Russula* in cellulose depolymerisation in forest soils (Weber *et al.*, 2012; Voriskova *et al.*, 2014). Moreover, a *Russula* sp. assimilated C from cellulose in the absence of mycorrhizal symbiosis (Stursova *et al.*, 2012). In this way, therefore, a shift to a more /russula-lactarius-dominated system may enhance decomposition of soil C; this response, however, is likely

limited to soil in direct contact with /russula-lactarius ECM roots in our system, since rhizomorphs that allow ECM hyphae to proliferate several dm from ECM roots (Agerer 2012) were absent members of /russula-lactarius detected in our study system. Hence, the increase of enzymes on the surface of ECM roots is unlikely to have a significant effect on respiration compared to the overall reduction in hyphal growth in the soil that was observed.

Inorganic N availability in soils, specifically NH_4^+-N, increased where *B. pubescens* was defoliated. The positive relationship between the extent of defoliation and NH_4^+ in the soil observed in midsummer was still observed at the same magnitude in autumn, although there was more variation in the latter. Other work from similar forests showed that excess NH_4^+ was present in defoliated plots over a year after the defoliation event (Kaukonen *et al.*, 2013). Based on our own results, and the findings by Kaukonen *et al.* (2013), there is reasonable evidence that the N flush is caused by the caterpillar outbreak. This could be driven by direct frass inputs from the caterpillars (Lovett *et al.*, 2002) and equally though reduced N uptake by defoliated trees relating to reduced root growth (Kosola *et al.*, 2001; Cigan *et al.*, 2015; Saravesi *et al.*, 2015). Although neither of these processes were measured, the severe defoliation of the trees would suggest that their roots have been negatively affected. The N response persisted throughout the remainder of the growing season, three months after the defoliation event finished, and potentially beyond. Similar results were measured in sub-alpine forests which saw a concurrent reduction in ecosystem respiration (Moore *et al.*, 2013) with tree mortality an increase in bioavailable soil N (Trahan *et al.*, 2015). These results are in contrast to more productive systems where excess N as a result of severe defoliation of oak

woodlands was retained in the ecosystem (Lovett & Ruesink, 1995). This can be either by immobilisation of free N by the microbial community stimulated by a co-increase in labile C (Lovett & Ruesink, 1995) or by a rapid recovery of N by the trees (Russell *et al.*, 2004). Neither of these processes are like likely to be significant in this case as a reduction in respiration was observed (which includes root and microbial components) and reduction in fungal growth, suggesting that neither plant or microbial groups were stimulated by the addition of frass. Instead, the evidence suggests that in this ecosystem, soil processes and biological uptake are slowed to a point where excess N builds up in the soil.

On balance, these data imply a slow-down of biogeochemical cycling in this sub-arctic birch forest. This is in contrast to other work in the sub-arctic which argued that defoliation could enhance C cycling by effectively 'bypassing' the fungal decomposition pathway (Kaukonen *et al.*, 2013). This study measured an increase in bacteria and enchytraed worms in response to N from added frass in the soil. The current work shows defoliation slows C and N cycling, partly by a change in the ECM community. These two mechanisms therefore need resolving. A combination of measurement of fungal: bacterial ratios with the ECM community composition would allow assessment of their relative effects on C and N fluxes A further research priority remains to measure the duration of response measured here to better understand the balance of C in the wake of these important disturbances.

Moth outbreaks in this area are known to be limited by minimum over-winter temperature, with a temperature of about – 35 °C known to freeze and kill over-wintering eggs (Tenow & Nilssen, 1990). Increases in temperature and concurrent

reductions in the number of days below – 35 °C have been shown to increase the range of both *E. autumnata* and *O. brumata* (Jepsen *et al.*, 2008). The latter has seen particularly large increases in its range as it is more sensitive to cold temperatures than *E. autumnata*. Herbivorous insect distributions and populations are known to be particularly responsive to winter and summer temperature increases (Bale *et al.*, 2002) and with this in mind, along with the observed past changes in moth ranges in relation to warming (Jepsen *et al.*, 2008), it seems likely that this kind of disturbance will increase in severity and magnitude in the coming century (Bale *et al.*, 2002).

To conclude, a large-scale defoliation event by *Operophtera brumata* and *Epirrita autumnata* caused a number of cascading effects in subarctic mountain birch forests. This work has highlighted that a reduced delivery of autotrophic C to the rhizosphere may contribute towards accumulation of mineral N, altering the diversity and composition of the ECM community and altering the potential activity of organic matter-degrading enzymes. The ECM community is shown to be a key link between changes in autotrophic C supply and cycling of C and N in the soil. Defoliation events are a feature of many forest ecosystems (particularly in sub-arctic, boreal and temperate regions), yet little is known of the complex processes and cascading interactions that they drive. In an era of rapid environmental changes where short-lived and mobile insect species are able to respond rapidly to new opportunities for range expansion, the severity of 'outbreak' years will likely have more profound ecosystem impacts. Further work needs to focus on the interactions and potentially non-linear effects of defoliation events to better understand how

these important events exert control over ecosystem processes and the C balance of these forests

Chapter 5: Biological environment and litter quality drive fast decomposition in sub-arctic birch forests in contrast to adjacent heaths

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Author notes
The snow fences were established by RDH who kindly allowed TCP to place litter bags alongside his own experiments. In addition to this, in collaboration with TCP, RDH measured snow depth at transects and at the snow fences.

5.1 Abstract

Decomposition of litter is one of the key controls over carbon (C) storage in the soil. The biochemistry of the litter and the environment in which it takes place are the most important factors affecting decomposition rates. Deciduous shrubs and trees are predicted to expand rapidly across tundra ecosystem as a result of regional climate warming. This change in vegetation represents a change in litter input into tundra soils and a change in the environment in which litter decomposes. At a sub-arctic treeline in Sweden, a litter transplant experiment between important vegetation communities was used to test the importance of litter biochemistry and environment in determining litter mass loss. In addition to this, a snow fence experiment was used in a tundra environment to understand the relative importance of snow cover in the decomposition of litter across the treeline. *B. pubescens* and *B. nana* litter decomposed at faster rates than *E. nigrum* across all environments and all litter types decomposed at faster rates in

the forest and shrub environments than the heath. The effect of increased snow on decomposition was minimal, leading us to conclude that microbial activity over summer in the shrub and forest plots is driving increased mass loss compared to the heath. CPMAS ^{13}C NMR (cross-polarization/magic angle spinning ^{13}C nuclear magnetic resonance spectroscopy) was used to show that degradation of carbohydrate-C is one of the important drivers of mass loss in the forest. This pathway was less prominent in the heath which therefore explains why tundra soil typically have high concentrations 'labile' C. This experiment suggests that further expansion of shrubs and trees may stimulate the loss of undecomposed decomposition of carbohydrate-C in the tundra.

5.2 Introduction

Climate warming in the Arctic of between 1 – 4 °C since 1960 (Serreze & Francis, 2006; Serreze & Barry, 2011) has resulted in areas of tundra becoming more productive, with some landscapes increasing biomass by 10 g m^{-2} yr^{-1} (Epstein *et al.*, 2012). In many of these areas experiencing increased productivity, shrubs and trees have also been observed to be increasing in cover and height (Elmendorf *et al.*, 2012b) as has been predicted by *in situ* warming experiments (Walker *et al.*, 2006; Elmendorf *et al.*, 2012a) and have been expanding onto arctic tundra in response to warming (Tape *et al.*, 2006; Myers-Smith *et al.*, 2011).

Earth system models have predicted that increased productivity in arctic ecosystems will increase carbon (C) sequestration at whole ecosystem level (Cramer *et al.*, 2001; Qian *et al.*, 2010; Todd-Brown *et al.*, 2013). However, empirical data suggest that the relationship between productivity and whole-ecosystem C sequestration is not linearly positive (Todd-Brown *et al.*, 2013). Indeed, there is a growing body of evidence showing that, once soil C densities are taken into account, the most productive ecosystems, although high in productivity may store less C than less productive systems (Wilmking *et al.*, 2006; Kane & Vogel, 2009; Hartley *et al.*, 2012).

Litter from plants is the primary input of C into the soil (Brovkin *et al.*, 2012; Todd-Brown *et al.*, 2013), of which, in forests, approximately 52 % comes from the aboveground biomass (Freschet *et al.*, 2013). Litter decomposes and contributes towards humic substances in the soil which can lead to stabilisation of soil organic matter (SOM) (Melillo *et al.*, 1989; Sollins *et al.*, 1996). However all chemical fractions of SOM are ultimately degradable and require physical protection to be stored for long

periods of time (Dungait *et al.*, 2012). The species identity and functional type of litter input is key to determining its rate of decomposition and eventual contribution to SOM (Dorrepaal *et al.*, 2005; Cornelissen *et al.*, 2007; Cornwell *et al.*, 2008; Brovkin *et al.*, 2012); this has been shown to be more important in influencing SOM composition than climate controls (Quideau *et al.*, 2001).

Aboveground biomass allocation by arctic plants is low compared to more productive biomes due to high allocation of C into roots (Iversen *et al.* 2014). There is not yet an estimate of an arctic-wide above:belowground allocation but there is a large amount of variation between species (Iversen *et al.* 2014). Changes in plant functional type (PFT) could change the balance of this ratio. The change in PFTs in arctic communities due to shrub expansion (Tape *et al.*, 2006; Myers-Smith *et al.*, 2011) was hypothesised to result in a negative feedback to climate change (Cornelissen *et al.*, 2007) as the litter of more productive shrub species was shown to decompose at slower rates than other arctic species. This is at odds with observations of lower SOM storage under such shrub and tree species than adjacent tundra systems (Wilmking *et al.*, 2006; Hartley *et al.*, 2012), suggesting that identity of the species in these studies is all important in determining the C balance of the study systems. Chapter 2 shows that productive *Betula pubescens* forests and shrub dominated plots (*Salix* and *Betula*) store significantly lower C than adjacent heaths, prompting the hypothesis that decomposition of their litter is more rapid than the *Empetrum nigrum*-dominated heaths.

E. nigrum is widespread across alpine tundras of Fennoscandia and boreal forests across Eurasia (Bell & Tallis, 1973; Büntgen *et al.*, 2014) where it is present on heathlands from the Netherlands to Svalbard (Buizer *et al.*, 2012). In Fennoscandian tundra heaths

it dominates vegetation through forming dense mats, excluding other plant species through production of allelopathic, phenolic compounds (Gallet *et al.*, 1999). The complex organic litter input in heath ecosystems favours a decomposition pathway through ericoid mycorrhizal fungi (ERM) (Read & Perez Moreno, 2003). Nitrogen in these systems is bound in organic molecules which are predominantly taken up directly by ERM fungi (Tybirk *et al.*, 2000; Read & Perez-Moreno, 2003; Talbot et al. 2008), giving their ericaceous hosts a competitive advantage over fungi and roots and reinforcing their dominance in the organic-rich soil. The dominance of ericaceous shrubs in tundra and boreal forest ecosystems is further reinforced by allelopathic action by *Empetrum* which reduces tree seedling establishment and growth of their mycorrhizal symbionts (Nilsson *et al.* 1993; Nilsson & Wardle 2005).

Decomposition of *E. nigrum* is very slow due to its high phenolic content (Gallet *et al.*, 1999) and high levels of the lipid polymer cutin, which is particularly slow to break down (Tegelaar *et al.*, 1989; Rasse *et al.*, 2005) as a result of a well-developed waxy cuticle (Bliss, 1962; Hetherington *et al.*, 1984). This retards decomposition and leads to build-up of humic-rich organic horizons in the soil (Tybirk *et al.*, 2000). In contrast, deciduous shrubs and trees decompose faster than evergreen species such as *E. nigrum* (Aerts *et al.*, 2006; Cornwell *et al.*, 2008). It has previously been found that high litter quality (low carbon: nitrogen ratio) can stimulate decomposition processes (Subke *et al.*, 2004; Knorr *et al.*, 2005) in a similar way to addition of inorganic N (Stark *et al.*, 2014).

For the forest - tundra heath ecotone, it was found that the most important factor controlling decomposition of the deciduous *Betula pubescens* was the environment it

was present in, with higher rates of decomposition in Scandinavian birch forests than nearby tundra heaths (Sjögersten & Wookey, 2004). This was more important than differences in regional climate (in contrast to other studies (Dorrepaal *et al.*, 2005; Cornelissen *et al.*, 2007)) and experimental warming. The authors hypothesised that litter moisture in the birch forest was important in enhancing decomposition rates; however, other abiotic factors, such as deeper snow cover (and therefore warmer winter soil and more active microbial community (Grogan & Jonasson, 2006)), can also contribute to this.

The biota present in the decomposition pathway are important in influencing the rate of decomposition. In boreal forests there is a large biomass of free-living fungi concentrated in the litter layer of the soil (Lindahl *et al.*, 2007; Clemmensen *et al.*, 2013). In the humic and mineral horizons in boreal and sub-arctic forests there is also a strong ectomycorrhizal (ECM) fungal community (Lindahl *et al.*, 2007) which has been shown to have the capacity to degrade structural C polymers using a white rot mechanism (Bödeker *et al.*, 2014). The fungi use this to extract nitrogen (N) from organic complexes which in turn enhances decomposition rates (Talbot *et al.*, 2008; Lindahl & Tunlid, 2015). These fungi grow and explore the upper horizons of soil at faster rates in forest and shrub-dominated soils than in tundra heath systems (Chapter 2).

ECM and saprotophic fungi have the capacity to degrade a large range of simple and complex plant-derived structural C (Hatakka, 1994; Talbot *et al.*, 2008; Rytioja *et al.*, 2014). It is therefore probable that all the chemical fractions of litter in forest ecosystems are decomposed to some extent, especially bearing in mind that all C is

considered chemically ‘available’ if the appropriate microbial community is present (Dungait *et al.*, 2012). This may not be the case in tundra soils, where strong environmental controls, such as low temperature (Robinson, 2001) and dominance of ERM fungi with their closed N and C cycle (Read & Perez-Moreno, 2003), may restrict the growth and activity of other fungi.

When the chemical composition of C in forest and tundra soils was measured across a climatic gradient, it was consistently found that the tundra had more ‘labile’ fractions of carbon (Sjögersten *et al.*, 2003). This is counterintuitive because the input of litter from the dominant plant species, *Empetrum*, has a strong phenolic signature (Tybirk *et al.*, 2000) yet there was no difference in phenolic compounds in the soil between forest and heath, only in compounds related to polysaccharides (Sjögersten *et al.*, 2003). Decomposition rates were higher in the forest (Sjögersten & Wookey, 2004). This prompts the hypothesis that these high rates of decomposition were due to loss of simpler, cellulose and hemi-cellulose-related products (Kögel-Knabner, 2002; Simpson & Simpson, 2012) which will then be followed by more complex compounds (Melillo *et al.*, 1989). In the tundra, conversely, a different decomposition pathway may be present, with C flowing through a more closed ERM-dominated system (Read & Perez-Moreno, 2003); in which case, lignin-derived compounds would initially be decomposed preferentially, and more ‘available’ forms of C would be still present, as was observed in the humic layer of heath systems (Sjögersten *et al.*, 2003). In addition, enhanced decomposition of phenolics in the heath, through an ERM pathway, would explain why no difference in phenolics between forest and heath has been observed previously (Sjögersten *et al.*, 2003).

Using a decomposition experiment whereby litter was transplanted between key vegetation types at a sub-arctic treeline ecosystem, the aim was to understand the key drivers of decomposition rates in this ecosystem. The following hypothesises were tested:

1. Litter from the most productive vegetation types (forest and shrub) decomposes at the fastest rates, regardless of environment type;
2. The forest and shrub environments stimulate decomposition, regardless of litter type, including increased snow depth;
3. All fractions of C (simple through to complex) in both litter types are decomposed more in forests due to a more diverse decomposer community;

5.3 *Materials and methods*

Sites description

Twelve independent, short (<100 m) transects were established within a permafrost-free landscape (c. 2 km^2) spanning the sub-arctic/alpine treeline at Nissunsnuohkki (Abisko area, Sweden; ca. 68°18'N 18°49' E, 600 m asl). In this study the terminology of Walker (2000) and Kaplan *et al.* (2003), presented in ACIA (2005) is adopted to distinguish tundra plant growth forms and to place the study into circumpolar context. The treeline is formed by mountain birch (*Betula pubescens* Ehrh. ssp *czerepanovii* (Orlova) Hämet Ahti), with an ericaceous understorey, and typically moves through a thick layer of shrub vegetation before becoming tundra heath dominated by *Empetrum nigrum* L. ssp *hermaphroditum* (Hagerup) Böcher and *Vaccinium vitis-idaea* L. The intermediate shrub zone is dominated by *Betula nana* L. and grey willow (*Salix*) species (specifically, *Salix glauca*, often accompanied by *Salix lanata*; other *Salix* spp., including *S. hastata* and *S. lapponum*, occur less frequently). This transitional shrub-dominated vegetation is similar to the 'low- and high-shrub tundra' ('Continuous shrubland, 50 cm to 2 m tall, deciduous or evergreen, sometimes with tussock-forming graminoids and true mosses, bog mosses, and lichens') referred to in ACIA (2005), although generally not exceeding 1.5 m height and with the only one evergreen shrub species, *Juniperus communis* L., at low abundances. Tundra heath is here similar to the 'erect dwarf-shrub tundra' ('Continuous shrubland 2 to 50 cm tall, deciduous or evergreen, with graminoids, true mosses, and lichens') of ACIA (2005). Soils in the forest are micro-spodosols with a thin O horizon (< 5 cm) underlain by glacial till on a bed-rock typically of hard-shale (Sjögersten & Wookey, 2002). Soil pH in the organic

horizon is 4.3 ± 0.1 at forest and 4.5 ± 0.1 at heath locations in the Abisko area (Chapter 2).

Transect lengths ranged from 52 to 97 m (Appendix 1) depending on the length-scale of the forest- heath ecotone. Care was taken to select vegetation transitions that were not present as a result of strong topographical influence; for example where water and snow accumulation due to dips and hollows dominate site conditions, and avoiding steep slopes (mean elevation change from heath to forest plots of -2.7 m). Transects were selected with a variety of contrasting compass bearings (Appendix 1) to ensure that there was no bias in the data due to shading or winter snow drifting. The 12 transects were grouped geographically into three blocks of four as shown in Figure 5.1.

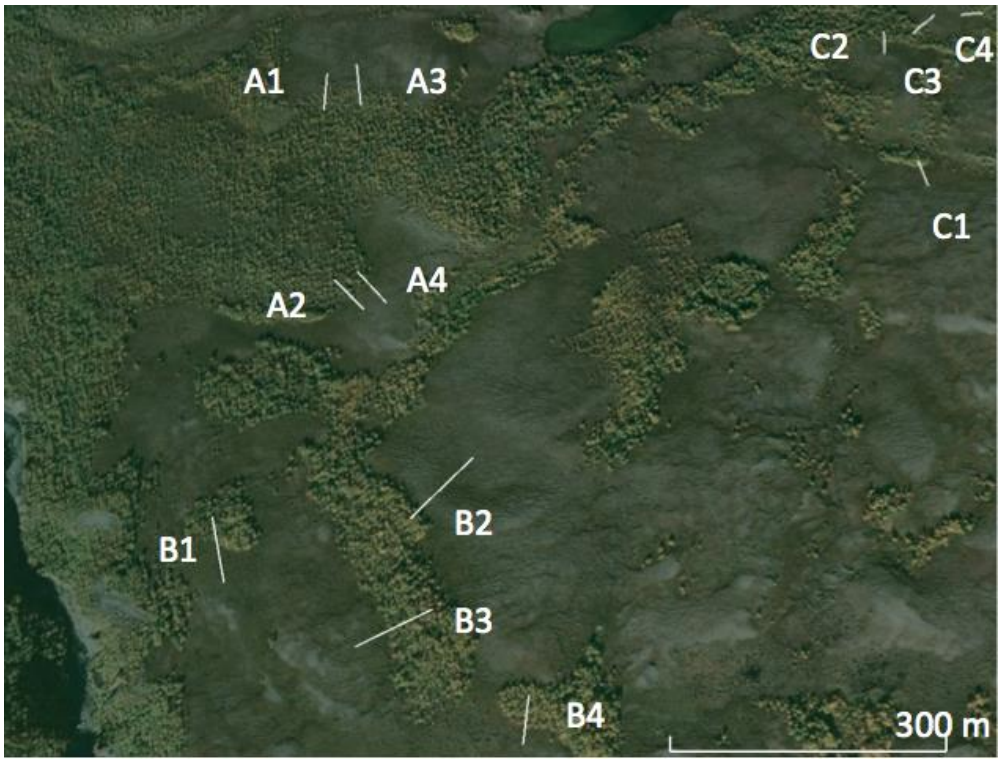

Figure 5.1: Google Earth images showing Abisko Ecotones, S plots are located approximately half way along each transect..

Three plots were established along each transect in order to represent best the transition in vegetation from heath to forest. These were; tundra heath (H), shrub (S) and forest (F) (see Table 5.1 for further site details). H plots were chosen for an open heath environment with low *B. nana* cover and a low canopy height, and with vegetation dominated by *E. nigrum*. S plots were identified as areas dominated by *B. nana* with shrub height characteristically between 40 and 60 cm. F plots were chosen to be in areas dominated by *B. pubescens,* approximately 10 to 15 m inside the forest edge.

Table 5.1: Vegetation characteristics along transects at Abisko across all blocks (means ± 1 SE, n = 12). 'Canopy height' refers to the actual vegetation canopy for Heath, Shrub-Heath and Shrub communities, and the understorey for the Forest Edge and Forest (where mountain birch trees - *Betula pubescens* ssp *czerepanovii* - comprise the canopy).

	Plot on transect		
	Heath	Shrub	Forest
Distance from Heath (m)	n/a	28.3 ± 2.9	67.6 ± 5.9
Canopy height (cm)	14.7 ± 0.7	32.0 ± 2.4	19.0 ± 1.7
B. pubescens density (trees m^{-2})			0.07 ± 0.01
B. nana cover (%)	21.2 ± 2.7	60.3 ± 4.8	8.0 ± 2.2
E. nigrum cover (%)	65.4 ± 3.3	66.9 ± 4.7	45.4 ± 4.2
pH (organic horizon)	4.3 ± 0.1	4.4 ±0 .1	4.5 ± 0.1

Snow fences

Five replicate 3.5 m wide, 1.5 m high snow fences were erected on tundra heath sites between 0.1 and 1 km N of the transect sites. They were erected before winter 2012 and 2013 (and lowered during the summer to avoid shading the vegetation and influencing evapotranspiration), designed to create snow drifts of comparable depth to the typical seasonal snow-cover at F and S plots on the transects. To replicate the snow at F plots, plots were set up 2 m to the leeward side of the fence, 7 m for the S plots and 20 m for the H plots (no extra snow). Snow depths were measured at both snow fence and

transect plots, once each between 14/3 and 29/3 in 2013 and between 29/3 and 30/3 in 2014. At each plot on the natural transects, five measurements were taken within 3 m of a central point (which was the accuracy of the GPS unit) at each plot. At the snow fences, the position of the plots was estimated based on the known distance from the snow fence and one measurement was taken per plot in 2013. In 2014 the exact position of the litter bags was marked using a pole so the measurement was more accurate. Snow depths in both years are summarised in Figure 5.2.

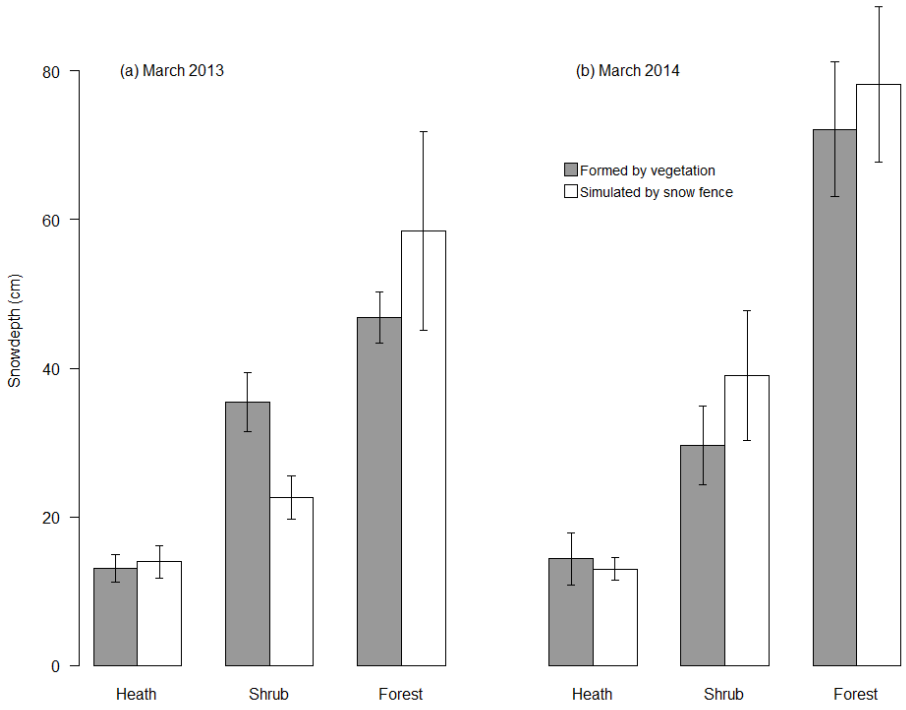

Figure 5.2: Snow depths measured at transects through heath, shrub and forest environments (Filled bars, n = 12) and on the leeward side of snow fences at plots designed to replicate snow depths at heath, shrub and forest plots (open bars, n = 5) in (a) March 2013 and (b) March 2014. Error bars indicate ± 1 SEM.

Litter bags

Litter was collected from four different transects at the Abisko study site from 2/9/2012- 12/9/2012. Freshly fallen *Betula pubescens* and *Betula nana* litter was collected from the top of the litter layer, taking care to exclude older litter. *Empetrum nigrum* litter was collected by carefully removing senesced leaves from the stem of extracted *Empetrum* shoots. Only recently senesced leaves were taken (light brown colour, 2-4 years old according to growth scars). Litter was collected from the 'home' plots in which each species is dominant, i.e. *B. pubescens* from F plots, *B. nana* from S plots, *E. nigrum* from H plots. All litter was brought back to the lab and sorted to remove any adhering particles or litter from other species, and then air dried at 40°C for 72 hours. For each species, 0.5 ± 0.01 g of litter was weighed into 7 x 7 cm polyester mesh bags with a 0.3 mm mesh size and sealed using hot melt adhesive. Litter bags were placed in the field on 17/9/12. Six bags of each species were placed at every plot on all 12 transects. Care was taken to ensure that every bag had good contact with the L horizon at each plot. Two corners of each bag were fastened to the ground using stainless steel pins and all bags were tied with nylon thread to nearby vegetation to prevent disturbance by grazing animals. Bags were also placed in the same manner on the leeward side of the snow fences were snow depth was estimated to be equivalent of snow collected at H, S and F plots. Retrospective measurements confirm that these were reasonable estimates (Fig. 5.2). Ten other samples of each species were oven dried at 60°C for 72 hours to calculate residual moisture of the air dried samples and the starting dry weight of the samples was corrected.

On 13/6/13, 24/7/13, 16/9/13 and 20/6/2014 one litter bag of each species at each plot at both transect and snow fence sites was chosen randomly to be extracted from the field and oven dried at 60°C for 72 hours. Once ingrown vegetation was removed, the remaining litter was extracted and weighed. From this dry weight and the residual moisture-adjusted air dried original mass, percentage mass remaining was calculated.

Solid state CPMAS ^{13}C NMR

Five randomly selected samples of each species/plot combination of *B. pubescens* and *E. nigrum* and H and F sites from the final harvest on 20/6/2014 (from a pool of 12 samples per combination) were used for solid state ^{13}C nuclear magnetic resonance CPMAS ^{13}C NMR (cross-polarization/magic angle spinning ^{13}C nuclear magnetic resonance spectroscopy) and elemental (C and N) analysis. CPMAS ^{13}C NMR spectra were obtained using a Bruker Avance 300 spectrometer (Bruker Analytik GmbH, Rheinstetten, Germany). For each sample 2500 scans were obtained from approximately 0.25 g of ball-milled leaf material that was packed into a cylindrical zirconia rotor with approximately 0.02 g Tetrakis (trimethylsilyl) silane (TKS) packed on top and sealed with a Bruker Kel-F drive cap (Bruker Analytik GmbH, Rheinstetten, Germany). The scanning parameters were as follows: frequency of 200 MHz, contact time of 1000 ms, relaxation time of 1.5 s, spinning speed of 5500 Hz and line broadening of 50 Hz. Chemical shift values were obtained compared to TKS. Peak areas of the following chemical shift areas were measured manually: 0-47 ppm (alkyl), 47-59 ppm (N-alkyl and methoxl-C), 59-92 ppm (O-alkyl), 92- 112 ppm (acetal), 112-139 ppm (aromatic), 139-162 ppm (phenolic) and 162-220 ppm (carboxyl) according to

Sjögersten et al. (2003). Note that the N-alkyl region may be over-represented by a contribution from methoxyl-C (Sjögersten *et al.*, 2003).

After analysis by CPMAS ^{13}C NMR, samples were separated from TKS, ensuring no contamination of the sample, and were analysed for carbon and nitrogen content after combustion in a Vario EL Cube elemental analyser (Elementar, Hanau, Germany). The carbon content data were then applied to the actual mass of the litter remaining and the spectra to calculate the mass of carbon in each region of the spectrum and the mass compared to the replicated (n =3), undecomposed control samples of each species.

5.3.5 Statistical analysis

Final percentage mass remaining data (harvest four, 642 days after placement in the field) were analysed using two-way ANOVAs, testing the effect of species (*E. nigrum*, *B. nana* or *B. pubescens*) and environment (heath, shrub or forest). Data were arcsin square-root transformed to meet the assumptions of the parametric analysis. The same analysis was carried out for the effect of different snow depths on percentage mass remaining of litter but with different snow depths as the 'environment' factor.
Mass of different C classes after 642 days was analysed using two-way ANOVAs testing the effect of species (*E. nigrum* or *B. pubescens*) and environment (heath or forest). Each C class was considered separately and differences between combinations of environment and litter species were identified using a Tukey's Honestly Significant Differences (HSD) test. All analyses were carried out using R studio V 0.98.501.

5.4 Results

Litter mass loss

Species identity and decomposition environment both had highly significant effects on percentage mass remaining of litter on the final harvest (642 days) on the transects from H plots, through S plots to F plots, according to a two way ANOVA (Fig. 5.3, Table 5.2). Species identity had the strongest effect ($F = 109.2$, $P < 0.001$) on decomposition, regardless of the environment; *Betula pubescens* consistently had the lowest mass remaining (Fig. 5.3a), followed by *Betula nana* (Fig. 5.3b) then *Empetrum nigrum* (Fig. 5.3c). The decomposition environment also had a highly significant effect on percentage mass remaining of litter ($F = 38.8$, $P < 0.001$). Mass remaining at H plots was significantly higher than at S plots ($P < 0.05$) which, in their turn, were significantly higher than F plots, with the least mass remaining ($P < 0.05$). There was also a significant interaction between species of litter and decomposition environment ($P = 0.002$), driven by a disproportionately low mass remaining of *B. pubescens* in its 'home' environment, the F plots (Fig 5.3a).

At the snow fence plots, there was again a significant effect of species identity on percentage mass remaining of litter according to a two-way ANOVA on the final harvest at 642 days ($F = 109.2$, $P < 0.001$, Fig. 5.4, Table 5.2). Again, *B. pubescens* had the lowest mass remaining at all snow treatments (Fig. 5.4a), followed by *B. nana* (Fig. 5.4b) then *E. nigrum* (Fig. 5.4c). At the final harvest, however, there was no effect of decomposition environment (level of winter snow cover) on decomposition ($P = 0.2$,

Table 2, Fig. 5.4) and no interaction present between species and environment (P = 0.9, Table 5.2).

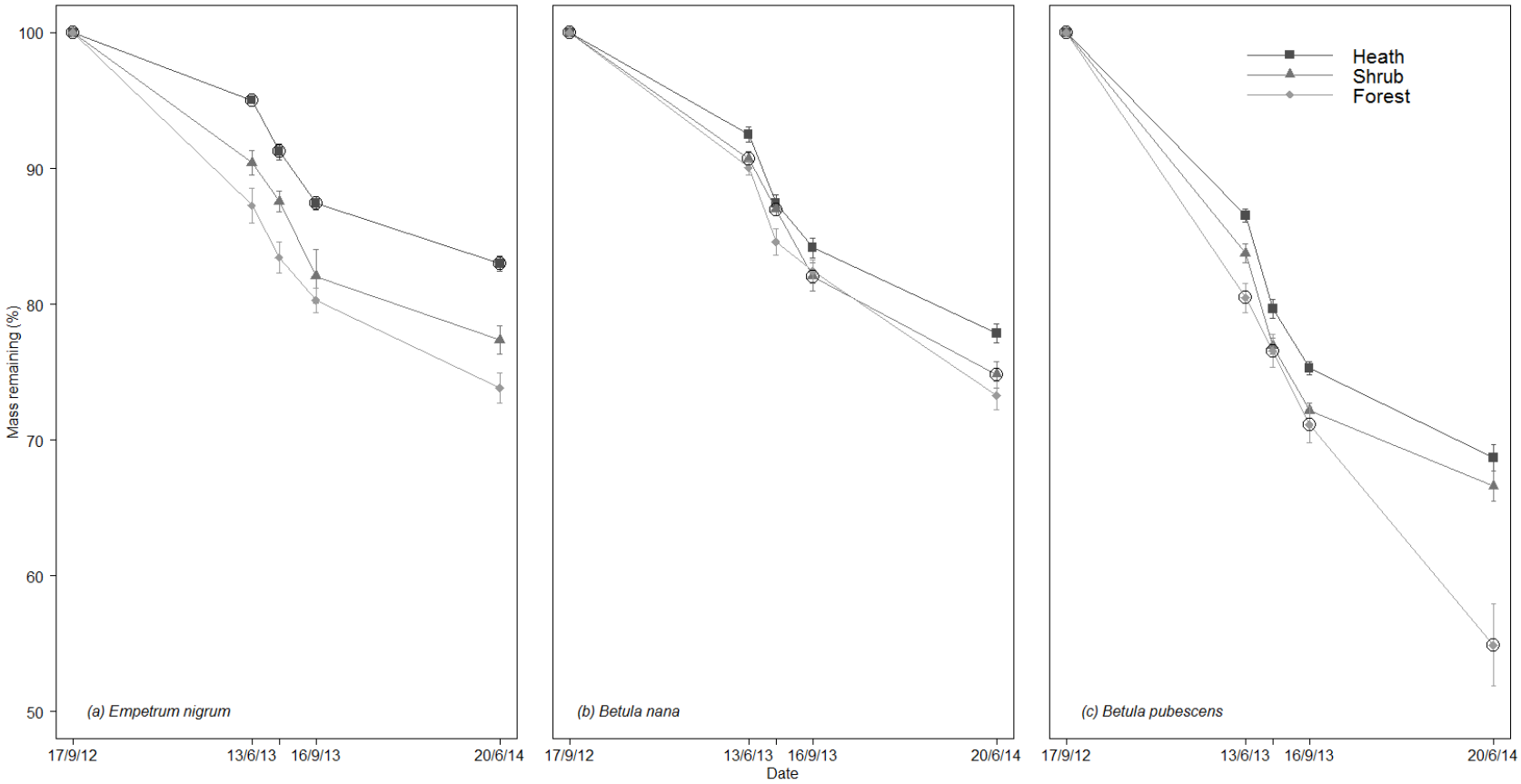

Figure 5.3: Mass remaining of litter over time of three different species: (a) *Empetrum nigrum*, (b) *Betula nana*, (c) *Betula pubescens* ssp *czerepanovii* in three different environments (heath, shrub and forest). Circled points indicate the 'home' environment for each species. Error bars represent ± 1 SEM (n = 12).

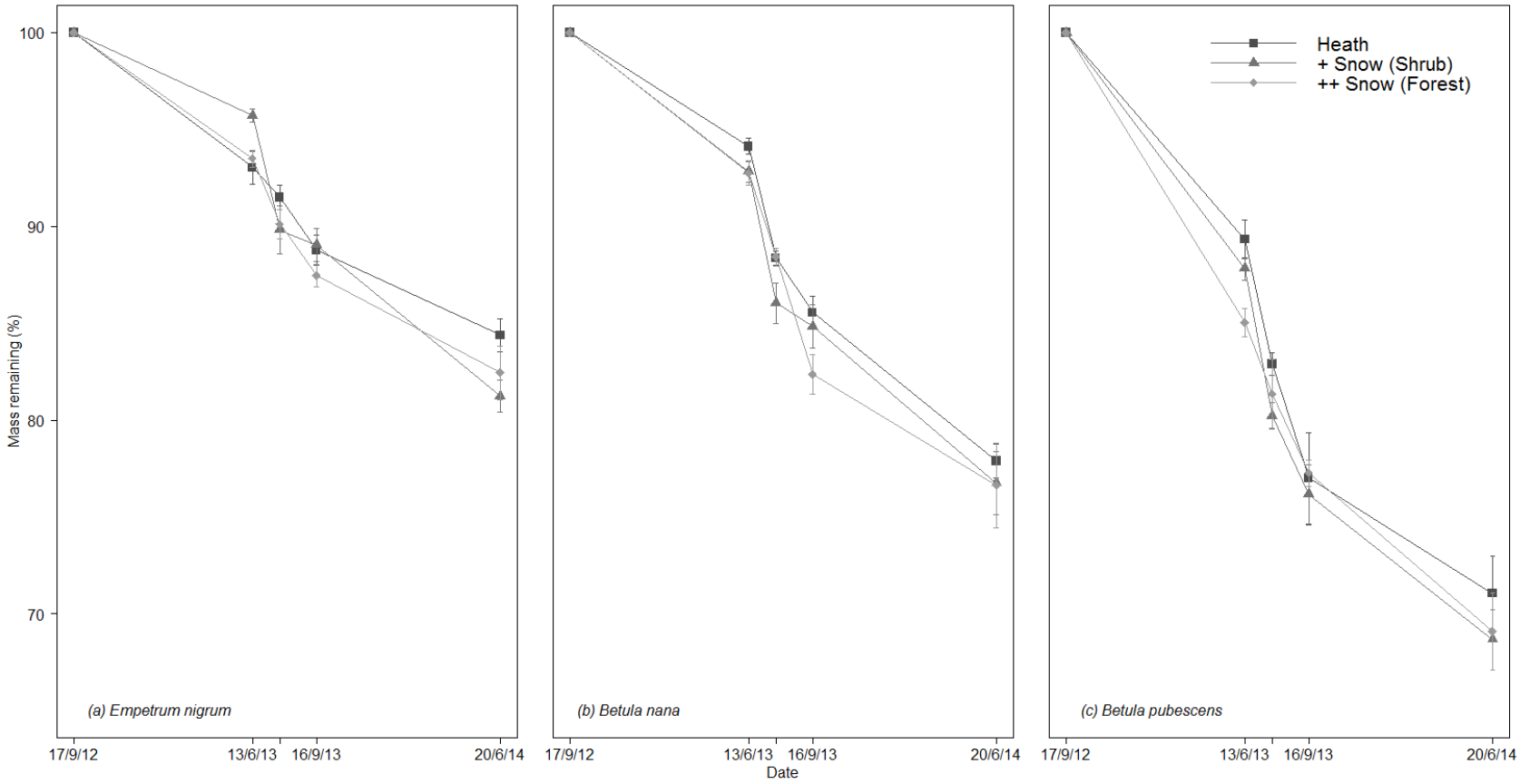

Fig. 5.4: Mass remaining of litter over time of three different species: (a) *Empetrum nigrum*, (b) *Betula nana*, (c) *Betula pubescens* ssp *czerepanovii* under three different snow depths simulating snow accumulation found at different vegetation types: Heath (control), + Snow (Shrub) and ++ Snow (Forest). Error bars represent ± 1 SEM (n = 5).

CPMAS ^{13}C NMR results

For the CPMAS ^{13}C NMR analyses we found clear peaks at ~ 30-32 ppm (alkyls), shoulder regions or peaks at ~ 56 ppm (N-alkyls), clear peaks at ~ 72 ppm (O-alkyls), peaks at 104-105 ppm (acetals), peaks at 115 and 130 ppm (aromatics), peaks at 145 and 153 ppm (phenolics) and a peak at 175 ppm (carboxyls), in all samples (Fig. 5.5). These are all in general agreement with Sjögersten *et al.* (2003).

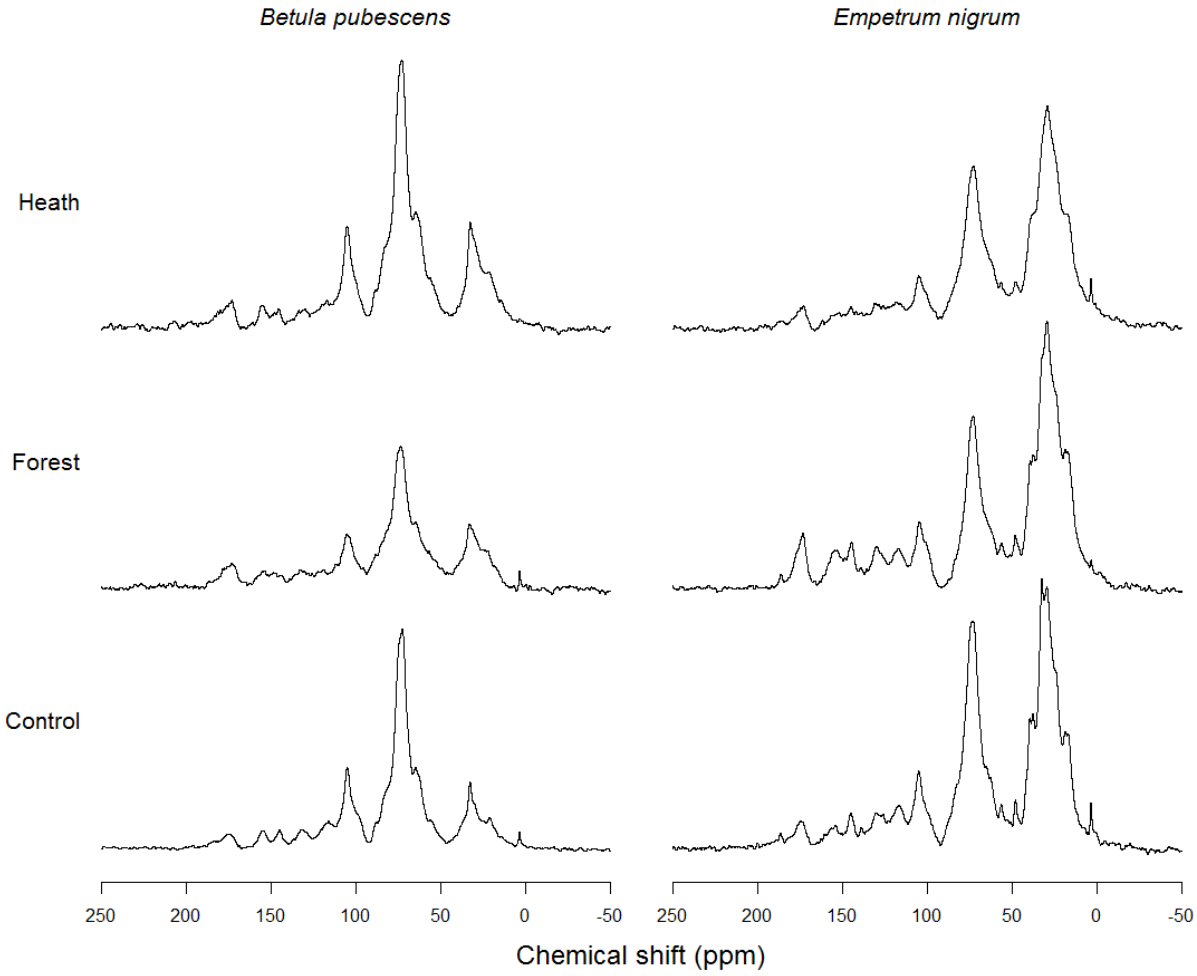

Figure. 5.5: Examples of solid state CPMAS ^{13}C NMR spectra of two litter types in two different environments (including undecomposed control samples). See Table 3 for correspdence of peaks to SOM constituents

CPMAS ^{13}C NMR spectra were divided into regions as set out by Sjögersten *et al.* (2003) and averaged for litter of *B. pubescens* and *E. nigrum* in forest and heath environments (n =5 each) and undecomposed controls (n = 3). Results show clear differences between litter species and environment treatments (Table 5.3); the clearest difference is that the spectra from *B. pubescens* are dominated by O-alkyls (48.8 ± 0.6 % for control) and *E. nigrum* is dominated by Alkyls (45.5 ± 1.1 % for control). This results in substantially different Alkyl:O-Alkyl ratios, which carry through to samples that have been decomposing in the field. Therefore in this case Alkyl:O-Alkyl ratios of samples from the field cannot be compared between species, only between environments. Decomposition in the forest plots increases the ratio markedly in both species compared to the controls, and litter that has decomposed at heath plots. The reason for this is reduction in O alkyls at the forest plots of both *B. pubescens* (down to 40.7 ± 0.9 %) and *E. nigrum* (down to 22.1 ± 1.2 %) and increases in signal from Alkyls with decomposition at both sites (Table 5.3). There were small changes in signals from N- alkyls, acetals, aromatics, phenolics and carboxyls but these were minor in comparison with changes in O-alkyls and Alkyls (Table 5.3).

C:N ratios of *E. nigrum* (control: 138.3 ± 3) were more than double those of *B. pubescens* (control: 60.8 ± 4.3) which both decreased substantially after decomposing in the forest environment (74.6 ± 4.5 in *E. nigrum*, 31.5 ± 1.9 in *B. pubescens*). This was far lower than samples decomposing in the heath environment for both *E. nigrum* and *B. pubescens* (111.6 ± 5.0 and 49.7 ± 0.9 respectively, Table. 5.3).

Table 5.3: Percentage contributions of functional C classes and C to N ratios of litter samples of *Betula pubescens ssp czerepanovii* and *Empetrum nigrum* that were decomposing in forest or heath environments, or un-decomposed control samples. Error values signify ± 1 SEM (n = 5 for decomposed field samples, n = 3 for controls).

	Species	*B. pubescens* ssp *czerepanovii*			*E. nigrum*		
	Plot	Control	Forest	Heath	Control	Forest	Heath
Alkyls (%)	0-47 ppm	16.8 ± 0.1	23.8 ± 1.1	20.4 ± 0.4	45.5 ± 1.1	52.5 ± 1.7	53.0 ± 1.1
N-Alkyls (%)	47-59 ppm	4.9 ± 0.3	6.3 ± 0.1	6.2 ± 0.1	4.1 ± 0.2	4.6 ± 0.2	4.8 ± 0.2
O-Alkyls (%)	59-92 ppm	48.8 ± 0.6	40.7 ± 0.9	46.7 ± 0.6	27.7 ± 1.2	22.1 ± 1.2	25.1 ± 0.6
Acetals (%)	92-112 ppm	12.6 ± 0.5	10.5 ± 0.7	12.1 ± 0.2	6.9 ± 0.2	5.4 ± 0.5	5.7 ± 0.1
Aromatics (%)	112-139 ppm	8.0 ± 0.4	7.0 ± 0.4	6.6 ± 0.3	7.5 ± 0.1	7.0 ± 0.2	6.1 ± 0.2
Phenolics (%)	139-162 ppm	4.6 ± 0.2	4.5 ± 0.4	3.4 ± 0.1	4.7 ± 0.4	4.3 ± 0.6	2.9 ± 0.2
Carboxyls (%)	162-220 ppm	4.3 ± 0.2	7.2 ± 1.0	4.7 ± 0.2	3.7 ± 0.1	4.2 ± 0.5	2.5 ± 0.3
Alkyl:O Alkyl ratio*		0.25 ± 0.0	0.42 ± 0.0	0.31 ± 0.0	1.18 ± 0.1	1.66 ± 0.1	1.49 ± 0.1
C:N		60.8 ± 4.3	31.5 ± 1.9	49.7 ± 0.9	138.3 ± 3.0	74.6 ± 4.5	111.6 ± 5.0

In the majority of functional C classes, the forest environment was associated with lower mass remaining compared to the heath environment, and mass loss/remaining was strongly influenced by litter species (Fig. 5.6, Table 5.4).

Mass remaining of the alkyl functional C class was most significantly related to species ($P < 0.001$) where initial input of alkyl mass was far higher in *Empetrum* (0.12 g) than *Betula* (0.037 g) (Fig. 5.6). The forest environment was also highly significant ($P = 0.0015$) in reducing alkyl mass compared to heath environment in *Empetrum* (0.12 g in the heath, 0.10 g in the forest) and in *Betula* (0.033 g in the heath, 0.026 g in the forest).

Mass of N-alkyls in the decomposed samples was slightly, but significantly higher in *Empetrum* compared with *Betula* ($P = 0.026$). There was a significant effect ($P < 0.001$) of decomposition environment on mass of N-alkyls with mass of *Betula*

lower in forests (0.0071 g) than in heaths (0.01 g); there was, however, no significant difference between mass from the *Empetrum* litter between forest and heath environments.

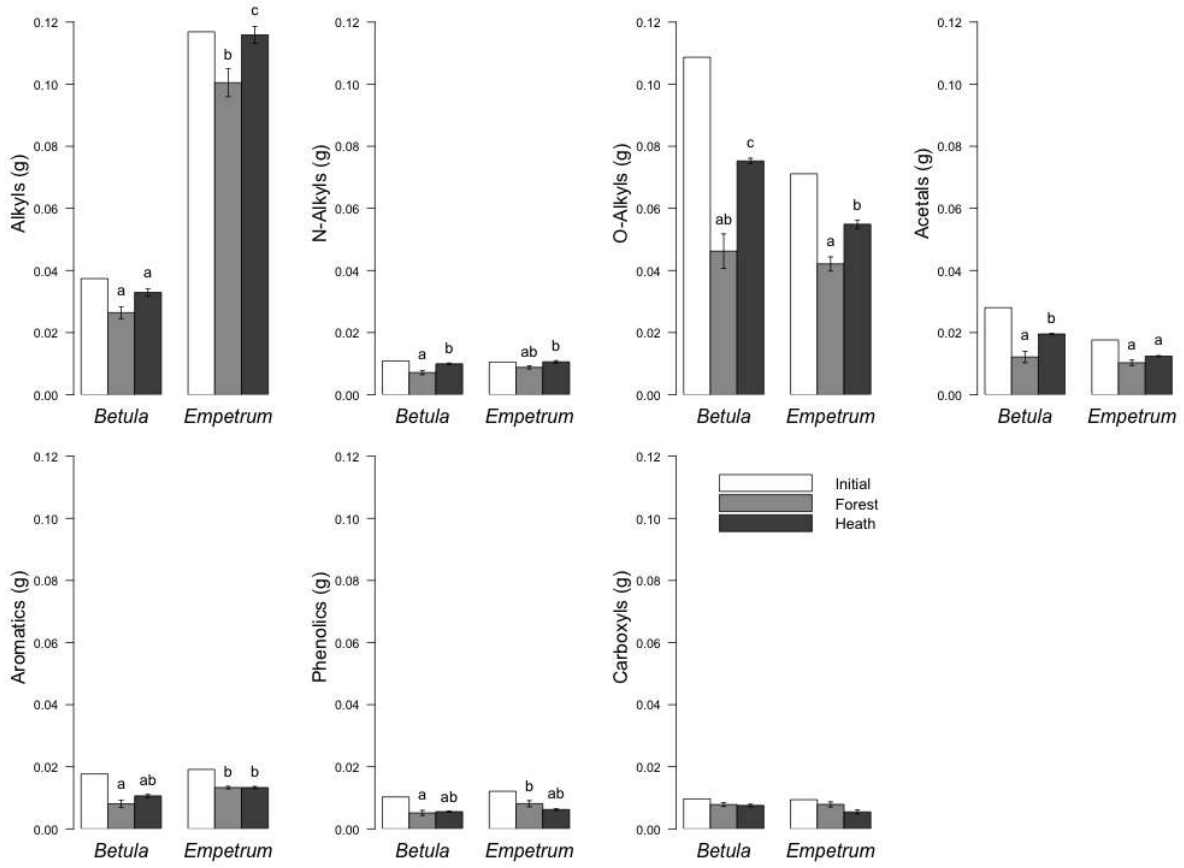

Figure 5.6: Mass of functional C classes of *Betula pubescens* ssp *czerepanovii* and *Empetrum nigrum* in forest and heath environments after 21 months of decomposition compared with undecomposed control samples. Different letters (only decomposed samples) are applied and show significant differences between means ($P < 0.05$) from Tukey HSD *post-hoc* tests. Error bars represent ± 1 SEM (controls: n = 3, decomposed samples: n = 5).

For mass of O-alkyls, there were important effects of litter species (P = 0.0011) and the decomposition environment (P < 0.001). O-alkyl mass was highest in *Betula* litter in the heath (0.075 g), which was significantly higher than in the forest (0.046 g). The same was true for *Empetrum* litter, where mass in the heath (0.042 g) was higher than in the forest (0.054 g); this difference was, however, less pronounced, which resulted in a significant (P = 0.018) interaction between species and environment (Fig. 5.6, Table 5.4).

Table 5.4: Test statistics for two way ANOVAs analysing the effect on mass input of different functional C classes by different species of litter (*Betula pubescens* ssp *czerepanovii* or *Empetrum nigrum*) in different decomposition environments (Forest or Heath).

Group	Factor	d.f	F	P
Alkyls	**Species**	**1,16**	**751.3**	**< 0.001**
	Environment	**1,16**	**14.6**	**0.0015**
	Species * Environment	1,16	2.4	0.14
N-Alkyls	**Species**	**1,16**	**6.0**	**0.026**
	Environment	**1,16**	**23.7**	**< 0.001**
	Species * Environment	1,16	1.3	0.28
O-Alkyls	**Species**	**1,16**	**15.5**	**0.0011**
	Environment	**1,16**	**45.4**	**< 0.001**
	Species * Environment	**1,16**	**6.9**	**0.018**
Acetals	**Species**	**1,16**	**19.3**	**< 0.001**
	Environment	**1,16**	**21.9**	**< 0.001**
	Species * Environment	**1,16**	**6.7**	**0.02**
Aromatics	**Species**	**1,16**	**33.0**	**< 0.001**
	Environment	1,16	3.4	0.082
	Species * Environment	1,16	3.4	0.083
Phenolics	**Species**	**1,16**	**7.5**	**0.015**
	Environment	1,16	1.2	0.29
	Species * Environment	1,16	2.9	0.11
Carboxyls	Species	1,16	2.5	0.13
	Environment	1,16	4.4	0.051
	Species * Environment	1,16	2.8	0.11

There was a highly significant effect of species ($P < 0.001$) and environment ($P < 0.001$) on the mass of acetal groups after decomposition. *Betula* litter in the forest (0.012 g) had significantly lower acetal-containing compounds than in the heath (0.020 g) which was also higher than *Empetrum* litter in the heath (0.012 g) and in the forest (0.010 g); there was no difference between the latter. As a result there was a significant interaction between species and environment ($P = 0.02$) as *Betula* litter responded more to the forest environment than the *Empetrum* litter (Fig. 5.6).

Mass input of aromatic functional C classes was higher in *Empetrum* litter ($P < 0.001$) with a higher mass in both environments (Heath: 0.013 g; Forest: 0.013 g) than *Betula* in the forest (0.008 g) and heath (0.011 g). As with aromatic mass input, species had a significant ($P = 0.015$) effect on mass input of phenolic functional C classes, with mass of *Empetrum* in the heath (0.008 g) the highest measured. There was no effect of species or environment on mass of carboxyl functional C classes.

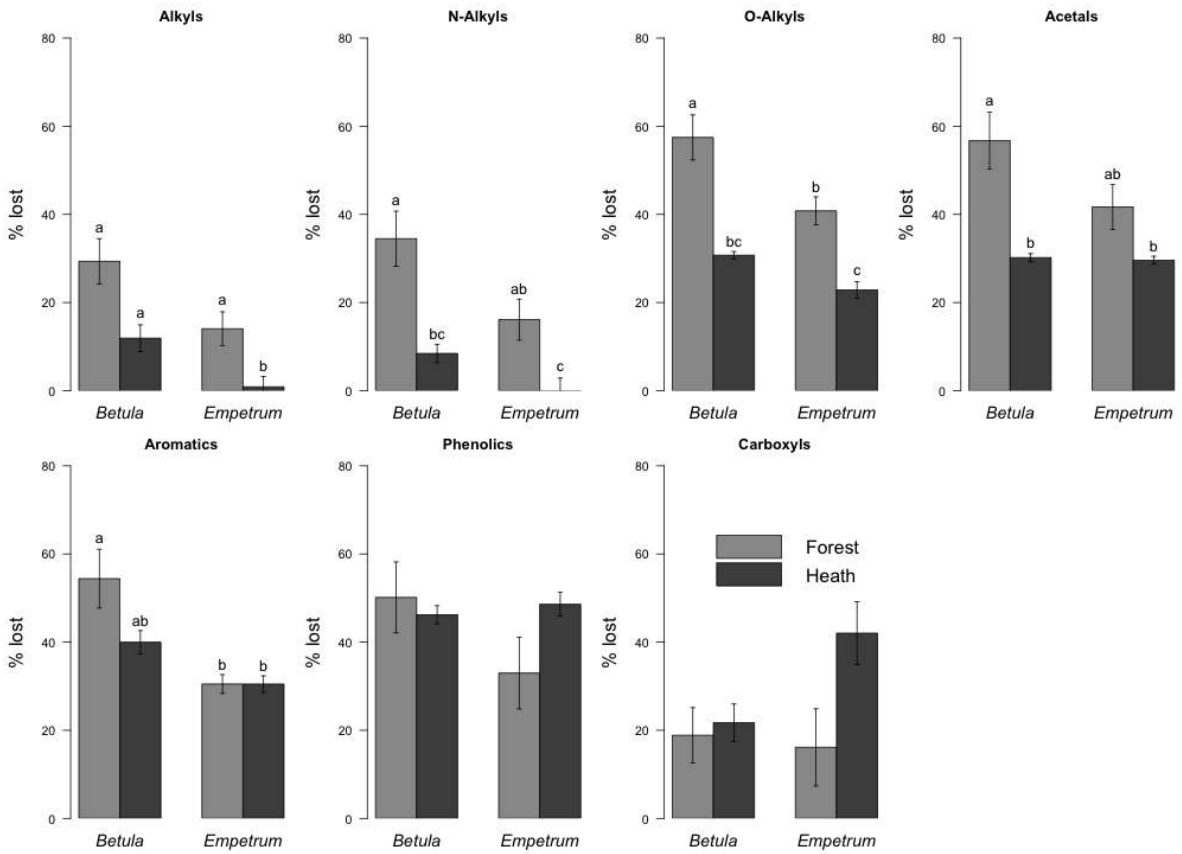

Figure 5.7: Functional C classes of *Betula pubescens* ssp *czerepanovii* and *Empetrum nigrum* in forest and heath environments expressed as percentage (%) lost compared to initial amounts prior to 21 months of decomposition. Different letters are applied and show significant differences between means ($P < 0.05$) from Tukey HSD *post-hoc* tests. Error bars represent ± 1 SEM (n = 5).

Of all the C fractions, O-alkyls showed the largest difference in percentage mass lost compared to initial amounts. Large differences are present between sites where there were large losses in the forest compared with the heath and between species where *Betula* lost more than *Empertum* (Fig 5.6, Table 5.5). Acetals showed a very similar pattern of percentage mass loss with the largest loss was from *Betula* litter in the forest. There were also important percentage mass loss effects in Alkyls and N-alkyls where there was consistently higher loss in the forest plots and more loss of C from *Betula* litter than *Empetrum* litter (Fig 5.6, Table 5.5). There was significantly more loss of aromatics from *Betula* litter in the forest than *Empetrum* in either of the ecosystems and no definite patterns with Phenolics and Carboxyls.

Table 5.5: Test statistics for two way ANOVAs analysing the effect on percentage mass loss of different functional C classes by different species of litter (*Betula pubescens* ssp *czerepanovii* or *Empetrum nigrum*) in different decomposition environments (Forest or Heath).

Group	Factor	d.f	F	P
Alkyls	**Species**	**1,16**	**9.8**	**0.006**
	Environment	**1,16**	**11.8**	**0.003**
	Species * Environment	1,16	2.1	0.16
N-Alkyls	**Species**	**1,16**	**8.9**	**0.009**
	Environment	**1,16**	**19.7**	**< 0.001**
	Species * Environment	1,16	0.13	0.72
O-Alkyls	**Species**	**1,16**	**15.5**	**0.001**
	Environment	**1,16**	**50.5**	**< 0.001**
	Species * Environment	1,16	1.5	0.25
Acetals	**Species**	**1,16**	**3.6**	**0.08**
	Environment	**1,16**	**21.7**	**< 0.001**
	Species * Environment	1,16	3.0	0.7
Aromatics	**Species**	**1,16**	**19.1**	**< 0.001**
	Environment	1,16	3.5	0.082
	Species * Environment	1,16	3.5	0.082
Phenolics	Species	1,16	1.6	0.22
	Environment	1,16	1.1	0.32
	Species * Environment	1,16	2.6	0.12
Carboxyls	Species	1,16	0.6	0.44
	Environment	1,16	4.4	0.051
	Species * Environment	1,16	2.7	0.11

A

5.5 Discussion

This decomposition experiment has highlighted a number of mechanisms at work which are fundamental in determining C storage and cycling in forest, shrub and heath ecosystems which can go a significant way in explaining why so little soil C is stored in sub-arctic birch forests (Chapter 2). Decomposition of dominant forest and shrub species' litter was higher than a dominant heath species regardless of decomposition environment and decomposition of all species was higher in forest and shrub environments than in the tundra heath.

This comparison of the most important litter types in their typical decomposition environments across the forest-heath ecotone reveals how large the difference in decomposition rates in these two systems is. Previous work had shown that decomposition rates were higher in the forest than tundra heaths (Sjögersten & Wookey, 2004), but this was only using *Betula pubescens* litter which would not typically be deposited (in substantial amounts) in tundra heath systems. After 21 months of decomposition, *Empetrum nigrum*, one of the dominant species on northern Fennoscandian heathlands, lost on average ~17 % of its mass when in the heath environment (Fig. 5.3). This is in contrast to the loss of *B. pubescens*, in the forest environment, which was 45 %. There are clearly large differences in decomposition rates between these important species, which will contribute significantly to C accumulation in their respective environments. Previous studies have shown that deciduous litter decomposes faster than evergreen (Cornwell *et al.*, 2008) and that decomposition occurs at a faster rate in forests than heaths (Sjögersten & Wookey, 2004) but to our knowledge the two factors have not been combined to represent better the decomposition rates in these two ecosystems.

The decomposition rates of *Betula nana* were examined. This is a shrub species which has been observed to be expanding its range over arctic tundra in response to climate change (Tape *et al.*, 2006; Myers-Smith *et al.*, 2011). This litter also has significantly higher decomposition rates than *Empetrum*, which could contribute towards observations of high C flux in these shrub systems (Chapter 2) and low standing litter stocks in plots that have undergone increases in shrub cover (Zamin *et al.*, 2014). However, *B. nana* decomposed at slower rates than *B. pubescens*. This could be due to a number of factors including specific leaf area, N content and structural C compounds although there is no data presented here to test this. Based on species specific decomposition rates, what are our data suggest is that expansion of *B. pubescens* forests will increase decomposition in tundra more than expansion of *B. nana* but both will increase C cycling rates through litter input.

Decomposition of all litter types was enhanced in forest and shrub environments compared with the ericaceous heath plots. This has previously been shown with *B. pubescens* litter (Sjögersten & Wookey, 2004) but the finding that the decomposition of ericaceous plants from a tundra environment can be stimulated in a forest environment is novel. Forest environments have a large community of saprotrophic fungi in the litter layer (Lindahl *et al.*, 2007) and below that, an ECM community, of which some have the same white and brown-rot abilities (Bödeker *et al.*, 2014; Lindahl & Tunlid, 2015). Although a decomposer community exists in tundra ecosystems (Robinson, 2002), this evidence clearly shows that it is not as effective at degrading any of the litter types as the community present in the forest.

The abiotic environment in which decomposition occurs in forests contrasts with tundra heath, especially in the winter, when deeper snowpacks accumulate in treeline forests, insulating the soil and creating both a warmer and more stable decomposition environment (Sturm *et al.*, 2005) with faster C cycling rates (Grogan & Jonasson, 2006). When snow was experimentally increased for two winters over litter on tundra soils, no increase in decomposition of any litter type was detected. The fastest-decomposing species, *B. pubescens*, saw initial increases in decomposition after the first winter, when snow was manipulated to the depth found in forest systems. This effect was lost, however, after only 41 days of decomposition during the growing season. These results are consistent with a similar decomposition experiment using a common substrate on Alaskan tundra which saw no increase in decomposition with increases in snow (DeMarco *et al.*, 2014). Another experiment in the same landscape as the present study, showed that decomposition was unaffected by a loss of snow under an extreme winter warming experiment (Bokhorst *et al.*, 2010). It can therefore conclude that, although snow-mediated winter processes are important for microbial community turnover (Schimel *et al.*, 2004) and contribute significantly to the C budget of tundra ecosystems (Fahnestock *et al.*, 1999; Oechel *et al.*, 2014), they may not have an important direct influence on decomposition of recently senesced litter.

Having ruled-out snow depth alone as an important control over litter decomposition at our sites, it is clear that other environmental factors associated with forest and shrub systems are more important in determining microbial growth and therefore decomposition rates (for a review see Rousk & Bååth (2011)). Temperature (Pietikainen *et al.*, 2005) and pH (Rousk & Bååth, 2011) are important

in determining fungal and bacterial growth rates, however both have been shown to be remarkably similar in the summer across the study ecotones (Table 1). This leaves differences in chemical environment between forest and heath as the key control of decomposition. In a controlled lab study it was found that fungal growth is stimulated by cellulose addition to soil (Meidute *et al.*, 2008) and this effect was amplified further when nitrogen was also added, which is consistent with field studies (Subke *et al.*, 2004; Knorr *et al.*, 2005). This may explain why there was a disproportionate decrease in mass of *B. pubescens* in the forest plots similar to 'home-field' advantage; this is where litter of dominant species decomposes fastest in their own environment due to a specifically-adapted microbial community (Ayres *et al.*, 2009).

Microbial 'home-field' advantage may be at work at our plots; however, there could be another mechanism is at play. *B. pubescens* produces litter rich in polysaccharides such as cellulose, which stimulates saprotrophic fungal growth and positively feeds back to further increase decomposition rates. In addition to this, *B. pubescens* produces litter which has far lower C:N than *Empetrum*, and this can also stimulate decomposition (Buckeridge *et al.*, 2010; DeMarco *et al.*, 2014; Stark *et al.*, 2014). This mechanism could be of particular importance in some areas, with further expansion of birch forest (Tommervik *et al.*, 2009; Rundqvist *et al.*, 2011; Hofgaard *et al.*, 2013). Our data show that colonization of tundra by forest could stimulate decomposition not only of *B. pubescens* litter but also litter of the dominant heath species. This, together with processes in the rhizosphere such as positive priming (Hartley *et al.*, 2012) and colonisation with ECM fungi (Chapter 2) could lead to a net loss of C from the system.

Using ^{13}C NMR, the hypothesis was tested that all fractions of C in both *B. pubescens* and *E. nigrum* would be more decomposed in the forest than the heath because all fractions of C should be available to decomposition if a suitable decomposer community is present (Dungait *et al.*, 2012). This is partially supported. There was significantly higher loss in the forest of compounds associated with carbohydrates (O-alkyl and acetal regions) and aliphatic C associated with fatty acids in cuticle compounds such as cutin (alkyls) (Kögel-Knabner, 1997; Sjögersten *et al.*, 2003; Simpson & Simpson, 2012). Together, these made up the majority of litter mass in both species and therefore their decomposition was the main driver of litter mass loss. We was, however, no difference between environment in the decomposition of fractions of C associated with lignin (aromatic and phenolic groups). There are two reasonable explanations for this: Firstly, that loss of these compounds takes place later in the decomposition process and our study was not of sufficient duration to capture this. This would be consistent with traditional theories of litter decomposition and accumulation of humus in soil (Melillo *et al.*, 1989). On the other hand, although there are no differences in lignin-derived compounds between sites, they have lost mass compared to the undecomposed controls. This may be due to two separate processes; (i) in the forest the saprotrophic community of fungi in the litter (Lindahl *et al.*, 2007) have all the enzymatic capabilities for white-rot decay of lignin (Hatakka, 1994), while (ii) in the heath, the primary decomposition pathway is through ericoid mycorrhizal (ERM) fungi associated with their dominant ericaceous hosts. ERM fungi have a greater capacity to degrade organic complexes than ECM fungi (Bending & Read, 1997; Read & Perez-Moreno, 2003) and their ability to take up organic N allows them to create a closed

N cycle to out-compete other fungal species (Read & Perez-Moreno, 2003). In this way, aromatic compounds may be decomposed at equal rates in both heath and forest, as was observed.

Interestingly, there were more lignin-derived compounds in *E. nigrum* litter than *B. pubescens* after 21 months of decomposition. Even though it only makes up small proportions of the litter recovered, these compounds will persist longer in the environment than others and therefore likely contribute towards SOM build-up observed at these sites (Hartley *et al.*, 2012). It was hypothesised that an expansion of deciduous shrubs and trees into arctic tundra would result in a negative feedback to climate change due to an increase in these slowly-decomposing compounds through litter fall (Cornelissen *et al.*, 2007). This could be true if the species being replaced are graminoid or forb species (Cornelissen *et al.*, 2007). However, if the tundra is dominated by *E. nigrum*, as is the case for much of Fennoscandia (Tybirk *et al.*, 2000), along with many other sites across the arctic circumpolar distribution (Walker *et al.*, 2002; Büntgen *et al.*, 2014) where northern limits of forest are interspersed with tundra (Payette *et al.*, 2001), this feedback is unlikely; in fact, the opposite may be true, with an acceleration of C cycling.

Previous measurements of the chemical fractions of C in the forest and heath at Abisko have shown a dominance of aliphatic compounds (alkyls) in the heath (similar levels have also been observed in another Fennoscandian tundra; (Väisänen *et al.,* Personal Communication)), but less so in the forest (Sjögersten *et al.*, 2003). It was shown that this is due to high concentrations of alkyls in *E. nigrum* with a well-developed waxy cuticle made up of cutin, a lipid polymer (Bliss, 1962;

Hetherington *et al.*, 1984), which decomposes slowly (Tegelaar *et al.*, 1989; Rasse *et al.*, 2005). This could therefore be a major fraction of stored C in tundra soils. Around a third of the alkyl input in *B.pubescens* litter, which corroborates with amounts observed in the soil (Sjögersten *et al.*, 2003). A significant proportion of alkyls observed in forest soils will not be directly due to the chemistry of the litter, rather due to the faster decomposition process; as O-alkyl-containing structures such as polysaccharides are decomposed, alkyl structures increase in their concentration as products of the decomposer community, or transformation of the organic compounds (Baldock *et al.*, 1997; Sjögersten *et al.*, 2003). This can be seen in both species; the alkyl: O-alkyl ratio is far higher in the forest than the heath, and this is another strong indicator of fast C cycling in the forest, resulting in low storage in these systems (Hartley *et al.*, 2012) in spite of relatively high primary productivity.

The decomposition environment, not the species of litter, was found to be the most important factor controlling O-alkyl input into the soil after 21 months of decomposition. This group of C, which makes up a core of plant structural compounds such as cellulose and hemicellulose (Kögel-Knabner, 2002; Simpson & Simpson, 2012; Rytioja *et al.*, 2014), was significantly more decomposed in the forest than the heath regardless of the species of litter. In the heath, with a hypothesised contrasting decomposer community, the polysaccharide constituent of litter has decomposed at a far slower rate. Labile C input by *B. pubescens* litter, together with an environment and microbial community conducive to cellulose decomposition, results in rapid decomposition of these compounds in the forest.

This gives a very good explanation as to why such high rates of C cycling in these deciduous stands of vegetation are observed (Chapter 2).

It was observed that in the organic matter of tundra heath plots similar to the present study there were consistently higher O-alkyl and acetal-related compounds than in near-by birch forests (Sjögersten *et al.*, 2003). Similarly high amounts of labile carbohydrate-derived compounds have been observed in a Norwegian heath (Väisänen *et al.,* Personal Communication). Tundra soils in Siberia (Uhlirova *et al.*, 2007) and Alaska (Dai *et al.*, 2002) have also been characterised as carbohydrate-rich and chemically accessible, should a suitable microbial community be present. The results from this study contribute towards our understanding of why these observations have been made when it had been expected that heaths would have had a more recalcitrant C signature. It is evident that the slow degradation of cellulose-related carbohydrates in the heath is causing this group that is traditionally considered to be easily accessible to decomposer communities to be left intact. This highlights that much of the large store of C in the Arctic (Tarnocai *et al.*, 2009) may be in forms relatively accessible to soil microbes, with a high potential to be metabolised rapidly.

The current limited decomposition of carbohydrates in the heath is significant for their future C balance in the context of the expansion of forests in some areas of the Sub-arctic (Tommervik *et al.*, 2009; Rundqvist *et al.*, 2011; Hofgaard *et al.*, 2013) and shrubs in large areas of the arctic tundra (Tape *et al.*, 2006; Myers-Smith *et al.*, 2011). If tundra heath soils, rich in undecomposed, labile forms of C (Sjögersten *et al.*, 2003), are colonised by forest, with its associated fungal community (including

ECMs), there will be a rapid metabolism of this C and a significant part of the stored C will be released to the atmosphere. This could lead to a positive feedback mechanism, contributing towards further warming of the Arctic and further shrub expansion.

In conclusion, it was shown, using a large-scale decomposition experiment involving transplants of dominant litter types across the forest-heath ecotone, that cycling of litter is extremely fast in the forest. This is due to a carbohydrate-rich input of litter from the birch canopy and the presence of a decomposer community that can metabolise this relatively labile source of C. An interaction between these two factors causes disproportionately higher decomposition rates in the forest compared to the tundra environment. Using a snow fence experiment on tundra soils it was shown that the effect of increased snow in the forest compared to the heath alone is minimal and that the effect of environment on decomposition rates in the forest is mostly due to microbial processes over summer. Reduced decomposition of polysaccharide-containing compounds in the heath explains why other studies have observed them to be rich in O-alkyl fractions of C. This prompts the prediction that this build-up of microbially accessible C will be vulnerable to decomposition should more productive deciduous species further expand onto the tundra, resulting potentially in a net emission of CO_2 to the atmosphere.

Chapter 6: General discussion and conclusions

6.1 Introduction: Climate, vegetation and soil carbon

Ecosystems in the Arctic and Sub-Arctic are experiencing rapid changes (Post *et al.*, 2009) due to regional climate warming of greater magnitude than the rest of the Earth (Serreze & Barry, 2011). One important change has been the expansion of shrub species (Myers-Smith *et al.*, 2011) which has been contributing towards a greening trend as northern latitudes become more productive (Epstein *et al.*, 2012). In addition to this, some treelines have been observed to move north, especially in the sub-Arctic (Harsch *et al.*, 2009). This change in vegetation dominance may have wide-reaching impacts if it interacts with the large stocks of carbon (C) stored in arctic soils (Tarnocai *et al.*, 2009).

The research contributing to this thesis demonstrates, using space-for-time transitions across treeline ecosystems, that expansion of shrubs and trees onto tundra may lead of a net loss of carbon (C), despite high productivity. It joins a growing body of work from northern latitudes that shows that more productive vegetation may store less C (Wilmking *et al.*, 2006; Kane & Vogel, 2009; Hartley *et al.*, 2012). Using field-scale experiments, however, together with process measurements, detailed soil organic matter characterisation, and fungal community analysis, it provides an improved mechanistic understanding of the controls of soil C dynamics and stocks. This gives us an understanding of their likely fate in the future, in forest and shrub systems near the arctic treeline.

6.3 Sampling design strengths

The transects approach across the forest-tundra ecotone puts a focus on differences in vegetation and their effects on soil processes, with the replication across numerous ecotones enabling a scaling of the dataset to the landscape scale (~ 2 km^2). This approach took into account noise in the data related to geographical variation in soil properties but retained the vegetation differences across 12 independent transects.

The space-for-time design of this work was critical because it under-pinned the sampling design in each of the chapters with the exception of chapter four. The transitions in vegetation over relatively short distances (< 100 m) provide controls for a number of co-variables, which can confound a more simplistic comparison of vegetation communities located further apart. Soil formation is dependent on five key factors: *time* to develop, *parent material*, *topography* of the land, regional or micro-*climate*, and the *organisms* present (Jenny, 1941). With these small-scale transitions in vegetation, one can be reasonably confident that bedrock and climate were similar between vegetation types across each ecotone. Organic-horizon pH across transects was found to be remarkably similar. This could suggest that the influence of the bedrock and glacial till was similar. Mineral soil pH, although not measured in this thesis, could have important influences on decomposition and soil processes. Low pH favours high fungal: bacterial ratios (Rousk *et al.*, 2009) and slows soil respiration rates (Andersson *et al.,* 2000). Therefore, more focus needs to be applied to the relative effect of mineral soil on pH along the transects compared

to the effect of vegetation on carbon cycling (e.g. acidification in heaths via ericaceous litter input (Tybirk *et al.*, 2000).

Transects were chosen to have no clear topographical contrasts between plots (the mean change in altitude from heath to forests was -2.7 m (Appendix 1)). With all the other soil forming factors considered (Jenny, 1941), it is reasonable to conclude that differences that were observed in soil C storage and cycling are principally a result of differences in vegetation and their associated microbial community.

6.3 Evidence for hypothesised differences in plan-soil interactions along tree-line ecotones

This body of work was set out to test specific hypotheses relation to the effect of different plant function types and ecosystem types on belowground processes and ultimately soil carbon storage. These are set out in Figure 6.1, as they were set out in the introduction (Fig. 1.5). The transition from heath to forest, through a thick shrub layer represented an increase in vegetation productivity as well as a shift in plant functional types, which are predicted and already observed with climate change (Myers-Smith *et al.* 2011). The overall and most important prediction, in contrast with predictions of earth system models (e.g. Todd-Brown *et al.* 2013) was that as vegetation increases in biomass along the transect, SOC storage would decrease as a result of fast C cycling. Strong evidence was found to support this notion in chapter 2 where a study of SOC stocks along the transition was found to rapidly decrease from heath to shrub to forest ecosystems. Low SOC storage in

more productive vegetation types coincided with rapid respiration rates, which further supported this hypothesis.

The graphics in Figure 6.1 go on to hypothesise that the increased the co-increases in ectomycorrhizal fungi (ECM) are important in the stimulation of decomposition and therefore low SOC storage in shrub and forest ecosystems. Two of lines of evidence support this. Firstly, a study of ECM hyphal production showed that ECMs growth was associated with the areas in which SOC storage was lowest and respiration rates highest. This therefore suggests that they are implicit in the fast turnover and low storage of SOC in sub-arctic forests and shrub tundra. The second line of evidence is that when trees were defoliated, therefore reducing C supply to ECMs, there was an observed reduction in respiration rates. This implies, not only that recently assimilated photosynthate is involved in the stimulation of respitation rates (Högberg *et al.*, 2001; Hartley *et al.* 2012) but also mycorrhizal fungi are important conduits for the stimulation of decomposition (Talbot *et al.* 2008).

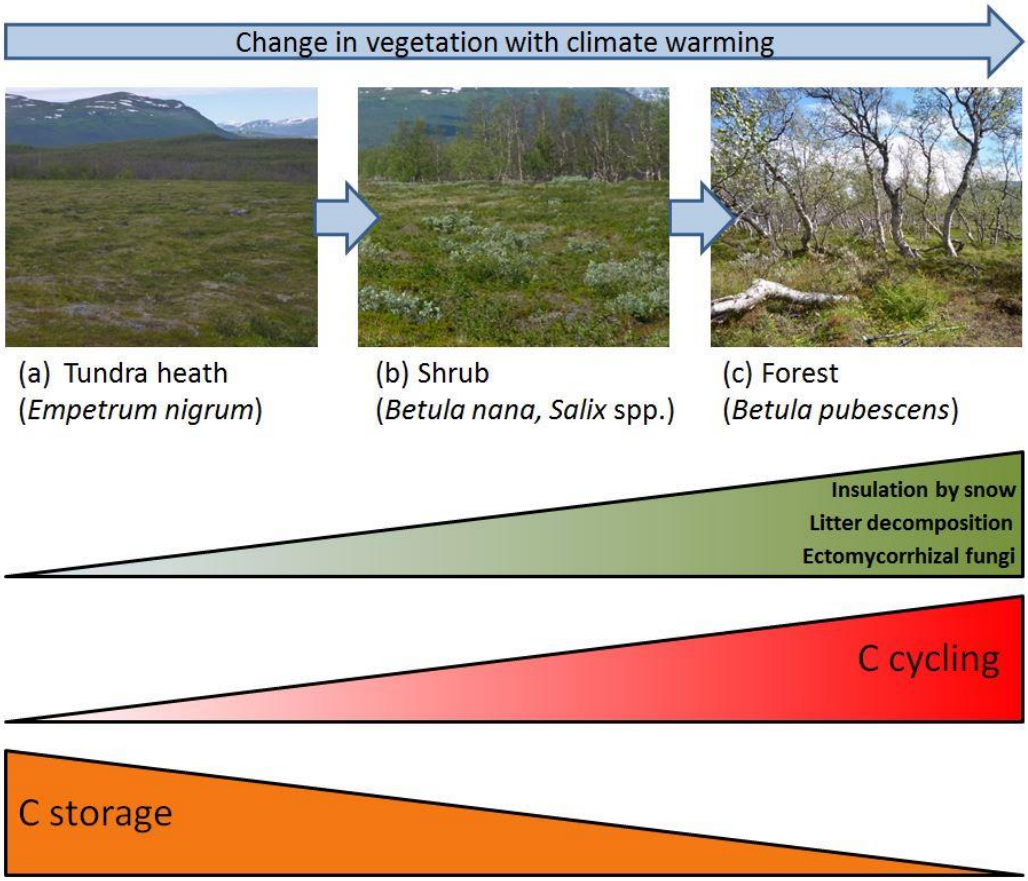

Figure 6.1: Conceptual diagram showing contrasting vegetation communities across ecotones: (a) Tundra heath, (b) Shrub, (c) Forest with shrubs and forests predicted to expand across tundra vegetation (Pearson *et al.*, 2013) this ecotone represents a 'space for time' transition. Hypothesised strength of ecological influences on C cycling and storage are represented below.

The second mechanism that was suggested to enhance C cycling rates and therefore reduced SOC storage in the most productive plots on the study transects was the accumulation of snow in the winter time. Evidence suggests that this affects carbon cycling throughout the year in a number of ways. Snow accumulations around tall vegetation insulate the soil from the extremes of winter air temperature. The data loggers deployed on six of the transects supported this comprehensively over two winters. Based on the measurements by others (e.g. Schimel *et al.,* 2004) which showed that tundra soil microbial communities remain active over winter and are responsive to increased temperature through snow addition, it is reasonable to suggest that winter carbon cycling rates are higher in forest and shrub plots compared with heath. The fluxes of C over winter, although unquantified in this work, may contribute significantly to low SOC stocks under forest and shrub vegetation. The soil transplant experiment between forest and heath plots suggested that deep cover of snow in forests also affected summer respiration rates by incubating the microbial community which in turn was more active over summer. An analysis of microbial community composition is needed to test this hypothesis however. In chapter 5, the effect of deep snow cover was found to be negligible in relation to surface litter decomposition. This therefore implied that effects of deep snow via differences in vegetation stature have a larger effect deeper into soil horizons. On balance, the evidence supports the hypothesis that deep snow cover is a mechanism by which decomposition of SOC under more productive species is enhanced.

The final hypothesis put forward by Figure 6.1 is that decomposition of litter is extremely fast in forests and shrubs and although litter fall is undoubtedly higher for

these species, it does not result in storage of C in the soil. The evidence supported this. Decomposition rates were highest in the forest plots, followed by the shrub plots and lowest rates at heath plots. The litter transplant experiment allowed a comparison of both environment and litter biochemistry in dictating these patterns of decomposition. It was clear that litter decomposition was particularly fast in the mountain birch forest because litter chemistry was carbohydrate rich with a low C:N ratio and that the summer environment was conducive to rapid decomposition.

Taken together, the various experiments and studies over the natural transitions of the Abisko tree-line allowed for a comparison of plant-soil interactions which all implied that more productive ecosystems store less carbon in the soil as a result of fast carbon-cycling. All imply that a continued expansion of shrubs and tree-lines around the Arctic will result in a shift in the carbon balance with a net loss of C from the soil.

6.4: Scaling to the rest of the Sub-Arctic and Arctic.

In Chapter 2, the surveys in soil organic carbon (SOC) were scaled to a different landscape, Vassijaure, which had contrasting climate but retained the same transitions in vegetation. The common pattern of low SOC in more productive ecosystems across two landscapes increases the confidence that can be taken in extrapolating these results across wider arctic ecosystems. The Sub-Arctic where patchy treelines form multiple transitions in vegetation, similar to those at Abisko (Payette *et al.*, 2001) are applicable here but also beyond. One key difference between the sites at Abisko and much of the rest of the Arctic is the presence of

continuous permafrost (Tarnocai *et al.* 2009). The sites at Abisko and Vassijaure had no such permafrost, therefore the applicability of these results to other such areas could be debated. Permafrost stores large amounts of carbon in frozen mineral horizons (Tarnocai *et al.,* 2009) and may slow decomposition through restriction of drainage and waterlogging (Natali *et al.*, 2011; Zona *et al.*, 2011). However, loss of organic horizon C has been found under an recently expanded spruce stand at a treeline on permafrost in Alaska (Wilmking *et al.*, 2006) and fertilisation of tussock tundra was found to increase shrub biomass, whilst reducing total soil C storage (Mack *et al.*, 2004). Although the direct plant-soil interactions leading to C loss in these studies are not well understood, the suggestion is that organic C underlain with permafrost may be degradable (Dai *et al*., 2002) and that vegetation change could be important in this process. Therefore, although the Abisko site is dissimilar to many other arctic regions in its lack of permafrost, the vegetation transitions that are present are representative of many arctic ecosystems (Walker et al. 2000, Kaplan *et al.* 2003). So far, the results form this work marries with studies over similar transitions (Wilmking *et al.*, 2006).

The scale of productivity increase (Epstein *et al.,* 2012) and shrub expansion (Myers-Smith *et al.,* 2011) is pan-arctic. Therefore the priority with this line of research is to make comparisons analogous to the work presented here but in other areas of the Arctic, specifically where abiotic constraints on decomposition differ. This has been achieved to an extent in the present work where areas of different precipitation levels were compared (Chapter 2) but this should be expanded across areas of the arctic in order to better understand the likely fate of SOC with continued vegetation change.

6.5: The influence of Ectomycorrhizal fungi on carbon cycling.

One of the strong themes to emerge from this work is the relationship between ectomycorrhizal fungi (ECM) abundance and speed of carbon cycling. This work would suggest that ECMs are central to the fast turnover of C in forest and shrub ecosystems. There is a growing body of evidence to suggest that ECM fungi enhance decomposition rates as a result of their scavenging for organically bound N (Talbot *et al.* 2008). This moves past the previous paradigm that ECM fungi have very little capability to degrade complex C compounds such as lignin and phenolics (Read & Perez-Moreno, Read *et al.,* 2004). Data presented here and in other studies (Bödeker *et al.*, 2014) show that ECM fungi (depending on lineage) have the capability to produce a range of important extracellular enzymes which can breakdown complex C compounds which in turn, liberates N for uptake. This ability may allow shrubs and trees to access N stored deep old horizons of the tundra heath should they expand further north and up mountain slopes (Myers-Smith *et al.,* 2011; Rundqvist *et al.,* 2011). It was shown that one of the dominant ECM genera, *Continarius*, expressed more perxodidase- coding genes when less mineral-N was present in the soil (Bödeker *et al.*, 2014). Therefore as ECM fungi expand their influence through the expansion of their hosts, expression of such genes may increase as C:N ratios of tundra heaths are known to be higher than forest ecosystems (Read & Perez-Moreno 2003).

The mycorrhizal community the heaths studied here are dominated by ericoid mycorrhizal fungi of the dominant ericaceous dwarf shrubs such as *Empetrum nigrum* (Tybirk *et al*., 2000; Read & Perez-Moreno 2003). These species and their

ericoid mycorrhizal fungi exert a dominance over heath systems which excludes other plants and mycorrhizae (Nilsson & Wardle 2005) through creating a closed N cycle and producing allelopathic chemicals (Tybirk *et al.* 2000). Ericoid mycorrhizal fungi are proficient at taking up organic N as well as producing a suite of extracellular enzymes which can break down complex C compounds in order uptake N ahead of other species (Bending & Read 1997; Read *et al.* 2004; Talbot *et al.* 2008). The high organic-N content of ericaceous species' litter gives their ERM fungi a competitive advantage over other mycorrhizal types which creates a 'closed' N cycle between ericaceous plants and their fungi (Read & Perez-Moreno 2003).

Although ERM fungi can produce the largest range of C-degrading enzymes and break down the most complex compounds, they lack the anatomy and energy to grow significantly into the soil (Smith & Read 2008). Heathlands are very unproductive with a strategy for slow growing, unproductive species (Tybirk *et al.* 2000). Therefore there is little energy in the form of fixed-carbon to seriously stimulate decomposition through supply to ERM fungi. There is no evidence of ERM hyphae extending past the influence of ericaceous root hairs (Smith & Read 2008). ECM fungi, although they have a smaller range of extracellular enzymes (Talbot et al. 2008), receive large proportions (up to 20 % (Hobbie 2006)) of fixed C by shrubs and trees by have a large capacity to forage the soil for nutrients. Shrubs are up to five times more productive than ericaceous heaths (Shaver, 2010). It is becoming clear that this ability contributes significantly to decomposition (Heinemeyer *et al.* 2007, 2012; Phillips *et al.* 2014).

The legacy effects of ERM and ECM fungi must be considered with regards to direct C storage though necromass inputs. In a Swedish boreal forest system it was found that ERM fungi on in old, undisturbed islands were dominant and produced slowly-decomposing, melanised necromass which contributed to storage of C in the soil (Clemmensen *et al.* 2015). The authors suggest that ERM fungi contribute more C and N to the soil than they degrade and therefore stabilise C in humic horizons (Clemmensen *et al.* 2015). In contrast, ECM fungi in younger boreal forests were found to be associated with faster C and N cycling. Rapid turnover of necromass was found to result in remineralisation of N and therefore low storage of C despite high investment of C into hyphal biomass (Clemmensen *et al.* 2015).

Interesting analogies can be drawn between the present study and studies of the Swedish boreal with differing ages of forest assemblages (Wardle *et al.* 2003). Here, it was shown that smaller islands were burnt less often due to lower chance of lightning strike, these islands had increased carbon storage due to lower decomposition rates despite lower productivity rates than large islands (Wardle *et al.* 2003). Empirical evidence from boreal forests and tundra ecosystems (including the present thesis) appears to support the hypothesis that more higher productivity aboveground results in lower long term soil storage (Wardle et al., 2003; Wilmking et al., 2006; Kane & Vogel et al., 2009; Hartley et al., 2012). In the Swedish island system it was also shown that high production rates of ECM fungi compared to slow-growing ERM fungi does not result in storage of C but the opposite (Clemmensen *et al.,* 2015). These results are analogous to the whole ecosystem patterns (Wardle et al., 2003) and it could be possible that the balance between

ECM and ERM fungi in soil is critical to the carbon balance of the whole ecosystem.

The patterns observed in this thesis and in others discussed (e.g. Phillips et al., 2014; Clemmensen *et al*., 2015) presents problems when scaling-up the effect of mycorrhizal fungi on carbon cycling and storage. Previous studies have grouped ECM and ERM fungi together because of their shared ability access organic N (Averill *et al.* 2014). This is reported to result in increased storage of C compared to arbuscular mycorrhizal (AM) systems as a result of decreased competition for N with free-living microbial communities. Work that makes the direct comparison between ERM and ECM communities, however shows that there are clear differences in carbon storage and cycling rates (Chapter 2; Clemmensen *et al*., 2015). This suggests that that these two groups of fungi interact with soil carbon in different ways and need to be considered separately. It may be that these differences are not as clear as when compared with AM systems but this needs to be investigated further and tree-line ecosystems such as at Abisko provides a useful model ecosystem to make these comparisons.

6.6 Advances in understanding and further steps

Taken as a whole, this thesis addresses shrub and tree expansion, a pan-arctic phenomenon (Myers-Smith *et al.*, 2011), and tackles important questions about the 'whole-ecosystem' implications of this process. Increases in productivity across the Arctic have been predicted to increase C sequestration (Qian *et al.*, 2010). On the face of it, it is reasonable to suggest that more photosynthesis would equate to more

storage of C in the ecosystem. This work takes the global issue of vegetation change and addresses some key plant-soil interactions which vary between plant functional types. These interactions were distilled to the influence of ECM fungi, defoliation events, snow accumulation and litter decomposition. All of these lines of evidence lead us to conclude that more productive vegetation does not store more C in the soil and that, via a number of pathways, C turnover can be stimulated by productive vegetation.

Chapter 2 made significant progress in showing that productive vegetation types were linked to fast soil carbon cycling and low storage. This had been previously shown in sub-arctic forests (Hartley *et al.*, 2012) but not in the shrubs. Shrubs are expanding at an expanding at a rapid rate in the Arctic (Tape *et al.*, 2006; Myers-Smith *et al*., 2011) whereas tree-lines do not appear to be as responsive to climate change (Harsch *et al*., 2009). The focus on shrubs as well as forest ecosystems in this work not only reinforces what was previously found but also makes significant steps to understand change in arctic tundra that is now colonised by shrubs. This has substantial implications for the rest of the Arctic and surveys of a similar nature should be expanded to other areas, especially those underlain with permafrost, to know whether this relationship holds across other Arctic ecosystems. Although the SOC stocks were quantified in detail, the resources were not available to complete an above-ground inventory of C. Although it is predictable that shrub plots will have more standing biomass than heath, the numbers should be known in order better to understand total C storage of the ecosystem. This was achieved in forest and heath plots previously, which demonstrated that although forested plots had more above-ground biomass, their total C storage was significantly lower than heath

plots (Hartley *et al.*, 2012). An equally important next step will be to measure rates of photosynthesis along-side respiration rates in order to understand better what the net flux of C over a season is.

The C stock data would suggest that C is not accumulating in the more productive plots but this needs to be tested. In this chapter it was argued that ECM fungi are key to the decomposition of SOM. Two field experiments are needed to fully test this hypothesis. Firstly, the exclusion of hyphae using regularly rotated cores of soil with mesh that restricts root growth (no fungi) and static (fungal in-growth) (Johnson et al., 2001). Secondly a girdling experiment (Högberg *et al.*, 2001) of *Betula pubescens* plots. The hyphal exclusion experiment, when applied with detailed soil respiration measurements, would test the effect of fungi (including saprotrophic fungi) on soil respiration. When combined in a factorial design with girdling hyphal exclusion, it would inform the effect on ECM fungi on soil respiration (assuming ECM fungi have little or no saprotrophic abilities). This is the clear next experiment and number one priority to follow up the present work.

It was shown, using a variety of methods, that sub-arctic forests influence soil microbial cycling throughout the year. This experiment with soil cores transplanted between forests and heaths showed that soils respire at faster rates in the forest than the heath. The experimental design isolated winter processes (i.e. snow depth) as the factor driving this. However, the interaction between snow cover and microbial processes is complex. Research should focus on the effect of longer snow cover on ecosystem processes and the effect of deep snow cover on nutrient flushes in the melt season (Buckeridge *et al.*, 2010) and on hydrology (Natali *et al.*, 2011). Heath

plots were found to be moister than forest plots over the growing season so the waterlogging effects of deep snow in the forest may only be short lived in this case.

Measurements after two winters of treatment forced us to reject the hypothesis that deep snow increased microbial biomass. This led to the hypothesis that a change in the composition of the microbial community had influenced respiration rates. The next logical step is therefore to measure the community composition of these soil cores to understand whether more insulated soils shift to more fungal dominated community than the soil that remained in the heath. If a significant fungal community develops in the heath soils which were transplanted into the heath as a result of insulation over winter, decomposition of C could be rapid, especially considering that heath soil is potentially very carbohydrate-rich (Sjögersten *et al.*, 2003) Enzyme assays will allow further work to understand whether insulation over winter in the forest allows for a more biologically active microbiota.

Chapter 4 examined how biogeochemical processes are affected by defoliation. It underlines how soil processes and cycles are dependent on autotrophic C supply and offers insights into how the microbial community responds to defoliation and how it feeds back to C and N fluxes. As the situation currently stands, reduction in autotrophic C supply seems to cause a slow-down in C cycling but the concurrent addition of N and C from the caterpillar frass (Lovett & Ruesink, 1995) confounds our understanding of the mechanisms at work. This work should, therefore, be followed-up by experiments to separate the relative importance of C supply, e.g. by girdling (Högberg *et al.*, 2001) and N additions via frass (via artificial addition).

One suggestion in this chapter is that the reduction in ECM growth observed beneath defoliated trees is due to a shift in ECM community composition to more conservative growth strategies, which was observed at the root tips. To follow this up, molecular techniques need to be used (e.g. TRFLP) to measure community composition of hyphae the as well as the root tips. This will better link hyphal growth to the interaction between tree and fungi and further test the hypothesis that change in ECM community influences carbon cycling during defoliation events.

The final data chapter of this thesis showed that litter decomposition is rapid in sub-arctic forests as a result of carbohydrate-rich litter input, which decomposes quickly and stimulates the decomposition the rest of the litter layer. The nature of this project only allowed for the decomposition study to continue for 21 months. This gave us a good understanding of the drivers of decomposition over this timescale but longer-term studies of the fate of litter are needed. To this end, there are two extra harvests of litter possible for later dates in this study (six harvests were deployed originally). It is important that these harvests are collected to understand decomposition at the treeline over longer time periods. Further ^{13}C NMR work on future harvests will inform us whether different decomposition pathways continue to exist between forest and heath, and how much litter input will contribute to humus formation in each system.

6.7 Conclusion

This body of work shows that in the landscape under investigation in sub-arctic Sweden, storage of soil carbon is low as a result of a number of ecological interactions between productive plants and the soil. The findings in this work can be applied at a number of different scales. At the microbiological scale, our data suggest that the action of ectomycorrhizal fungi can be key in regulating SOC. At the scale of a vegetation stand, snow accumulation can be an important regulator of microbial communities as can litter input. Due to the nature of the sampling strategy, the data that have been collected can be applied to large-scale biogeographic issues such as shrub expansion, as we observed consistent patterns holding true across landscapes.

The strong underlying theme of this work is the relationship between aboveground productivity rates and belowground C storage. The relationship between these two is globally important as tundra productivity rates are changing. The response of arctic ecosystems to changes in aboveground structure will have significant effects on the balance of C. This work has shown that plant-soil interactions are fundamental in interacting with SOC. Direct effects of climate change on SOC in tundra ecosystems are important, however, plant-soil interactions have a critical role to play in determining the fate of C in the Arctic.

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Appendices

Appendix 1

Table S1: Geographical details of each transect at Abisko and Vassijaure sites. 'Elevation change' and 'heading' refer to transects from heath to forest plots. Plots on the transects run approximately in a straight line.

Abisko	Transect length (m)	Elevation change (m)	Heading (degrees)
A1	74	+5	169
A2	70	-11	314
A3	87	+6	164
A4	71	-11	322
B1	82	-8	337
B2	97	+3	213
B3	77	+2	59
B4	52	-4	355
C1	59	-7	350
C2	53	-7	2
C3	60	+1	219
C4	31	-1	71
Average	67.8	-2.7	
Vassijaure			
1	77	4	124
2	22	-12	358
3	59	1	342
4	61	2	119
5	50	0	234
6	70	-8	30
7	67	4	153
Average	58	-1.2	

Appendix 2

Table S2. Reference sequence annotation, relative abundance, and frequency of occurrence of ectomycorrhizal (ECM) fungi in non-defoliated and defoliated *Betula* forests in Abisko, Sweden. Values for genus- or higher-level taxonomic groups are shaded.

Reference taxon	NCBI/UNITE Accession	Match (%)	UNITE Species Hypothesis	Mycorrhizal Exploration Type[#]	Non-defoliated $\hat{\mu}$[†] (%)	Non-defoliated $\hat{\sigma}$[†]	Non-defoliated f[‡] (/5)	Defoliated $\hat{\mu}$ (%)	Defoliated $\hat{\sigma}$	Defoliated f (/5)
ECTOMYCORRHIZAL FUNGI										
Basidiomycota										
Agaricales										
Cortinariaceae										
/cortinarius				MDF	50	± 12	5/5	55	± 18	4/5
Cortinarius alboviolaceus	JQ724019.1	99	SH221787.06FU	MDF	38	± 13	2/5			
Cortinarius armillatus	DQ114744.1	99	SH221779.06FU	MDF				21		1/5
Cortinarius atrocoeruleus	JQ724019.1	99	SH232829.06FU	MDF	12		1/5			
Cortinarius balaustinus	AF389153.1	98	SH220396.06FU	MDF	25		1/5	31		1/5
Cortinarius caperatus	FJ845425.1	99	SH191824.06FU	MDF	6		1/5	41	± 20	3/5
Cortinarius casimiri	HQ604710.1	99	SH232830.06FU	MDF	3	± 0.2	2/5	33		1/5
Cortinarius cedriolens	FJ552786.1	99	SH232841.06FU	MDF	7		1/5			
Cortinarius collinitus	DQ367896.1	99	SH191855.06FU	MDF	5		1/5			
Cortinarius delibutus	UDB002173	99	SH220385.06FU	MDF	8	± 3	2/5			
Cortinarius laetus	AF389170.1	99	SH192000.06FU	MDF				4		1/5
Cortinarius sp.	EU597035.1	96	SH191963.06FU	MDF	5		1/5			
Cortinarius sp.	FJ039656.1	99	SH192083.06FU	MDF				7		1/5
Cortinarius sp.	JN032537.1	99	SH191806.06FU	MDF	10	± 4	2/5			
Cortinarius sp.	UDB002209	99	SH191882.06FU	MDF	38	± 22	2/5			
Hygrophoraceae										
/hygrophorus				SD				7		1/5
Hygrophorus pudorinus	FJ845408.1	88	SH024898.06FU	SD				7		1/5
Inocybaceae										
/inocybe				SD	6	± 3	2/5			
Inocybe obscurobadia	AM882802.2	97	SH001193.06FU	SD	2		1/5			
Inocybe petiginosa	EF218781.1	99	SH208978.06FU	SD	9		1/5			
Tricholomataceae										
/tricholoma				MDF	15	± 4	2/5	7		1/5

	Tricholoma album	UDB002398	99	SH194000.06FU	MDF			4	1/5
	Tricholoma flavovirens	AF349689.1	99	SH192989.06FU	MDF	13 ± 1	2/5	4	1/5
	Tricholoma virgatum	UDB011594	100	SH194223.06FU	MDF	6	1/5		
Atheliales									
Atheliaceae									
	/amphinema-tylospora				SD	3	1/5		
	Tylospora sp.	HM189733.1	100	SH229868.06FU	SD	3	1/5		
	/piloderma				MDF	14 ± 9	2/5	4	1/5
	Piloderma olivaceum	JQ711859.1	98	SH212379.06FU	MDF	12 ± 8	2/5	4	1/5
	Piloderma sp.	JQ711935.1	99	SH212383.06FU	MDF	3	1/5		
Boletales									
Boletaceae									
	/boletus				LD	4 ± 1	2/5		
	Leccinum scabrum	UDB001608	99	SH197538.06FU	LD	4 ± 1	2/5		
Cantharellales									
Hydnaceae									
	/cantharellus				MDF, MDS	9 ± 3	2/5		
	Hydnum umbilicatum	AJ547885.1	97	SH214526.06FU	MDS	11	1/5		
	Sistotrema sp.	FN669254.1	99	SH219329.06FU	MDF	6	1/5		
Russulales									
Russulaceae									
	/russula-lactarius				C, SD	20 ± 6	5/5	70 ± 15	3/5
	Lactarius				C	8 ± 3	3/5	83 ± 17	2/5
	Lactarius pilatii	UDB018157	99	SH238107.06FU	C	5 ± 2	2/5		
	Lactarius rufus	KF241543.1	99	SH191391.06FU	C			67	1/5
	Lactarius tabidus	HM189825.1	99	SH193869.06FU	C			8	1/5
	Lactarius trivialis	UDB000365	99	SH238110.06FU	C	13	1/5	92	1/5
	Russula				C, SD	15 ± 5	4/5	48	1/5
	Russula gracillima	KF002779.1	99	SH224403.06FU	C	7	1/5		
	Russula nuoljae	UDB002530	99	SH207687.06FU	C	12 ± 6	4/5	48	1/5
	Russula versicolor	UDB001641	99	SH224391.06FU	SD	3	1/5		
Thelephorales									
Bankeraceae									
	/hynellum-sarcodon				MDM	7	1/5		
	Sarcodon sp.	UDB015699	100	SH227933.06FU	MDM	7	1/5		
Thelephoraceae					SD, MDS	13 ± 3	3/5	4	1/5
	/tomentella-thelephora				SD, MDS	8 ± 2	2/5	4	1/5
	Thelephoraceae sp.	U83467.1	99	SH195967.06FU	MDS	7	1/5		
	Tomentella lapida	U83480.1	98	SH199020.06FU	SD	6	1/5		
	Tomentella sp.	FJ553031.1	99	SH219847.06FU	SD	2	1/5	4	1/5

	/tomentellopsis				MDF/MDS	11 ± 8	2/5	
	Tomentellopsis sp.	UDB018589	99	SH199526.06FU	MDF/MDS	11 ± 8	2/5	
Ascomycota								
Mytilinidiales								
Gloniaceae								
	/cenococcum				SD		4	1/5
	Cenococcum geophilum	JN943891.1	99	SH196545.06FU	SD		4	1/5
Helotiales								
Incertae sedis								
	/meliniomyces				SD		7	1/5
	Meliniomyces bicolor	HM164675.1	100	SH207165.06FU	SD		7	1/5
				NON-ECTOMYCORRHIZAL FUNGI				
Basidiomycota								
Agaricales								
Mycenaceae								
	Mycena						5 ± 1	2/5
	Mycena simia	GU234138.1	99	SH237366.06FU			4	1/5
	Mycena sp.	HM069358.1	100	SH193138.06FU			7	1/5
Capnodiales								
Incertae sedis								
	Toxicocladosporium strelitziae	JX069874.1	98	SH196751.06FU		3	1/5	
Ascomycota								
Helotiales								
Vibrisseaceae								
	Phialocephala					2	1/5	10 ± 3 2/5
	Phialocephala fortinii	DQ497924.1	99	SH213468.06FU		2	1/5	7 1/5
	Phialocephala sphaeroides	JQ711837.1	99	SH213470.06FU				13 1/5
Incertae sedis								
Incertae sedis								
	Meliniomyces variabilis	FN565286.1	98	SH207164.06FU			6	1/5

[#]Abbreviations: C, contact; SD, short-distance; MDF, medium-distance fringe; MDM, medium-distance mat; MDS, medium-distance smooth; LD, long-distance. References: Agerer (2006), Tedersoo & Smith (2013), Beenken (2004), Weigt et al. (2011), and Jakucs et al. (2015).

[†]$\hat{\mu} \pm \hat{\sigma}$: Mean and standard error of taxon relative abundance in transect-replicate samples where it occurred; further detail in *Methods and Materials.*

[‡]f: Frequency of occurrence in five transect-replicate samples