Interactions between Ectomycorrhizal Associations and Bacteria

Srisailam Marupakula
Faculty of Forest Sciences
Department of Forest Mycology and Plant Pathology
Uppsala

Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2016

Acta Universitatis agriculturae Sueciae
2016:42

Cover: Mycorrhizal roots and mycelium from O horizon soil
(photo: S. Mahmood)

ISSN 1652-6880
ISBN (print version) 978-91-576-8586-5
ISBN (electronic version) 978-91-576-8587-2

Print: SLU Service/Repro, Uppsala 2016

Interactions between ectomycorrhizal associations and bacteria

Abstract

Boreal forest podzol soils have vertically stratified horizons with different physico-chemical characteristics and high microbial diversity. Ectomycorrhizal fungi play key roles in accessing nutrients from both organic and mineral substrates. The role of associated bacteria in these processes is still poorly understood. The aim of the studies described in this thesis was to improve understanding of the distribution, diversity and community structure of fungi and bacteria on roots and in soil and their responses to environmental perturbations such as N-fertilisation.

In two microcosm studies a single-root tip microbiome method was used to sample bacteria associated with different ectomycorrhizal roots at defined time-points, using high throughput sequencing of both fungi and bacteria The first study revealed highly dynamic patterns of assembly of bacterial communities associated with ectomycorrhizal roots at different time points in organic soil. Bacterial community structure differed between roots colonised by different species of ectomycorrhizal fungi from different genera. The second study extended these results to include both organic and mineral horizons, demonstrating significant differences between fungal and bacterial communities colonising soil from different horizons. Responses of both fungi and bacteria to short-term N additions were context dependent, influenced by both soil horizon and the dominant ectomycorrhizal fungi colonising the roots. Bacterial communities associated with roots colonised by pairs of closely related fungal species within the same fungal genera were also shown to be statistically distinct.

Field studies of fungi and bacteria in a forest fertilised with 150 kg N ha^{-1} 15 months previously, revealed 1017 unique fungal OTUs, (877 in the soil, 652 in the roots). N increased fungal diversity slightly in the O horizon soil but decreased it in the roots, particularly in the B horizon. Fungal community structure varied significantly between horizons and within each soil horizon the community structure of fungi colonising ectomycorrhizal roots was significantly different from that associated with the soil, suggesting that analyses of both soil and roots are necessary for accurate monitoring of environmental perturbations. 10925 unique bacterial OTUs were distinguished in total (8560 in the soil, 5512 in the roots). Bacteria displayed similar trends to the fungi but were less strongly influenced by N. These studies pave the way for more detailed functional studies of specific combinations of fungi and bacteria.

Keywords: Ectomycorrhizal fungi, Bacteria, Microbiome, Horizons, Nitrogen fertilisation, High throughput sequencing, Root tips

Author's address: Srisailam Marupakula, SLU, Department of Forest Mycology & Plant Pathology, P.O. Box 7026, SE-75007 Uppsala, Sweden
E-mail: Srisailam.Marupakula@slu.se

Dedication

To my loving Parents and Daughter …

Fill the brain with high thoughts, highest ideals, place them day and night before you, and out of that will come great work.
Swamy Vivekananda

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I **Marupakula S, Mahmood S, Finlay RD. 2015.** Analysis of single root tip microbiomes suggests that distinctive bacterial communities are selected by *Pinus sylvestris* roots colonised by different ectomycorrhizal fungi. *Environmental Microbiology* (In Press)**.** doi: 10.1111/1462-2920.

II **Marupakula S, Mahmood S, Jernberg J, Nallanchakravarthula S, Fahad Z, Finlay RD. 2015.** Bacterial microbiomes of *Pinus sylvestris* roots colonised by different ectomycorrhizal fungi: effects of soil horizon and N addition. (manuscript)

III **Marupakula S, Mahmood S, Santos J, Jacobson S, Högbom L, Finlay RD.** Analysis of fungal communities colonising mycorrhizal tree roots and soil in a nitrogen-fertilised boreal forest podzol. (manuscript).

IV **Marupakula S, Mahmood S, Finlay RD.** Analysis of bacterial communities colonising mycorrhizal tree roots and soil in a nitrogen-fertilised boreal forest podzol. (manuscript).

The contribution of Srisailam Marupakula to the papers included in this thesis was as follows:

I Planned the study together with the supervisors. Prepared the laboratory material for sequencing. Analysed the molecular data and performed the statistical analyses. Wrote the manuscript in collaboration with the supervisors.

II Planned the study together with the supervisors. Prepared the laboratory material for sequencing. Analysed the molecular data and performed the statistical analyses. Wrote the manuscript in collaboration with the supervisors.

III Planned the study together with the supervisors. Prepared the laboratory material for sequencing. Analysed the molecular data and performed the statistical analyses. Wrote the manuscript in collaboration with the supervisors.

IV Planned the study together with the supervisors. Prepared the laboratory material for sequencing. Analysed the molecular data and performed the statistical analyses. Wrote the manuscript in collaboration with the supervisors.

Abbreviations

AM	Arbuscular mycorrhizal
ANOSIM	Analysis of similarity
ANOVA	Analysis of variance
B horizon	Illuvial horizon
DGGE	Denaturing gradient gel electrophoresis
E horizon	Elluvial horizon
ECM	Ectomycorrhizal
ITS	Internally transcribed spacer
NCBI	National Center for Biotechnology Information
NMDS	Non-metric multi-dimensional scaling
NPMANOVA	Non-parametric Multivariate analysis of variance
O horizon	Organic horizon
OTU	Operational taxonomic unit
PAST	Paleontological Statistics
RDP	Ribosomal database project
SCATA	Sequence Clustering and Analysis of Tagged Amplicons
SIMPER	Similarity percentage analysis

1 Boreal Forests

The world's forests occupy 30% (3.9 billion ha) of global land area, and boreal forests in turn, occupy about 38% of this area (1.5 billion ha), constituting one of the largest terrestrial biomes. Globally, Sweden has the sixth largest boreal forest coverage representing 27.1 million hectares or 1.8% of the global boreal forestland area (Committee I.2014). Boreal forests are characterised by cool climates with low evapotranspiration and slow rates of decomposition. The growing season is generally three months or less, although the long summer daylight may compensate for the short season. Mean annual precipitation ranges from 20-200 cm and mean annual temperature between +5°C and – 5°C (Burton *et al.,* 2010; Lundström *et al.,* 2000; Taggart & Cross, 2009). Boreal forests sequester ~20% of the global C sink generated by forests (Pan *et al.*, 2011) and have been estimated to contain 367.3 to 1715.8 Pg (Bradshaw & Warkentin, 2015).

Boreal forests have low tree species diversity. *Pinus* and *Picea* are the dominant genera together with deciduous trees in the genera *Salix, Betula* and *Larix* (Read *et al.*, 2004). Boreal forests in the Scandinavian peninsula and Finland have a latitudinal extension from 56° N to 69° N, called Fennoscandia. The total forest area in Fennoscandia is more then 50 million ha, of which 47% is in Sweden, 40% in Finland and 13% in Norway (Esseen *et al.,* 1997). Fennoscandian shrubs include *Juniperus communis*, *Salix xerophila* and *S. starkeana*, as well as dwarf shrubs such as *Empetrum hermaphroditum, Calluna vulgaris, Vaccinium myrtillus, V. vitis idaea, V. uliginosum and Ledum palustre*. The common feather mosses are *Pleurozium schreberi* and *Hylocomium splendens* and the most common lichens belong to the genera *Sterocaulon* and *Cladonia* (Esseen *et al*., 1997).

1.1 Boreal forest soils

Accumulation of coniferous litter reduces the rate of decomposition, leading to low nutrient levels and acidic soil (Burton *et al.* 2010). The soils under boreal forest ecosystems are characterised by podzols (meaning "under ash" in Russian). Few burrowing animals thrive in these acid soils and mixing is thus limited, leading to the conservation of visible horizons in the soil profile. Podzols typically consist of an upper **organic** horizon (**O**) with partially decomposed organic matter with medium to coarse textured material; an **elluvial** horizon (**E**) of weathered, ash-grey soil, enriched in silicates and minerals with lower weathering rates compared to the parent material, containing lower concentrations of Fe, Al and base cations; and a darker, brown-red **illuvial** horizon (**B**), enriched in Al, Fe, base cations and P (Lundström *et al.,* 2000). The parent material below the B horizon is called the C horizon.

Podzol soils contain different organic acids such as citric acid, oxalic acid, malic acid, acetic acid, formic acid, succinic acid, vanillic acid, *p*-hydroxy benzoic acid, *p*-coumaric acid and other low molecular weight compounds (LMW), depending on the site and tree species. Decomposition of litter by microbes, leaching from plant root exudates, fungi and other microorganisms in the soil contributes to the organic acids (Lundström *et al.*, 2000). Boreal forest soil microorganisms – fungi & bacteria

1.2 Boreal forest soil microorganisms – fungi & bacteria

Fungi play crucial roles in many ecological and microbiological processes. They are heterotrophs and acquire their carbon either by establishing an intimate symbiotic association with host plants, or as saprotrophs by decomposing organic matter, or as pathogens - infecting plants and deriving their carbon. They are ubiquitous and it has been estimated that there are 1.5 million fungal species (Hawksworth, 1991). More recent estimates of fungal diversity using high throughput sequencing methods suggest that there may be as many as 5.1 million fungal species (O'Brien *et al.*, 2005).

Fungi play key roles in forest ecosystems, driving processes that are essential for sustainable growth and production (Read *et al.,* 2004). Saprotrophic fungi recycle recalcitrant woody residues from plant litter and possess unique enzymatic competence to degrade lignified material but it is becoming increasingly evident that ectomycorrhizal (ECM) fungi play pivotal, hitherto undescribed, roles in mobilisation and sequestration of N and P from organic matrices consisting of polymeric residues of both plant and microbial origin (Lindahl *et al.,* 2007). Symbiotic ectomycorrhizal mycelia can result in

significant transport of carbon belowground (Finlay, 2008; Jones *et al.,* 2009; Högberg *et al.,* 2001) potentially affecting C sequestration either negatively or positively (Godbold *et al.,* 2006, Heinemeyer *et al.,* 2007, Heimann & Reichstein, 2008). It has recently been revealed that ectomycorrhizal fungi can significantly retard decomposition, leading to substantial belowground sequestration of C (Clemmensen *et al.*, 2013, 2015) and several other studies have demonstrated that ectomycorrhizal fungi can increase the amount of belowground C sequestration (Averill *et al.*, 2014; Bradford, 2014) compared with other systems.

Apart from sequestration of N and P from organic polymers, a second paradigm that has gained increasing acceptance amongst researchers is that ectomycorrhizal fungi can play a role in mobilising nutrients such as base cations and P from mineral substrates. Early observations in Sweden (Jongmans *et al.,* 1997; van Breemen *et al.,* 2000; Landeweert *et al.*, 2001), followed by further experimentation (Rosling *et al.,* 2003, 2004; Smits *et al.*, 2012), have gained increasing acceptance by biogeochemists, ecologists and evolutionary biologists. New studies argue that vegetation growth and C allocation supporting mycorrhizal weathering of silicates has created a C sink explaining drawdown of global CO_2 levels during the late Cenozoic (Taylor *et al.,* 2009, 2012). Recent nanoscale studies of hyphal-mineral surface interactions involving hyphal exudates and deposition of extracellular polymeric substances (Saccone, 2011; Gazzè *et al.*, 2012) and etching effects related to CO_2 levels (Quirk *et al*., 2012) support the ideas put forward by Finlay *et al.* (2009) that weathering agents may be exuded into organic matrices in intimate contact with mineral surfaces that are effectively isolated from the soil solution. This complicates construction and interpretation of models that predict that current intensities of harvesting in Swedish forests may not be sustainable with respect to base cation supply (Akselsson *et al.,* 2007; Klaminder *et al.,* 2011).

Estimates of mycelial biomass (Ekblad *et al.,* 2013) suggest that it may be a significant source of nutrients that can be recycled during decomposition but information about turnover rates and the relative contributions of mycelial decomposition and biological weathering of different mineral substrates is still fragmentary and new stable isotope based methods need to be developed to address this question. High throughput sequencing methods (Clemmensen *et al.,* 2013; Ihrmark *et al.,* 2012; Lindahl *et al.,* 2013) can be applied to study fungal communities in boreal forests. Most of these studies have so far used DNA-based methods but Baldrian *et al.* (2012) recently showed, using both DNA- and RNA-based community analyses, that some low abundance fungal species make important contributions to decomposition in soils. New RNA-

based sequencing and stable isotope probing (SIP) methods enable examination of the metabolically active components of the fungal and bacterial communities. Fungi may have important effects on production of organic acids chelating Al^{3+} and PO_4^- (Ahonen-Jonnarth *et al.,* 2000), and leaching of base cations (Ahonen-Jonnarth *et al.,* 2003), as well as interactions with other fungi (Gadgil & Gadgil, 1971, 1975; Lindahl *et al.*, 2010).

Bacteria represent another important component of boreal forest ecosystems. Soil contains diverse bacterial communities occupying different niches and it has been estimated that one gram of soil contains 10^{10} bacterial cells (Torsvik *et al.*, 1996). Bacterial communities in boreal forests are diverse and recently Vik *et al*. (2013) have reported 27 phyla in soil and 25 phyla in ectomycorrhizal roots. *Armatimonadetes*, *Chloroflexi*, and *Actinobacteria* were more frequent in the plant root systems, whereas *Chlorobi*, *Firmicutes*, *Planctomycetes* and *Acidobacteria* were more frequent in the soil samples. Apart from abovementioned genera *Bacteroidetes*, *Gemmatimonadetes*, *Verrucomicrobia* frequently appear in different studies in the coniferous soils (Baldrian *et al.*, 2012; Hartmann *et al.,* 2009).

1.3 Fungal and bacterial communities in boreal forest podzols

Boreal forests have acidic soils with litter containing phenolic material and lignin that makes it difficult to degrade (Aerts, 1995). Nutrient availability is very limited and plants and microbes compete for the available nutrients (Lindahl *et al.*, 2002). There is a clear functional stratification of the ectomycorrhizal and saprotrophic communities in boreal forest podzols. Saprotrophic fungi with litter degrading enzymes are active during the initial stages of litter degradation but mycorrhizal fungi appear to obtain N from more decomposed organic matter lower in the soil profile (Lindahl *et al.*, 2007). Saprotrophs depend on the litter as a carbon source and are unable to compete with the ectomycorrhizal fungi in the more decomposed substrates with lower C:N ratios since the ectomycorrhizal fungi obtain their carbon directly from their plant hosts (Yarwood *et al.*, 2009). Previous studies of soil ectomycorrhizal community profiles have shown that the organic horizon harbours high species richness (Dickie *et al.*, 2002) and higher densities of root tips (Tedersoo *et al.*, 2003; Genney *et al.*, 2006) than in lower horizons.

Earlier molecular studies of ectomycorrhizal distribution using RFLP and T-RFLP (Heinonsalo *et al.*, 2001, 2007; Dickie *et al*., 2002; Lindahl *et al*., 2007) have shown that different ectomycorrhizal fungi dominate in different soil horizons. Heinonsalo and Sen (2007) showed higher fungal diversity in the O and E horizons than in the B horizon. *Suillus variegatus* was found in all 3

horizons but some other taxa were exclusively present in different horizons (O horizon – *Cortinarius* sp., *Cenococcum geophilum*, *Tomentellopsis* sp.; E horizon – *Suillus bovinus*, *Lacacaria laccata*; B horizon – *Rhizopogon* sp., *Tylospora* sp.). Similarly other studies using Sanger sequencing (Shahin *et al.*, 2013; Rosling *et al.*, 2003) have shown that ectomycorrhizal fungal composition is different in different horizons.

To date most published studies of fungal communities in forest soils using pyrosequencing have been confined to the upper, organic and mineral soil horizons and have examined soil but not roots (Baldrian *et al.*, 2012; Sterkenburg *et al.*, 2015; McGuire *et al.*, 2013; Voříšková *et al*., 2014; Uroz *et al.*, 2013). Rosling *et al.* (2003) demonstrated that 66% of the ectomycorrhizal root tips and over half of the identified fungal species were present in the mineral horizons, suggesting that sampling of the whole soil profile is necessary to capture the full diversity of species present.

Soil stratification and particularly soil resource availability also impact the functional and taxonomic diversity of bacterial communities (Uroz *et al.*, 2013). The *Scleroderma citrinum* ectomycorrrhizosphere of oak roots significantly structures the culturable bacterial communities along the two horizons by selecting very efficient strains for Fe and P mobilization with bacterial isolates from the lower horizon that are more efficient than those from the upper horizon (Calvaruso *et al.*, 2007). In another study by Eilers *et al.* (2012) bacterial diversity was highest in the upper 10 cm horizon and declined by 20-40% in the lower horizons. Other pyrosequencing based studies (Baldrian *et al.*, 2012; Lopez-Mondejar *et al.*, 2015; Turlapati *et al.*, 2013; Koyama *et al.*, 2014) have also examined bacterial communities in different soil horizons but neither of the above studies were conducted in boreal forests and none of them compared bacteria associated with roots and those associated with soil.

1.4 Forest N fertilisation – effects on fungi and bacteria

Soil microbial communities play fundamental roles in biogeochemical cycling of nutrients in forest ecosystems. Investigating their responses to long- and short-term anthropogenic disturbances is therefore critical for sustainable forestry. In boreal forests nitrogen is the main factor limiting tree growth (Tamm, 1991; Vitousek & Howarth, 1991; LeBauer & Treseder, 2008). Forest fertilization with N to increase timber production is a common practice in northern Europe and North America (Jones *et al.*, 2012; Bergh *et al.*, 1999; Jacobson & Pettersson, 2010). In northern Sweden a single dose of 150 kg N ha^{-1}, 10 years before harvest, generally increases stem production by 10–20 m^3

(Demoling *et al.*, 2008). It has been shown in a number of studies that trees decrease below ground carbon allocation to ectomycorrhizal fungi after N fertilization (Lilleskov *et al.*, 2011; Wallander *et al.*, 2011; Kjøller, 2012; Ostonen *et al.*, 2011). Högberg *et al.* (2010) showed that below ground plant allocation of carbon to soil a microbial community was reduced to around 60% one year after fertilization.

Many earlier studies of ectomycorrhizal responses to N fertilisation have been based on fruitbodies, identification of ectomycorrhizal root morphotypes (Fransson *et al.*, 2000) or molecular methods with lower resolution, such as RFLP or T-RFLP (Cox *et al.*, 2010; Kjøller *et al.*, 2012). Several studies have reported a reduction in fungal species richness and a change in community composition following N fertilisation (Jones *et al.*, 2012; Avis *et al.*, 2003; Lilleskov *et al.*, 2011; Parrent & Vigalys, 2007; Hasselquist & Högberg, 2014).

Nitrogen addition often has a negative effect on production of ectomycorrhizal mycelium (Bahr *et al.*, 2013; Nilsson & Wallander, 2003), and reduces the biomass of fungi (Mayor *et al*., 2015). In another experiment, after termination of nitrogen addition it took 15 years to restore the ectomycorrhizal fungi and their functional roles to that of N-limited control plots (Högberg *et al.*, 2014; Högberg *et al.*, 2011). Annual fertilisation changes fungal species richness and composition (Fransson *et al.*, 2000; Jones *et al.*, 2012), and colonisation are negatively affected (Berch *et al.*, 2006). Furthermore, ectomycorrhizal species vary in their response to nitrogen fertilisation (Lilleskov *et al.*, 2011; Kaiser *et al.*, 2011).

Most earlier studies showing the effects of forest fertilisation on bacterial communities in soil have been conducted using TRFLP (Högberg *et al.*, 2014; Krumins *et al.*, 2009; Feng *et al.*, 2010) or PLFA based methods (Demoling *et al.*, 2008; Blasko *et al.*, 2013) that are known to have poor taxonomic resolution. Both fungi and bacteria were negatively affected by the nitrogen addition and microbial biomarkers were reduced to 40% in the nitrogen added plots compared to control plots (Blasko *et al.*, 2013). After termination of a high N deposition treatment ectomycorrhizal fungi recovered but not the bacterial communities (Högberg *et al.*, 2014; Högberg *et al.*, 2011), suggesting that bacterial communities are probably more sensitive to nitrogen addition than fungal communities (Krumins *et al.*, 2009). In another study, Turlapati *et al.* (2013) reported a shift in community composition and diversity of bacteria following N addition.

Only a few studies of the effects of N fertilisation on bacteria have been conducted using high throughput sequencing methods (Turlapati *et al.*, 2013; Koyama *et al.*, 2014). These two studies were conducted in geographically

distinct places and were limited to humus and mineral horizon soils, no B horizon was included and root associated communities were not analysed.

1.5 Ectomycorrhiza–bacteria interactions

Plant roots harbour rich, non-random communities of fungi and bacteria from the surrounding soil (Bulgarelli *et al.*, 2013; Nallanchakravarthula *et al.*, 2014) and identification of these plant-selected communities is important since they are likely to influence both plant health and growth promotion. The ancient origin of mycorrhizal fungi over 450 million years ago, early during the evolution of terrestrial plants, is likely to have had a strong influence on bacterial niche development leading to both antagonistic and mutualistic strategies (de Boer *et al.,* 2005). This must have necessitated bacterial adaptation to fungal symbionts as well as to the roots themselves. Tripartite associations between roots, fungi and bacteria are therefore probably the norm rather than the exception (Bonfante & Anca, 2009) but, remarkably, we still know very little about the factors influencing their taxonomic composition, distribution and responses to different environmental parameters.

Ectomycorrhizal roots and mycelia should constitute an attractive niche for bacteria since the hyphae receive a continuous supply of energy-rich, photosynthetically derived carbohydrates and have a large surface area for bacterial colonisation. Endosymbiotic associations with bacteria have been reported and Bertaux *et al.* (2003, 2005) identified a number of Alphaproteobacteria and Gram-positive bacteria in the genus *Paenibacillus* in the mycelium of *Laccaria bicolor* S238N. Izumi *et al.* (2006, 2007) demonstrated that bacteria belonging to the genera *Pseudomonas*, *Burkholderia* and *Bacillus/Paenibacillus* were the most abundant cultivable bacteria associated with *Pinus sylvestris* ectomycorrhizal roots colonized by *Suillus variegatus* and *Tomentellopsis submollis.* Species of *Rahnella, Janthinobacterium* and *Rhodococcus* were exclusively present in roots colonised by *S. variegatus, R. paludosa and Russula* spp. respectively. Some of the isolated endobacteria utilised fungal sugars more readily then plant sugars.

The detailed functional significance of ectomycorrhiza-bacteria interactions is still not fully understood but different authors have suggested various functional roles. The role of so-called ‘helper’ bacteria in facilitating ectomycorrhizal colonisation of seedling roots in forest nurseries has been widely discussed (Garbaye, 1994; Frey-Klett *et al.*, 2007; Labbé *et al.*, 2014) and may depend upon stimulated germination of fungal propagules, promotion of mycelial growth/ branching, reduced soil-mediated stress, modification of root system architecture or effects on host recognition or receptivity (Tarkka

and Frey-Klett, 2008; Aspray *et al*., 2013). *Pseudomonas fluorescens* BBCc6R8 has a specific priminig effect on growth, morphology and gene expression of its fungal associate *L. bicolor* (Deveau *et al.*, 2007).

Possible roles in bioremediation of oil contaminated soil have been discussed by Sarand *et al.* (1998, 1999, 2000) who showed that certain fluorescent pseudomonads with plasmids for m-toluate degradation were associated with ectomycorrhizal mycelium. Production of compounds that are antagonistic to plant pathogens has also been demonstrated in mycorrhiza-associated bacteria (Riedlinger *et al.*, 2006; Tarkka & Frey-Klett, 2008) and the biocontrol potential of *Streptomyces* strains that are not general antagonists of fungi has been discussed in relation to control of pathogens such as *Fusarium* and *Heterobasidion* in silviculture (Schrey *et al.*, 2012). *Amanita muscaria* and *Suillus bovinus* growth was promoted by *Streptomyces* sp. AcH 505 whereas *Hebeloma cylindrosporum* growth was inhibited. (Frey- Klett *et al.*, 2007). This specificity was linked to the antibiotic WS-5995 B produced by the *Streptomyces* sp. AcH 505 (Keller *et al.*, 2006; Riedlinger *et al.*, 2006). *A. muscaria* inhibits the production of a AcH 505 antibiotic by *Streptomyces* sp. AcH 505 by secreting organic acids (Riedlinger *et al.*, 2006). Fluorescent pseudomonads that are active against fungal root pathogens have also been shown to be higher in abundance in the rhizosphere of Douglas fir roots colonised by *Laccaria bicolor* than in bulk soil (Frey-Klett *et al.*, 2005).

Bacteria-mycorrhiza interactions may also play a role in associative N-fixation in nitrogen poor environments (Perez-Moreno & Read, 2000; Paul *et al.*, 2007), and the presence and expression of bacterial *nifH* nitrogenase genes has been shown by Izumi *et al.* (2006, 2013). The role of bacteria associated with ectomycorrhizal fungi and roots in weathering of minerals and dissolution of P from apatite in forests has also been discussed (Uroz *et al*., 2009, 2011a,b; Lepleux *et al*., 2012). Mycorrhiza-associated bacteria may play an important role in mineral weathering by secreting protons, LMW organic anions and siderophores (Frey-Klett *et al.*, 2007). Uroz *et al.* (2007) showed that bacteria able to release iron from biotite are more abundant in ectomycorrhizas than in bulk soil.

Provision of carbon compounds by fungal mycelia may promote bacterial fitness (Nazir *et al*., 2010), and there is some evidence for co-migration of bacteria and non-mycorrhizal fungi in soil (Warmink & Van Elsas, 2009; Warmink *et al*., 2009, 2011; Nazir *et al*., 2012). In addition to the fungi-facilitating bacterial migration through the soil, the bacteria may have reciprocal effects on the fungi, and *Burkholderia terrae* BS001 has been shown to protect its fungal host *Lyophyllum* sp. strain Karsten (DSM2979) from

several antifungal agents such as *P. fluorescens* strain CHA0 metabolites, as well as from the anti-fungal agent cycloheximide (Nazir *et al*., 2014).

Sun *et al.* (1999) examined the composition of exudates from ectomycorrhizal fungal mycelia and found that they contained a range of sugars, sugar alcohols, amino acids, peptides and organic acids. These compounds may support diverse communities of bacteria associated with mycorrhizal roots or hyphae and are consistent with the observations of Warmink *et al.* (2009) who demonstrated selection of bacterial 'fungiphiles' in the mycosphere that was associated with BIOLOG substrate utilization profiles involving L-arabinose, L-leucine, m-inositol, m-arabitol, D-mannitol and D-trehalose. There is also some evidence that Type III secretion systems may be involved in some bacterial interactions with fungal hosts (Warmink & van Elsas, 2008).

Recent DNA-based studies have provided conflicting evidence concerning the effect of different ectomycorrhizal species on bacterial community structure. Izumi and Finlay (2011), using Denaturing Gradient Gel Electrophoresis (DGGE) and 16S/ITS sequencing, reported some selective effects of particular ectomycorrhizal symbionts on associated bacteria in *Betula pubescens* roots. Nguyen and Bruns (2015) sequenced bacterial communities associated with *Pinus muricata* roots using 454 pyrosequencing and found some evidence that bacterial communities in the roots were affected by fungal species identity. Several studies have demonstrated that the bacterial communities associated with ectomycorrhizal roots differ significantly in structure from those in the adjacent soil (Uroz *et al*., 2010, 2012; Izumi and Finlay, 2011; Vik *et al*., 2013; Antony-Babu *et al.*, 2014; Nguyen and Bruns, 2015). However, many studies have failed to demonstrate any significant effect of the ectomycorrhizal fungi colonising plant roots on the bacterial communities associated with them (Izumi *et al.*, 2007b, 2008; Burke *et al.*, 2008; Kataoka *et al.*, 2008; Tanaka & Nara, 2009; Uroz *et al.*, 2012).

Identification of bacteria associated with roots colonized by particular mycorrhizal fungi is often complicated by the use of pooled root samples containing morphologically characterized root tips of indeterminate age. In field studies, additional variation is introduced by soil heterogeneity and variation in other environmental parameters. In study I, we tried to overcome this problem by examining individual root tips of *Pinus sylvestris* sampled at defined time points between 5 days and 24 weeks. The dominant fungi colonising each root tip were identified using Sanger sequencing, and the bacterial communities colonising individual roots were then identified using 454 pyrosequencing.

The main aims of the laboratory studies described in this thesis were to use these single root tip microbiomes to (1) examine temporal and spatial patterns of colonisation of *P. sylvestris* roots by fungi and bacteria at different stages of root development and in soil from different horizons of a podzol, (2) to determine whether particular bacterial taxa or distinctive communities of bacteria were associated with roots colonized by different ectomycorrhizal fungi and (3) to examine responses to short-term additions of N. Two field studies were conducted in a N fertilisation experiment located in a boreal forest at Lamborn. In these studies patterns of distribution and abundance of fungi and bacteria associated with roots or soil in different podzol horizons were examined using high throughput sequencing. Responses to N fertilisation (150 kg N ha^{-1}) 15 months after fertilizer application were also examined.

2 Experimental objectives

Studies of microbial communities in forest soils have repeatedly revealed high levels of diversity (Baldrian *et al.*, 2012; Vik *et al.*, 2013; Voříšková *et al.*, 2014; Uroz *et al.*, 2013; Lopez-Mondejar *et al.*, 2015; Turlapati *et al.*, 2012) but we still have a very incomplete picture of how the distribution of different fungal and bacterial taxa is influenced by different environmental variables. Boreal forest soils are strongly stratified due to the absence of burrowing animals that would otherwise mix the different soil horizons. The interactions of fungi and bacteria with tree roots in these different horizons are still poorly understood on a functional level and better information on their spatial and temporal distribution is necessary before we can formulate hypotheses concerning their functional roles in nutrient acquisition, alleviation of stress and cycling of carbon and organic matter.

The overarching objective of my work has been to improve our understanding of factors influencing the diversity and community structure of bacteria colonising tree roots colonised by different ectomycorrhizal fungi. The work described in this thesis is based on a series of laboratory (**I, II**) and field (**III, IV**) experiments designed to investigate different aspects of the distribution, interactions and functioning of bacteria and mycorrhizal and saprotrophic fungi in boreal forest soils. These include 1) temporal variation (**I**) and ectomycorrhizal fungal–related variation (**I**, **II)** in the structure and diversity of bacterial communities associated with individual ectomycorrhizal roots, as well as 2) soil horizon-related spatial variation and effects of short term N addition influencing bacterial community structure and diversity associated with individual roots (**II**), and 3) spatial variation between different podzol horizons and effects of longer term N fertilization influencing overall community structure and diversity of fungi (**III**) and bacteria (**IV**) in a boreal pine forest.

To achieve these aims I used single root tip microbiomes to investigate temporal and ectomycorrhizal fungal–related variation in bacterial community structure and diversity associated with *Pinus sylvestris* roots colonised by different fungi. Fungi colonising individual roots were first identified by Sanger sequencing, then patterns of bacterial colonisation were studied using 454 pyrosequencing.

The specific aims of the first study (I) were

- To examine temporal patterns of colonisation, including changes in diversity and community structure, as well as
- To determine whether particular bacterial genera or bacterial microbiomes were associated with different dominant ectomycorrhizal fungi colonising single root tips of *Pinus sylvestris*.

In the second study (II) a new type of microcosm system, based on 50 ml Falcon tubes, was developed

- To examine patterns of ectomycorrhizal fungal and bacterial colonisation of *P. sylvestris* roots in soil from different horizons of a boreal podzol soil
- To test whether inter-specific variation in patterns of bacterial colonisation could be observed in individual roots colonised by ectomycorrhizal fungi of the same genus, and
- To examine whether and how short-term N fertilisation influenced patterns of bacterial colonisation in roots colonised by different dominant ectomycorrhizal fungi.

In the final study (Papers III and IV) a long-term field experiment was used

- To examine spatial patterns of ectomycorrhizal fungal and bacterial colonisation in different podzol soil horizons under an 85 year old *P. sylvestris* forest at Lamborn, Sweden
- To compare patterns of fungal and bacterial colonisation associated with roots and soil, and
- To examine the effects of fertilisation, with 150 kg N ha^{-1} 15 months prior to the sampling, on fungal and bacterial colonisation.

Fuller details of the experimental objectives and methods are given in the individual papers and manuscripts, but the projects are described briefly below.

3 Project Description

3.1 Single root tip microbiomes – temporal dynamics (Study I)

Single root tip microbiomes were analysed to study temporal variation in patterns of bacterial diversity and composition and how these are affected by different dominant ectomycorrhizal fungi colonising individual roots. In order to distinguish the effects of ectomycorrhizal fungi from other environmental variables, the experiment was conducted in soil-plant-microcosms in a phytotron. *Pinus sylvestris* seeds were surface sterilised and grown in sterile vermiculite in magenta boxes for 2 months. Mor layer soil from a mixed coniferous forest at Lunsen, Sweden (59°47′ N, 17°40′ E, altitude 64 m) was collected and homogenised using a 5 mm mesh sieve to remove roots, coarse woody fragments and rock particles. Seedlings were planted in the microcosms measuring 6 x 6 x 7 cm, containing 80 g soil. Sampling was performed at 0, 1, 3, 5 days and 1, 2, 4, 8, 16 and 24 weeks and three plants were harvested at each time point. Roots were cleaned with sterile, double distilled water and stored at −20°C. Root tips were separated from the main root system and cleaned of adhering soil particles and organic debris under a dissecting microscope in a laminar hood to prevent laboratory contamination. DNA from each root tip was extracted and the identity of the dominant fungi was established by Sanger sequencing and bacterial community composition analysis was carried out using 454 pyrosequencing.

3.2 Single root tip microbiomes – horizon differences (Study II)

Single root tip microbiomes were also used in a second study designed to examine variation in bacterial colonization in relation to i) different mycorrhizal fungi, ii) different soil horizons and iii) short-term additions of N. Soil collected from the O, E and B horizons of a boreal forest in Jädraås,

central Sweden (60° 49′ N, 16°30′) (Persson, 1980) was sieved and roots and stones were removed. Microcosms (50 ml Falcon tubes) were filled with 13.5 g and 26 g (fresh weights) of O and E or B horizon soils respectively (**Figure 1**). Soils in all microcosms were gently packed to 25 ml volume. *Pinus sylvestris* seedlings were planted in the tubes, allowing the shoots to protrude through a hole in the tube (**Figure 1**). After six months of growth in a phytotron, urea was added as a nitrogen source (176 µg N cm^{-3} of each soil substrate) to three replicates of each horizon and control microcosms were left unamended. Two weeks following nitrogen addition, plants were harvested, root tips were cleaned (as described above) and DNA from each root tip was extracted for fungal and bacterial community composition analysis using 454 pyrosequencing

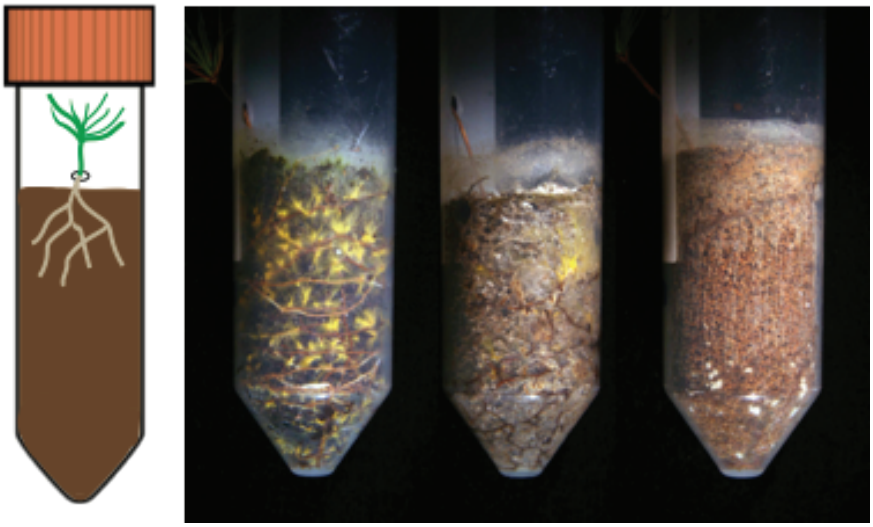


Figure 1. Schematic diagram of the Falcon tube microcosm system and three accrual microcosms containing soil from the O, E and B horizons.

3.3 Fungal and bacterial communities in soil and roots in a boreal forest podzol (Studies III and IV)

In papers **III** and **IV**, a long-term forest fertilization experiment at Lamborn, Sweden, (N 60° 58', E 15° 48') was used to investigate the fungal and bacterial communities in different soil horizons of a podzol under an 85-year-old *Pinus sylvestris* forest. Half of the experimental plots had been fertilized with 150 kg N ha^{-1} 15 months prior to the sampling (**Figure 2 a, b**). Ten soil cores were taken in each plot and were divided into O, E and B horizon material before

pooling (**Figure 2c**). Pooled samples from each of the horizons in three replicate plots of both treatments were analysed. DNA was extracted from both soil and roots, amplified using PCR and community analysis of fungi (paper **III**) and bacteria (paper **IV**) colonising both roots and adjacent rhizosphere soil was carried out using 454 pyrosequencing. The effects of N fertilisation and of different soil horizons on fungal/bacterial community structure and diversity were evaluated.

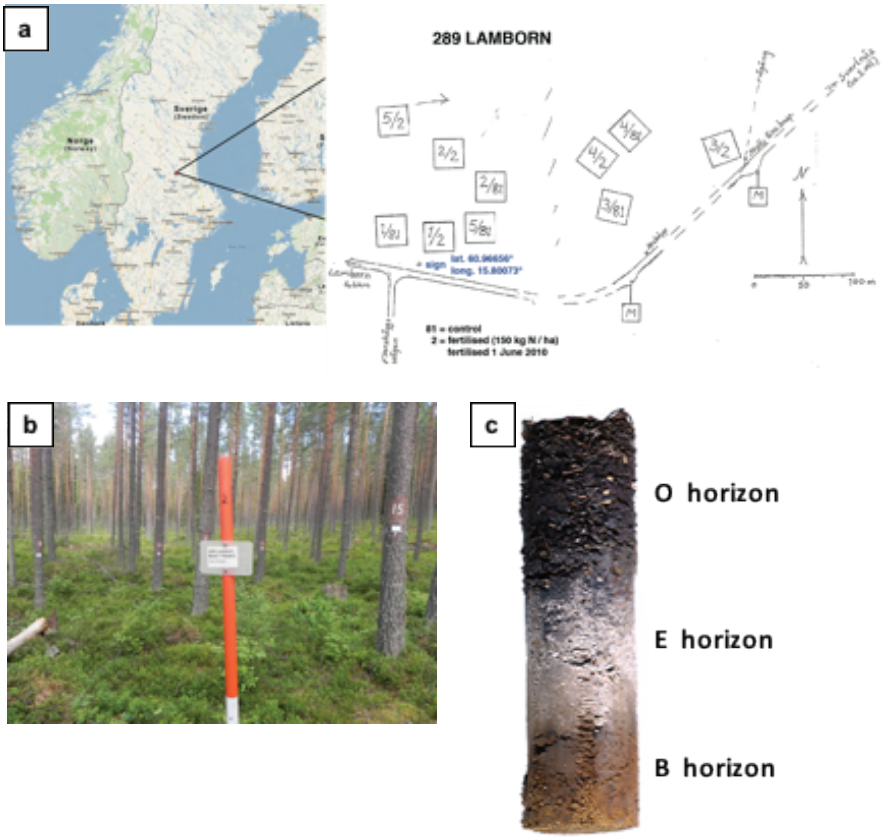


Figure 2. (a) Maps showing the location of the Lamborn field site, as well as the layout of the experimental plots. Each plot measures 30 x 30 m. (b) One of the experimental plots at the Lamborn field site. (c) Podzol soil horizons in a soil core from Lamborn showing the O, E and B horizon.

4 Materials & Methods

An overview of the main methods used is given below. More detailed descriptions of the methods are presented in the individual papers.

4.1 Laboratory growth systems and field sampling

4.1.1 Study I

Pinus sylvestris seeds were sterilized with 37% hydrogen peroxide for 1 h and washed three times with sterile ddH_2O for 30 min. Seeds were planted in sterile vermiculite in magenta boxes (30 seeds per box) and placed in a growth chamber with a 16 h photoperiod at 18°C and a photon flux density of 250 µmol m^{-2} s^{-1} and an 8 h dark period at 16°C. Soil was collected from the mor layer at a mixed coniferous forest at Lunsen, Sweden. Freshly collected soil was homogenised using a 5 mm mesh sieve and stored at 4°C for 24-48 h prior to transplanting individual pine seedlings into plastic pots (6 x 6 x 7 cm) containing 80 g soil. At this stage the seedlings were 8 weeks old with main root axes 2-3 cm in length and no lateral roots. The moisture content of soil was maintained gravimetrically at 65% by weekly addition of ddH_2O. In total 148 pots were used and their location within the growth chamber was randomized each week in conjunction with harvesting. Three plants were harvested at each harvest after 0, 1, 3, 5 days and 1, 2, 4, 8, 16 and 24 weeks. At each harvest soil attached to the roots was carefully removed by washing with sterile ddH_2O. The cleaned entire root systems were stored at -20°C until further processing.

4.1.2 Study II

Soil was collected from Jädraås forest in central Sweden. O, E, B-horizon soil was separated and sieved to homogenize the soil. Pine seedlings (grown on vermiculite) were planted into 50 ml Falcon tubes containing either O, E or B

horizon soils (**Figure 1**). The shoots were allowed to protrude through a hole in the tube. In total 18 microcosms were set up with 6 replicates of each (O, E, B) horizon soil. The microcosms were watered gravimetrically by weekly addition of ddH_2O. After six months, when the seedlings had developed ectomycorrhizal roots and established extensive mycelial networks, urea was added to triplicate microcosms containing, either O, E or B soils. Urea application resulted in a concentration of 176 µg N cm^{-3} of each soil substrate. No N was added to the respective control microcosms. After 2 weeks of nitrogen addition plants were harvested and the roots were carefully separated from the adjacent rhizosphere soil and stored at -20°C until further processing.

4.1.3 Studies III and IV

From each plot 10 replicate cores were sampled randomly and they were dived into O, E and B horizons in the laboratory. Soil and roots were separated and the roots were cleaned thoroughly to remove adhering soil and organic matter particles under a dissecting microscope in a laminar flow hood. Before extracting DNA, O, E and B horizon soils from 10 replicate cores of each plot were pooled together to generate three composite samples representing podzol layers (O, E, B) of each plot. The same approach was used for the root samples from O, E and B horizons. All soil and root samples were freeze dried and homogenised prior to DNA extractions.

4.2 DNA extraction and PCR

Root tips separated from the main root system were carefully cleaned with sterile, double distilled water under a dissecting microscope in a laminar hood and on ice to maintain integrity and community composition of the root tips. DNA extraction from single root tips was challenging and critical for the community analysis of single root tip microbiomes. The same method should extract DNA from both fungal and bacterial communities. Initially, we tested a number of different methods and checked the efficiency of each extraction method by amplifying the extracted DNA using PCR and analysing the fungal and bacterial communities using denaturing gradient gel electrophoresis (DGGE) as a rapid screening method. Root tip harvesting, cleaning, DNA extraction and PCR reaction set up were all done in a laminar hood to prevent contamination.

After testing different protocols, we came to the conclusion that DNeasy plant kit (Qiagen, Germany) gave the best amplification efficiency of both fungal, as well as bacterial communities. We also improved and optimized the DNA extraction protocol by making some modifications to the existing

protocol. We used sterile 0.5 ml self-standing screw cap tubes (VWR 211–3236), 2 mm zirconium beads (MOBIO laboratories, CA, USA) and Buffer AP1 from the DNeasy plant kit to homogenise the root tips. The advantage of using metal beads is that they are heavy and are better able to homogenise the root tips with no formation of sediment after the bead beating. We used half the volume of Qiagen DNeasy plant kit reagents that are specified in the manufacturers' protocol (Papers **I** and **II**). To extract microbial DNA from roots collected from different soil horizons, the standard Qiagen DNeasy plant kit protocol was used, and to extract microbial DNA present from different horizon soils, FastDNA Spin Kit for Soil (MP Biosciences, USA) was used (Papers **III** and **IV**).

To identify the dominant fungi associated with individual root tips, in the first study we used Sanger sequencing. Root tip template DNA was amplified using universal fungal primers ITS1F and ITS4 (White *et al*., 1990; Gardes and Bruns, 1993). In total over 392 root tips were harvested, cleaned and sequenced. In Paper **II** the root tip-associated fungal communities were amplified using ITS4 primer with unique 8-base pair identifying tags attached and fITS9 primer (Ihrmark *et al.,* 2012). In total 323 root tip samples were harvested, cleaned and 454 pyrosequenced. Similarly in Paper **III**, fungal communities in soil and on roots were amplified using the same primers and the resulting PCR products were subjected to 454-pyrosequencing. Bacterial community composition associated with individual root tips was investigated using 454-pyrosequencing. After removing the mixed, low homology and non-ectomycorrhizal fungal root tips, 175 samples with best ectomycorrhizal fungal sequence identities were selected for bacterial community analysis in paper **I** and 245 root tips in paper **II**. In paper **III** and **IV,** soil and root samples from different podzol soil horizons from fertilised and unfertilised control plots resulted in a total of 34 samples that were subjected to 454 pyrosequencing analysis. Template DNA was amplified using 515F and 816R primer set (Bates *et al*., 2011; Caporaso *et al.*, 2011). 816R contained a unique 12-base pair tag to distinguish between the different samples (Papers **I**, **II** and **IV**).

4.3 Pyrosequencing and bioinformatic analysis

The principle behind the pyrosequencing is that DNA polymerase binds to the immobilized single stranded DNA and nucleotides of same base pair are sequentially released into the medium and when an appropriate match occurs they are added to the growing strand, resulting in the release of pyrophosphate. This is converted to ATP by sulfurylase. Luciferase will use the ATP to oxidise luciferin to oxyluciferin and generating chemiluminescence. The light

generated is recorded on the pyrogram as a peak for nucleotide incorporation. If multiple nucleotides of the same type are inserted the peak height will be higher and if no match is found there will be empty space in the pyrogram. A pyrase enzyme removes unincorporated nucleotides. This method with a GS FLX Titanium Pico Titer Plate (Roche, Branford, CT, USA) generates around 1 million amplicon reads for a complete plate.

Fungal reads obtained from the 454 pyrosequencing were filtered and assigned to respective samples based on unique barcode sequences using SCATA (Sequence Clustering and Analysis of Tagged Amplicons) pipeline (https://scata.mykopat.slu.se). During the filtering step, lower quality, short and missing primer read sequences were filtered out and the remaining sequences were clustered at the 98.5% cluster distance into OTUs (operational taxonomic units) by single linkage clustering method. NCBI Blastn search tool (Altschul *et al.*, 1990) was used to identify the fungal taxa (Paper **II** and **III**). Rarefaction curves were drawn using Analytic Rarefaction tool (v. 1.3, www.uga.edu/strata/software/Software.html). Diversity indices were calculated using PAST v. 3, (PAleontology STatistics) software, Øyvind Hammer, Natural History Museum, University of Oslo, Norway (www.folk.uio.no/ohammer/past/index.html).

Bacterial reads obtained from the 454 pyrosequencing were processed using the RDP (Ribosomal data base project) pipeline (Cole *et al*., 2014). We used supervised and unsupervised methods to process the 454-pyrosequencing reads. In both methods the first two steps, initial processing and chimera removal steps, are common. In the initial processing step the 454-reads were filtered based on primer mismatch, short and low quality reads were discarded and remaining sequences were sorted and binned to samples tagged with unique barcodes. Primers were removed at this stage. In the next step the chimera check function in the Fungene pipeline USEARCH tool (Edgar *et al*., 2011) was used to remove chimera sequences. In supervised mode the sequences were classified up to genus level using the RDP classifier tool (Wang *et al*., 2007) and the rest of the beta diversity analysis and other statistical and exploratory analyses were performed on the classified sequences. The RDP unsupervised method was used to calculate the bacterial species richness, rarefaction curves and diversity indices, which require the clustering step. After the chimera removal step, sequences were aligned using the RDP infernal aligner tool (Nawrocki & Eddy, 2013). Aligned sequences were subjected to clustering using the RDP mcClust complete-linkage-clustering tool. We used 0.03 as the maximum distance so the sequences were clustered at 97% cluster distance into OTUs. The cluster file generated was used in the calculation of species richness and diversity indices using RDP

pipeline diversity index tools. Rarefaction curve values were generated using rarefaction tools (RDP database, www.pyro.cme.msu.edu) and the curves were drawn in Microsoft Excel.

4.4 Statistics

Nonmetric multidimensional scaling (NMDS) using the Bray-Curtis distance measure was used to explain the beta diversity patterns in all the papers. NMDS shows the relationship between the objects graphically in multidimensional space. It uses the rank orders to plot the objects with several iterations to reduce the stress value in minimal number of dimensions. The distances between the samples in the NMDS are not their original distances but are rank order distances. In NMDS plot the more similar objects are closer and the more dissimilar objects are further apart. The stress is the measure of goodness of fit; multiple iterations were performed to reduce the stress value (Ramette *et al.,* 2007).

Non-parametric statistics were used to analyse the variation in community structure. The significance of each ordination was measured using ANOSIM (analysis of similarity) and NPMANOVA (nonparametric ANOVA) tests. Both of these tests can be used to test the null hypothesis. ANOSIM compares the ranks of distances within groups and between groups. When multiple comparisons were performed the significance of differences between each treatment was measured using NPMANOVA pairwise comparisons (Ramette *et al.,* 2007). The species causing the variation (dissimilarity) between the treatments were extracted using the SIMPER (similarity percentage) analysis. We selected all taxa contributing more than 1% of the total variation and plotted them in Microsoft Excel to show the patterns of community composition in different treatments (Paper **I, II, III** and **IV**).

Venn diagrams were based on presence or absence data to show the bacterial core and specific taxa associated with different ectomycorrhizal fungi. Venn diagrams were generated using the VENNY online program (http://bioinfogp.cnb.csic.es/ tools/venny/index.html).

5 Results and Discussion

5.1 Single root tip microbiomes – temporal dynamics (Paper I)

In this project we were interested in the ectomycorrhizal fungal and bacterial community development during the root development of *Pinus sylvestris*. The questions addressed were: 1) *Fungal specificity*: Do different ectomycorrhizal fungi harbour distinct bacterial communities at different sampling points during root development? 2) *Temporal bacterial community dynamics on ectomycorrhizal fungi*: Do individual ectomycorrhizal fungi harbour distinct bacterial microbiomes at different time points during root development?

Previous studies of fungal selectivity on bacterial communities have produced contrasting conclusions. Some studies provide some evidence of significant differences in the bacterial communities associated with different ectomycorrhizal fungi (Izumi & Finlay, 2011; Nguyen & Bruns, 2015). But many studies have failed to show any differences (Izumi *et al.*, 2007b, 2008; Burke *et al.*, 2008; Kataoka *et al.*, 2008; Tanaka & Nara, 2009; Uroz *et al.*, 2012). Environmental variation and pooling of morphologically identified root tips of indeterminate age complicate correct interpretation of field data, and in the present study we conducted the experiment in a phytotron, using sieved mor layer soil and analysed bacterial community composition on individual root tips to avoid these problems.

5.1.1 Fungal colonization

Fungal sequences were analysed by Sanger sequencing and it was found that *Phialocephala fortinii, Meliniomyces variabilis* and an unidentified *Russula species* colonised the roots at all sampling points. *Rhizoscyphus ericae* colonized the roots from day 5 until week 8. *Piloderma* spp. and *Paxillus involutus* appeared from week 2 onwards until week 24. *Cenococcum*

geophilum appeared later from week 8 until week 24. *Tomentellopsis* spp. was present at weeks 4, 8 and 16 (**Figure 3**).

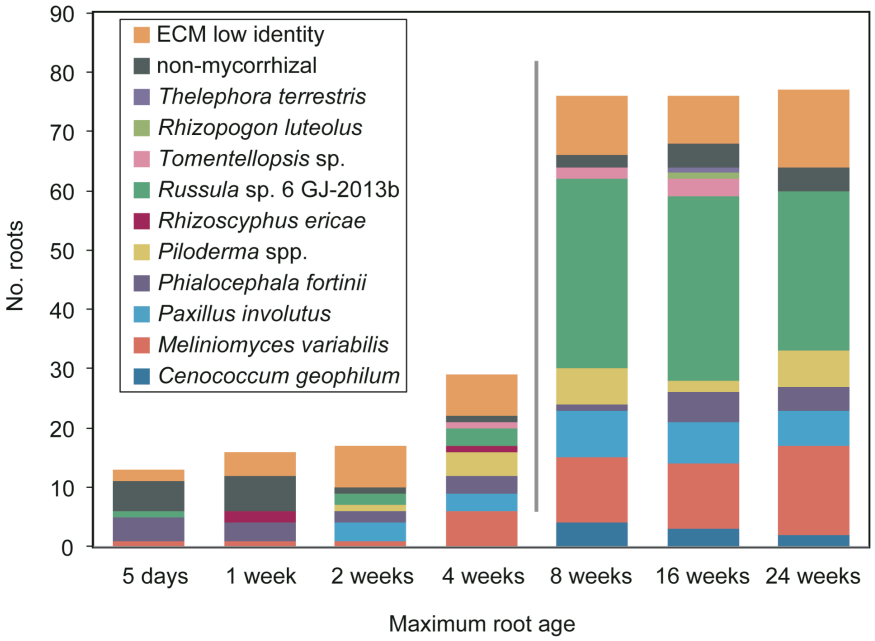


Figure 3. Fungal colonization patterns in root systems of microcosm-grown *Pinus sylvestris* seedlings at different time points following planting in soil from a boreal forest at Lunsen, Sweden. Data up to and including week 4 are based on Sanger sequencing of all roots and root primordia, data from 8 weeks to 24 weeks are based on subsampling of all unique morphotypes. In total over 392 root tips were analysed.

Ectomycorrhizal root tips associated bacterial communities were analysed using 454 pyrosequencing. Rarefaction analysis and Chao diversity indices suggested that bacterial species richness increased during root development from week 1 to week 16 and then declined significantly at week 24 (**Figure 4**). The reason for this decline is not clear but could be due to a decline in pH that sometimes occurs in pot systems, to increasing allocation of assimilates to hyphae rather than roots as the roots age, or to decreased overall C allocation due to apical bud formation and dormancy. In total 5694 operational taxonomic units (OTUs) were distinguished from the 239159 sequences in the dataset and between 60% and 70% of the expected species richness was captured suggesting that more sampling was necessary to achieve saturation.

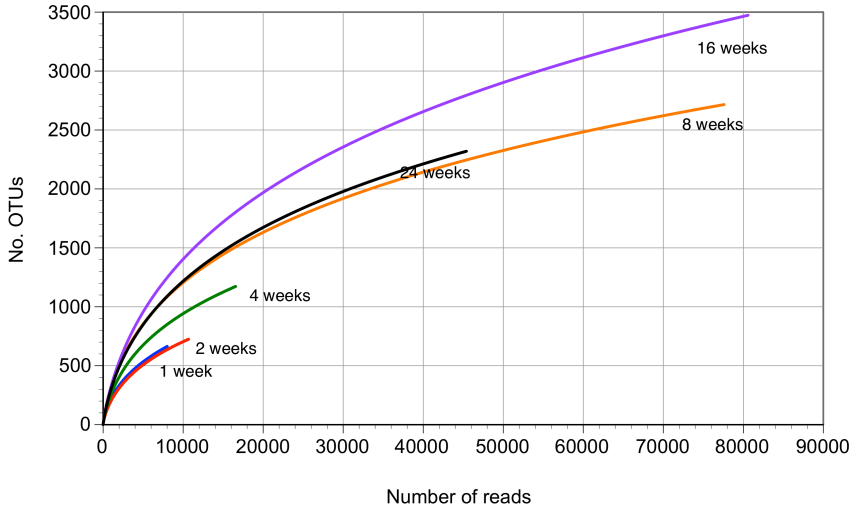


Figure 4. Rarefaction curves illustrating the numbers of bacterial OTUs associated with mycorrhizal root tips of *Pinus sylvestris* at different maximum ages. Error bars illustrate 95% confidence intervals. n = 41 (week 8, 16, 24).

5.1.2 Temporal changes in bacterial community structure

The analysis was based on non-metric multidimensional scaling (NMDS) and significant ANOSIM values suggested that the *Russula* sp. 6 GJ-2013b, *M. variabilis, P. involutus* and *Piloderma* spp. associated bacterial communities changed significantly with time between 4 and 24 weeks (**Figure 5**).

Pairwise comparisons using non-parametric multivariate analysis of variance (NPMANOVA) showed that, except for *P. involutus* at week 16 and week 24, all other fungal comparisons at different time points were statistically significant. Similarity percentage analysis (SIMPER) using the Bray–Curtis dissimilarity measure was used to show the taxa contributing to the differences between different sampling points. Only the taxa causing more than 1% of the variation are shown. The most common genera causing the differences in all the ectomycorrhizal fungi studied were *Burkholderia*, *Sphingopyxsis*, *Dyella*, *Pseudomonas*, *Acinetobacter*, *Actinospica*, *Aquaspirillum*, *Acidobacter* Gp1, *Sphingomonas*, *Terriglobus*, *Enhydrobacter*, *Herbaspirillum* and *Bradyrhizobium*. *Burkholderia* and *Sphingopyxsis* were the dominant genera. Many genera had high initial abundance at week 8, declining with time, but several genera such as *Dyella* and *Terriglobus* increased in abundance at later time points. In roots colonized by *Piloderma* spp. several other bacterial genera, such as *Actinospica*, *Bradyrhizobium*, *Acidobacter* Gp1 and *Rhizomicrobium* appeared to increase in abundance at later sampling points.

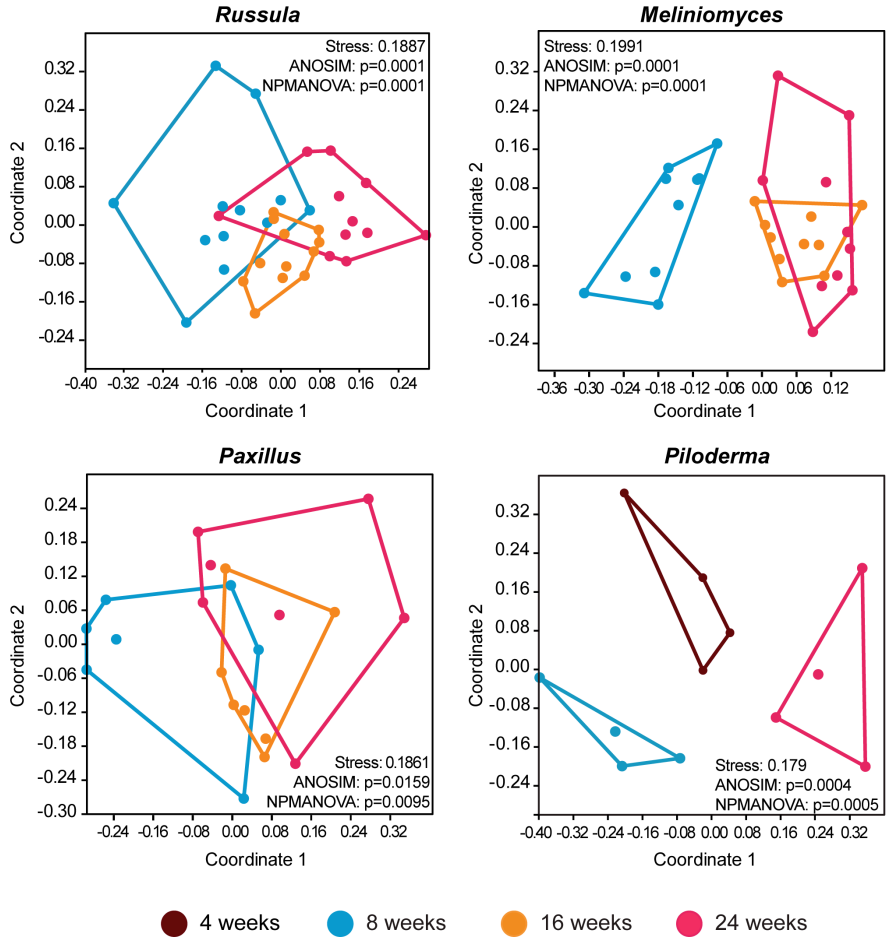
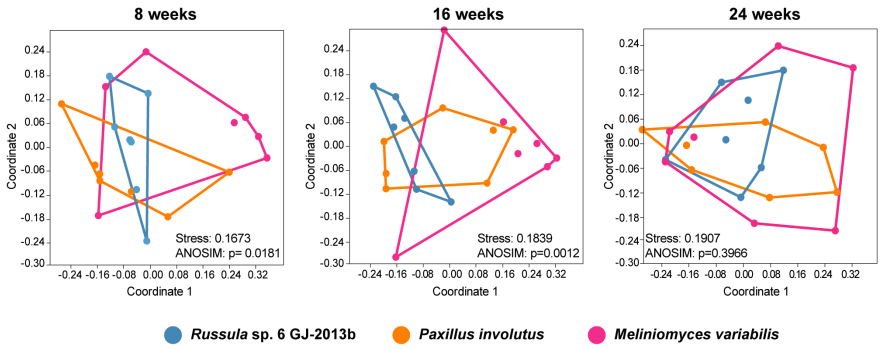


Figure 5. Non-metric multidimensional scaling ordinations of temporal changes in bacterial community structure associated with mycorrhizal root tips colonized by different ectomycorrhizal fungi. (A) *Russula* sp. 6 GJ2013b n = 12, (B) *Meliniomyces variabilis* n = 10, (C) *Paxillus involutus* n = 7, (D) *Piloderma* spp. n = 4.

These results have implications for sampling of field material where root age cannot be determined accurately and suggest that temporal variation may confound systematic effects of the dominant mycorrhizal host fungi on bacterial community structure. This may explain the failure of some previous field studies to reveal any systematic variation in bacterial community structure related to the presence or absence of particular ectomycorrhizal fungal symbionts.

5.1.3 Influence of mycorrhizal fungi on bacterial community structure

Non-metric multidimensional scaling analysis of bacterial community structure associated with root tips colonized by different ectomycorrhizal fungi showed that the effect of the dominant fungi colonising the roots varied with time. Two-dimensional ordinations are shown in **Figure 6**, and the ANOSIM values indicate a significant effect of the fungal species on bacterial community structure at weeks 8 ($P = 0.018$) and 16 ($P = 0.001$), but no significant effect at week 24 ($P = 0.397$).



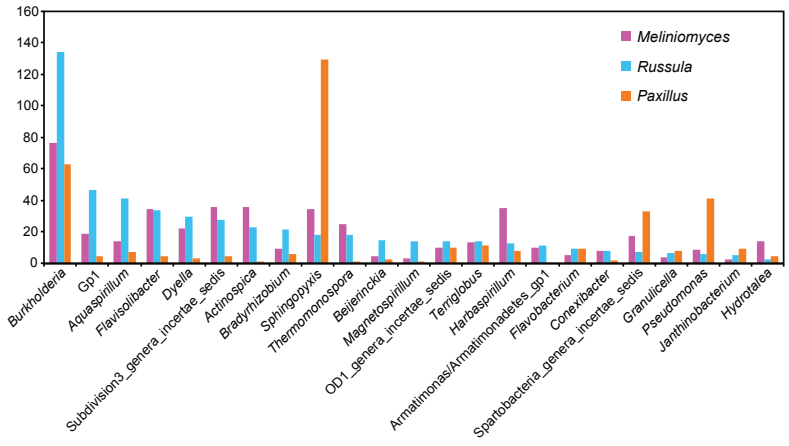
Figur 6. Non-metric multidimensional scaling ordinations of bacterial community structure associated with root tips colonized by the ectomycorrhizal fungi *Russula* sp., *Meliniomyces variabilis* and *Paxillus involutus* (n = 7) at 8, 16 and 24 weeks.

NPMANOVA pairwise comparisons showed that at week 8, the bacterial community composition of roots colonized by *P. involutus* was statistically distinct from that of roots colonized by *Russula* sp. 6 GJ-2013b and *M. variabilis*. At week 16, all pairwise comparisons were statistically significant, but at week 24, none of the comparisons was significant.

SIMPER analysis was used to elucidate the bacterial taxa causing (> 1%) variation at week 16 since it gave the strongest (ANOSIM $P < 0.0012$). All three mycorrhizal types were associated with high abundance of *Burkholderia* (**Figure 7**) but, whereas roots colonised by *Russula* sp. 6 GJ-2013b were associated with higher abundance of *Acidobacter* Gp1, *Aquaspirillum* and *Dyella*, roots colonised by *Meliniomyces variabilis* were associated with higher abundance of *Herbaspirillum* and *Actinospica*. Both *Russula* and *M. variabilis* were associated with intermediate abundance of *Flavisolibacter*.Roots colonized by *P. involutus* were associated with high abundance of *Sphingopyxsis*, *Pseudomonas* and an unidentified genus of *Spartobacteria*.

We identified a core microbiome of approximately 20 bacterial genera that were consistently present in all ectomycorrhizal types at all time points. The

common taxa were *Burkholderia*, *Dyella*, *Pseudomonas*, *Flavisolibacter*, *Beijerinckia*, *Actinospica*, *Janthinobacterium*, *Aquaspirillum*, *Acidobacter* Gp1, *Sphingomonas*, *Terriglobus*, *Enhydrobacter*, *Magnetospirillum* and *Bradyrhizobium*. Certain genera were exclusively present with specific ectomycorrhizal fungi (e.g. *Rhodobacter* and *Rhodopirellula* with *M. variabilis*, *Naxibacter* and *Pelomonas* with *P. involutus*, *Herminiimonas* and *Corynebacterium* with *Piloderma* spp.). Some bacterial genera occurred in association with all mycorrhizal types exclusively at early (*Herminiimonas*) or late (*Acidobacterium* Gp2, *Dechloromonas*) time points.



Figur 7. Mean abundance (no. of sequences) of different bacterial genera on roots colonized by three different ectomycorrhizal fungi at 16 weeks. The bacterial genera shown are those contributing more than 1% dissimilarity in pairwise SIMPER comparisons between individual fungi.

The differences observed in the bacterial community composition on specific ectomycorrhizal fungi at different time points and different ectomycorrhizal fungi at particular time points, as well as overall levels of bacterial diversity, might be related to quantitative and qualitative variation in the composition of different carbon compounds produced by different mycorrhizal fungi (Ahonen-Jonnarth *et al.*, 2000; van Hees *et al.*, 2005; 2006a,b; van Schöll *et al.*, 2006; Toljander *et al.*, 2007; Johansson *et al.*, 2008a,b, 2009).

Sphingopyxis and *Sphingomonas* belonging to the family Sphingomonadaceae were abundant in association with all four dominant ectomycorrhizal fungi in our study, it has also been been reported in *Laccaria proxima* and *Russula exalbicans* ectomycorrhizal fungi (Boersma *et al*., 2009) and similar observations were made by Uroz *et al.* (2007) in *S. citrinum*

ectomycorrhizal fungi and postulated that the bacteria could supplement the weathering activity of their host fungi. Other abundant bacterial genera in our study (e.g. *Burkholderia*, *Herbaspirillum*, *Janthinobacterium*, *Dyella*) have also been identified in association with ectomycorrhizal roots and linked to mineral weathering and P solubilisation/Fe chelation (Uroz *et al.*, 2011b). Bacteria in the Burkholderiales and Rhizobiales were the dominant associates of the *P. muricata* ectomycorrhizal roots studied by Nguyen & Bruns (2015). These two orders were also abundant in the present study and include the genera *Burkholderia, Herbaspirillum, Herminiimonas, Naxibacter* and *Janthinobacterium*, *Bradyrhizobium* and *Beijerinckia* (Steenhoudt & Vanderleyden, 2000; Estrada-de Los Santos *et al.*, 2001). These genera include strains with the ability to fix N, but also other strains may not have this ability.

Analysis of the core microbiomes associated with different roots is helpful in identifying stable and consistent components across complex microbial assemblages (Shade & Handelsman, 2012). The core microbiomes we identified suggest that many of the bacterial genera were persistent across different sampling occasions and in different ectomycorrhizal roots, although there were changes in their relative abundance (**Figure 8 a, b**).

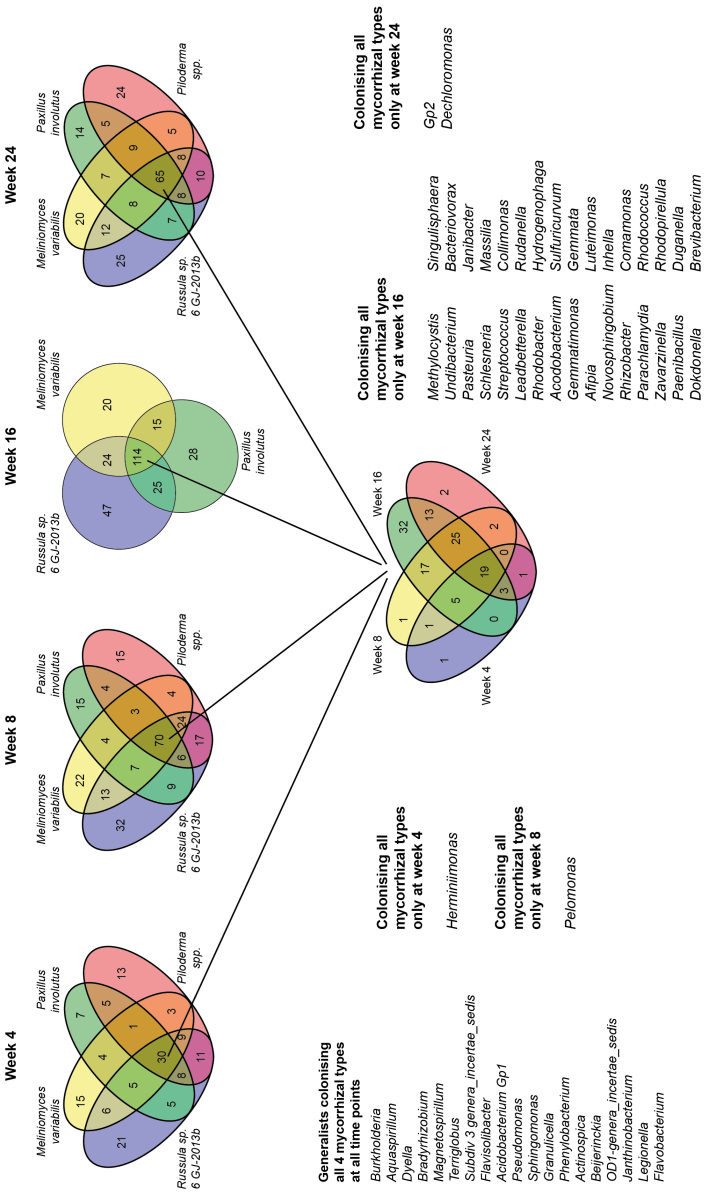


Figure 8a. Venn diagrams showing numbers of bacterial genera associated with roots colonized by different dominant ectomycorrhizal fungi at different time points. The core microbiomes colonising all mycorrhizal types at each time point are shown. The overlap of the genera constituting these cores and their distribution between different time points is shown in a second Venn diagram. The bacterial genera colonising all mycorrhizal types at all time points are shown on the left. The unique bacterial assemblages consistently colonising all mycorrhizal types, but exclusively at individual time points, are also shown.

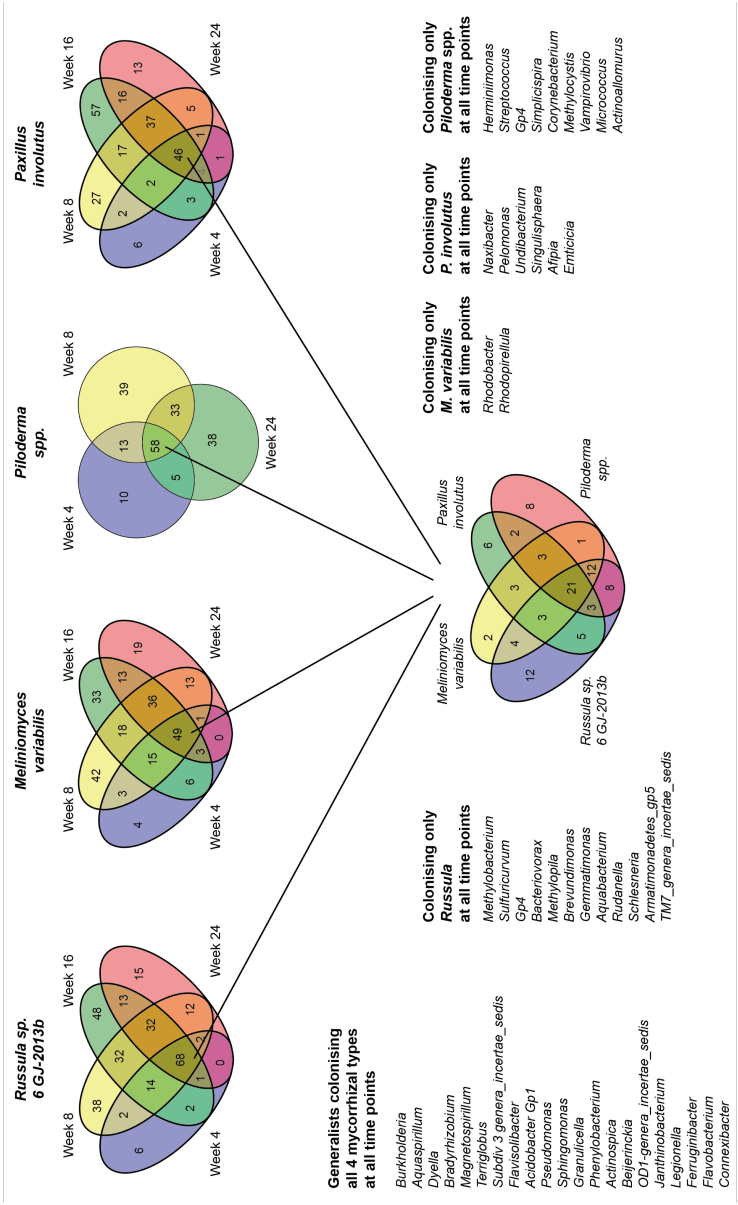


*Figure 8b.*Venn diagrams showing numbers of bacterial genera associated with roots at different time points and colonized by different dominant ectomycorrhizal fungi. The core microbiomes colonising at all time points for each mycorrhizal type are shown. The overlap of the genera constituting these cores and their distribution between different ectomycorrhizal types is shown in a second Venn diagram. The bacterial genera colonising at all time points and all mycorrhizal types are shown on the left. The unique bacterial assemblages consistently colonising at all time points, but exclusively for individual mycorrhizal types, are also shown.

5.2 Paper II

Boreal forest podzol soil has distinct horizons with different chemical and physical characteristics and nutrient availability. In this project pine seedlings were grown in microcosms with either O, E, or B horizon forest soil separately. In one treatment urea was added as a nitrogen source two weeks before harvesting. In this study we are interested in understanding the specific bacterial and fungal communities associated with dominant ectomycorrhizal fungi colonising the single root tips of pine seedlings in different podzol horizons and effect of nitrogen addition on the ectomycorrhizal fungi-bacteria interactions.

Previous studies, mainly based on profiling soil based communities, have shown that different soil horizons harbour distinct fungal (Dickie *et al.*, 2002; Lindahl *et al.*, 2007; Coince *et al.*, 2013) and bacterial communities (Uroz *et al.*, 2013; Baldrian *et al.*, 2012; Fransson & Rosling, 2014; Calvaruso *et al.*, 2007; Eilers *et al.*, 2012). There is one study that has focused on ectomycorrhizal root tips growing in different soil horizons and report that there is a relationship between soil horizon and ectomycorrhizal fungi colonising the roots (Rosling *et al.*, 2003). Most of the studies have concentrated on sampling the soil rather than tree roots however it has been shown that the soil and root communities differ significantly (Vik *et al.*, 2013; Uroz *et al.*, 2012; Nguyen *et al.*, 2015). Two recent studies suggest that the bacterial community composition on individual root tips colonised by different dominant ectomycorrhizal fungi in the organic soil layer is distinct (Nguyen *et al.*, 2015; Marupakula *et al.*, 2015) but to date there are no studies of bacteria associated with roots in the deeper mineral horizons.

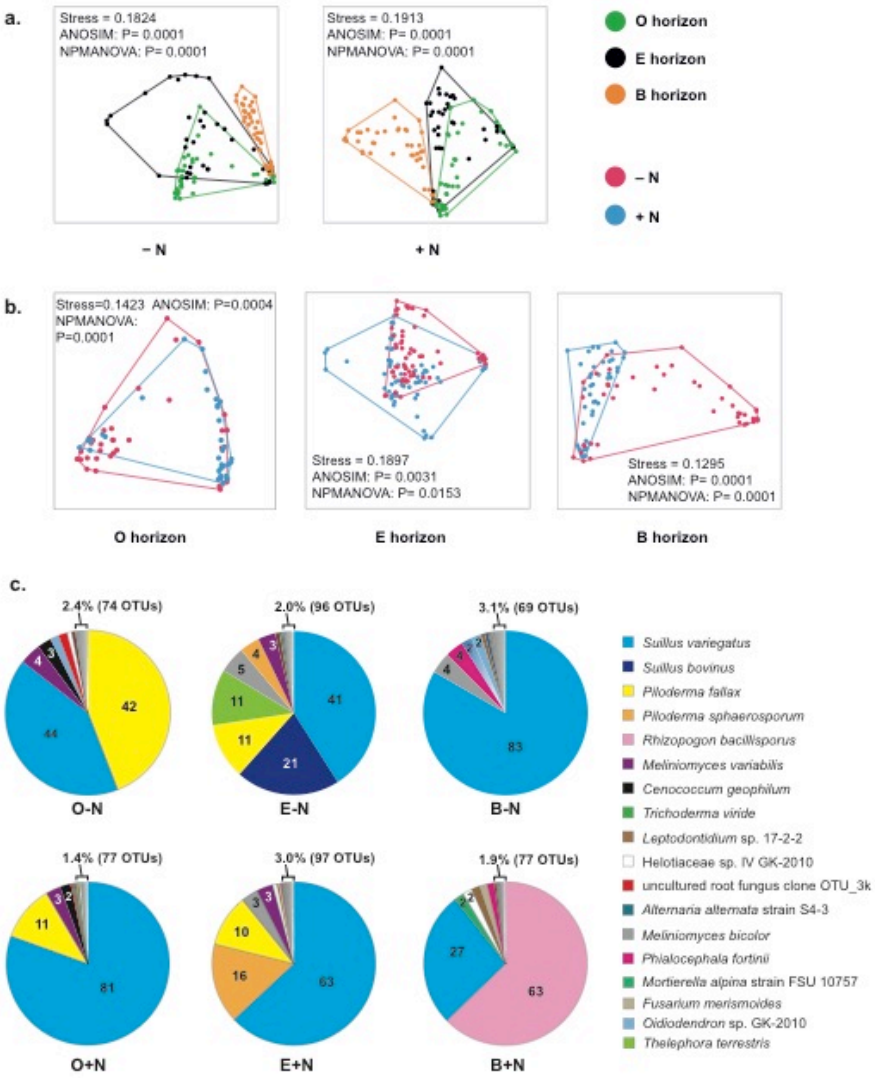
5.2.1 Fungal community structure on root tips growing in different soil horizons

To study the fungal community structure in different soil horizons, non-metric multidimensional scaling (NMDS) was used and significant ANOSIM values suggested that *Pinus sylvestris* root tips in different soil horizons (O, E and B) harbour statistically distinct fungal communities irrespective of the N addition (**Figure 9a**). Our results are in agreement with Shahin *et al.*, (2013) and Rosling *et al.*, (2003) who, using root tip isolation and Sanger sequencing, showed that ectomycorrhizal fungal composition was different in different soil horizons. However these authors only examined the root tip associated ectomycorrhizal fungi not the entire fungal communities colonising the root tips. Recently Coince *et al.* (2013) and Marupakula *et al.* (study III) have showed that ectomycorrhizal roots in different soil horizons harbour distinct fungal communities. Apart from these studies, other studies have examined the bacterial communities residing in different soil horizons using high throughput

sequencing (Baldrian *et al.*, 2012, McGuire *et al.*, 2013, Variskova *et al.*, 2013) and they found significant differences in the community structure in different horizons.

The horizon effect was greater than the effect of N addition but when compared within the horizon, fungal communities exhibited statistically significant differences in all the horizons, suggesting that N addition has an effect on the fungal communities within the horizons. The effect of short-term urea addition (176 µg N cm^{-3} of each soil horizon substrate) on fungal community structure was also statistically significant within each soil horizon (P <0.003 – P <0.0001) (**Figure 9b)**. It has been shown in number of previous studies (Fransson *et al.*, 2000; Cox *et al.*, 2010; Kjoller *et al.*, 2012; Jones *et al.*, 2012; Avis *et al.*, 2003; Lilleskov et al 2011; Parrent and Viglays, 2007; Hasselquist & Högberg, 2014) that fungal community composition changes when fertilised with N, but all the above mentioned studies are based on low through put methods.

When N was added in the O horizon *P. fallax* abundance was reduced from 41% to 11% and *M. variabilis* and *C. geophilum* were also negatively affected whereas *S. variegatus* abundance increased from 41% to 81%. In the E horizon *S. bovinus* (21%) and *Thelophora terrestris* (11%) were reduced to below the detection limit by the nitrogen addition. Nitrogen had a negative effect on *P. fallax, M. bicolor, M. variabilis* whereas *S. variegatus* and *P. sphaerosporum* increased in relative abundance. In the B horizon nitrogen fertilisation severely affected the *S. variegatus*, reducing its relative abundance from 83% to 27%. *M. bicolor and P. fortinii* were also decreased in abundance. In contrast *Rhizopogon bacillisporus*, completely absent in the unfertilised B horizon treatment, was most abundant in the N added microcosms (**Figure 9c**). Previous studies also showed that different ectomycorrhizal fungi, including *Cortinarius* (Lilleskov *et al.*, 2002, Avis *et al.*, 2003) and *Piloderma* (Cox *et al.*, 2010, Lilleskov *et al.*, 2002), respond differently to N addition. *Tricholoma* abundance was decreased by N fertilisation (Lilleskov, 2011) whereas *Russula decolorans* was not affected by N addition (Cox *et al.*, 2010). Fransson *et al.* (2001) found greater abundance of *Cenococcum geophillum* colonised root tips in the fertilised plots.

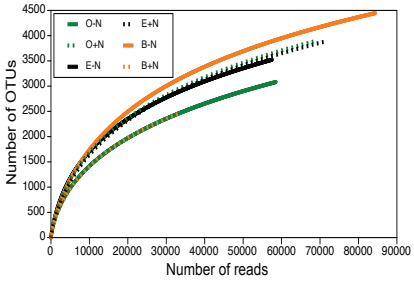


Figur 9. Nonmetric MultiDimensional Scaling ordinations showing differences in community structure of fungi colonising *Pinus sylvestris* roots growing in soil from different podzol horizons (O, E, B) from a boreal forest at Jädraås, Sweden. The soil was unfertilised (-N) or fertilised with urea as an N source (+N), (b) differences in community structure of fungi colonising roots between -N and +N treatments within each soil horizon, (c) pie charts showing relative abundance of different fungal taxa in the system described above.

5.2.2 Species richness and alpha diversity patterns of bacterial communities associated with ectomycorrhizal fungi colonising root tips

In total 11033 bacterial OTUs were distinguished in this study. Rarefaction analysis (**Figure 10**) and diversity indices suggest that the B horizon harbours maximum bacterial diversity than O and E horizons. Nitrogen addition had increased the number of OTUs in O horizon and there was no effect on E horizon whereas a negative effect in the B horizon was observed. Chao diversity index, suggest that between 66% and 71% of the expected species richness was captured suggesting that more sampling was necessary to achieve saturation. This finding is in agreement with our field study (study IV) suggesting that deeper mineral horizons harbour significantly more bacterial OTUs than the upper O horizon. In fact, B horizon (1922) harbours significantly more bacterial OTUs than E (1657) or O horizon (1578) (B>E>O).

Nitrogen addition effects in the O and B horizons are contrasting with respect to bacterial species richness. One possible reason might be that the bacterial communities in deeper soil horizons are dependent on ectomycorrhizal fungi and root-derived carbon whereas the O horizon has abundance of organic matter, that bacterial communities decompose for carbon acquisition. There are reports that N fertilisation decreases allocation of plant-derived carbon to belowground ectomycorrhizal fungi and roots (Lilleskov *et al.,* 2011; Wallander *et al.*, 2011; Kjoller 2012; Ostonen *et al.*, 2011; Högberg *et al.,* 2010).



sampleID	distance	No. reads	clusters	chao	LCI95	UCI95	H'	varH	E
O-N	0.03	58538	3082	4582	4367	4834	5.25565	0.00011	0.65423
O+N	0.03	68364	3884	5464	5256	5705	5.65093	0.0001	0.68375
E-N	0.03	57538	3524	5015	4806	5258	5.59251	0.00012	0.68474
E+N	0.03	70971	3877	5503	5287	5752	5.53118	0.00009	0.66941
B-N	0.03	84408	4447	6219	5993	6477	5.67909	0.00007	0.67608
B+N	0.03	33011	2448	3673	3484	3896	5.31548	0.00017	0.68121

Figure 10. Rarefaction curves showing the numbers of bacterial OTUs associated with ectomycorrhizal root tips of *Pinus sylvestris* plants growing in fertilised and unfertilised soil from different podzol horizons (O, E, B) from a boreal forest at Jädraås, Sweden. The soil was unfertilised (-N) or fertilised with urea as an N source (+N).

5.2.3 Bacterial community structure associated with root tips growing in different soil horizons

As in the case of fungi, the structure of bacterial communities differed significantly (P = 0.0001) between soil horizons, irrespective of short term N addition (data not shown). However NMDS ordinations of bacterial communities within each soil horizon and ANOSIM and NPMANOVA P values suggest that the effect of short term N addition on bacterial communities was only statistically significant in the B horizon (**Figure 11**).

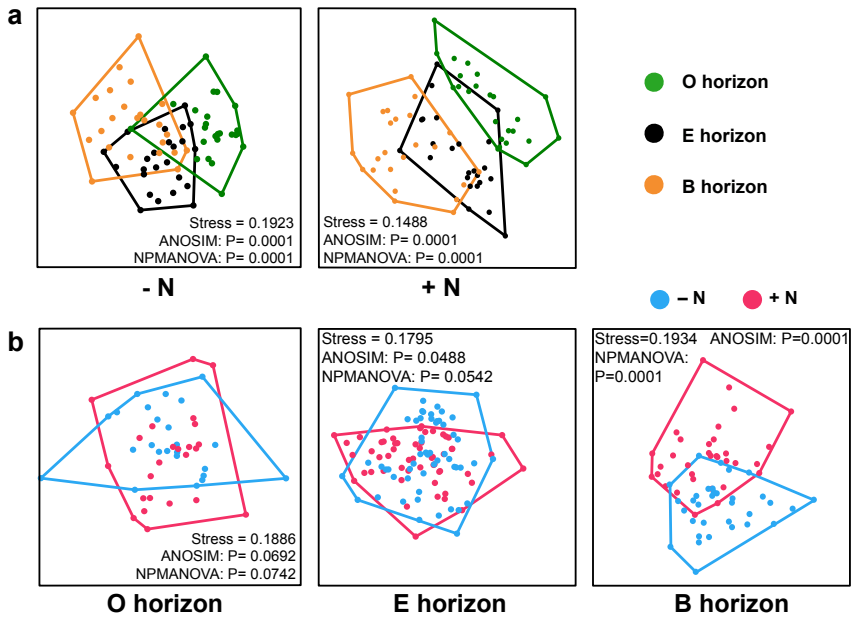


Figure 11. (a) Nonmetric MultiDimensional Scaling ordinations showing differences in community structure of bacteria associated with ectomycorrhizal root tips of *Pinus sylvestris* plants growing in soil from different podzol horizons (O, E, B) from a boreal forest at Jädraås, Sweden. The soil was unfertilised (-N) or fertilised with urea as an N source (+N), (b) differences in community structure of bacteria associated with ectomycorrhizal root tips of *P. sylvestris* plants growing in -N and +N treatments within each soil horizon.

NMDS ordinations of bacterial community structure associated with root tips growing in the E horizon and colonised by five different ectomycorrhizal fungi, *Suillus variegatus*, *Piloderma fallax*, *Piloderma sphaerosporum*, *Meliniomyces bicolor* and *Meliniomyces variabilis*, revealed that the bacterial communities were statistically distinct from each other, both in the presence and absence of N addition, although the statistical separation was much stronger in the control treatment without N addition (**Figure 12a**).

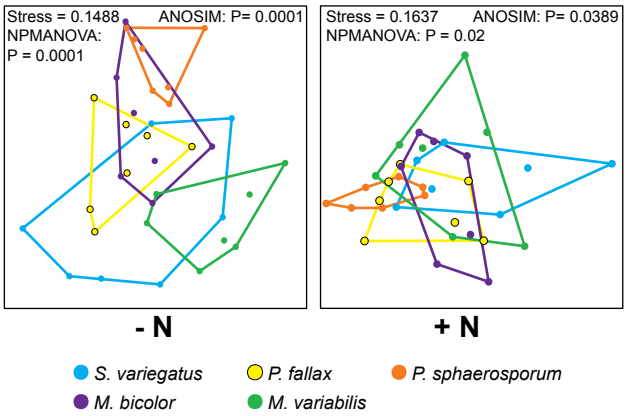


Figure 12. (a) Nonmetric MultiDimensional Scaling ordinations showing differences in community structure of bacteria associated with root tips of *Pinus sylvestris* plants colonised by five different ectomycorrhizal fungi and growing in the E horizon of a podzol from a boreal forest at Jädraås, Sweden. Patterns of bacterial community structure are shown for unfertilised (-N) soil as well as soil exposed to a short term N addition of urea (+N).

The mean bacterial abundance data for roots colonised by the individual ectomycorrhizal fungi are displayed in **Figure 12b.** Although the overall abundance of *Burkolderia* bacteria on roots of *P. sylvestris*, irrespective of ectomycorrhizal species, increased by 80% in response to N addition in the E horizon, the different bacterial genera showed variation in abundance that was strongly influenced by the individual ectomycorrhizal fungi colonising the roots. The average number of *Burkholderia* sequences associated with roots colonised by *Suillus variagatus decreased* by 63% in response to N addition but was *increased* in roots colonised by *P. fallax*, *P. sphaerosporum*, *M. bicolor* and *M. variabilis* by 51%, 2100%, 56% and 300% respectively. The abundance of bacteria in the genera *Flavisolibacter*, *Bradyrhizobium*, *Granulicella*, *Steroidobacter*, *Dyella* and *Acidobacter* Gp 1, associated with roots colonised by *P. sphaerosporum*, was strongly stimulated by N treatment. There was also some evidence of a similar but somewhat weaker effect on *Parcubacteria*, *Flavobacterium*, *Granulicella* and *Steroidobacter* in roots colonised by *P. fallax*. Conversely the abundance of bacteria in the genera *Flavisolibacter*, *Parcubacteria*, *Bradyrhizobium* and *Flavobacterium*, associated with roots colonised by *S. variegatus* appeared to be reduced by N addition. Bacteria in the genus *Sphingomonas* also appeared to be increased by N addition when associated with *M. variabilis*.

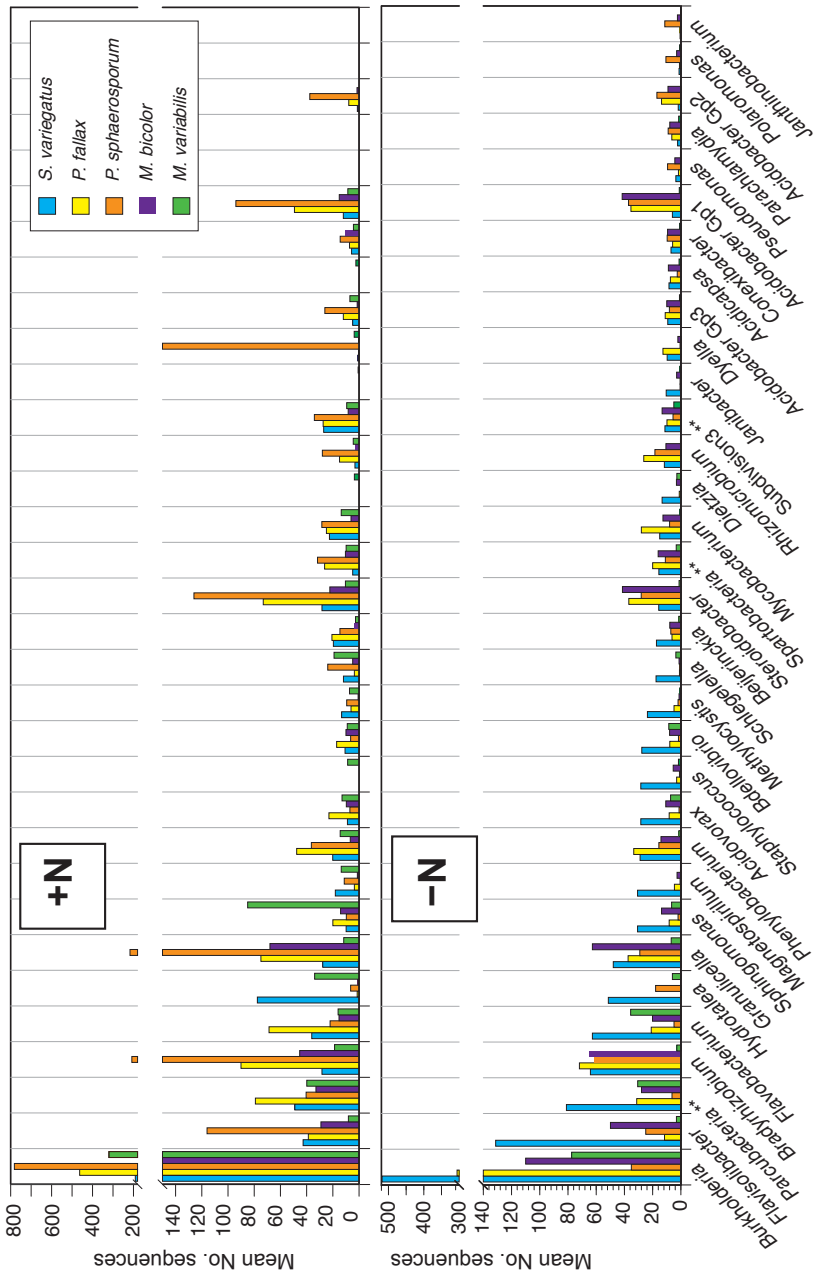


Figure 12b. (b) Mean abundance (no. sequences) of different bacterial genera associated with *Pinus sylvestris* root tips colonised by five different ectomycorrhizal fungi and growing in E horizon soil of a podzol from a boreal forest at Jädraås, Sweden. The bacterial genera shown are those contributing more than 1% dissimilarity in pairwise similarity percentage analysis (SIMPER) comparisons between different horizons. The soil was unfertilised (-N) or fertilised.

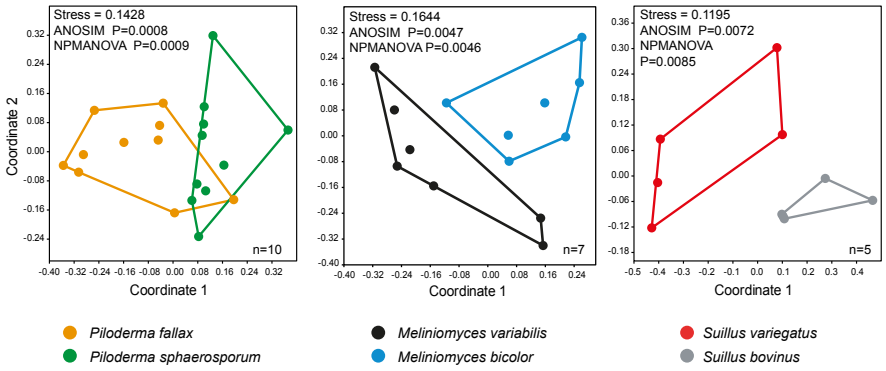


Figure 13. Non-metric multidimensional scaling ordinations of bacterial community structure associated with *Pinus sylvestris* roots colonized by three different pairs of ectmycorrhizal fungi growing in E horizon soil from a podzol at Jädraås, Sweden.

NMDS analysis of the structure of bacterial communities associated with roots colonised by three pairs of fungal species belonging to three different genera, *Suillus variegatus* and *S. bovinus, Piloderma fallax* and *P. sphaerosporum*, *and Meliniomyces bicolor and M. variabilis*, revealed statistically significant (P = 0.0008-0.007) differences between individual species within the same genus in the absence of N addition (**Figure 13**). *Suillus bovinus* colonised roots could not be found in E horizon soil following short tern N addition but the differences observed between bacterial communities associated with roots colonised by *P. fallax* & *P. sphaerosporum, and M. variabilis* & *M. bicolor* disappeared following addition of nitrogen (data not shown).

The bacterial microbiomes associated with most of the ectomycorrhizal fungi (*S. variegatus, P. fallax, M. variabilis* and *M. bicolor*) in the E horizon soil were not sensitive to N addition, however the bacteria associated with *P. sphaerosporum* harboured distinct communities depending upon whether the soil had been fertilised or not (**Figure 14**).

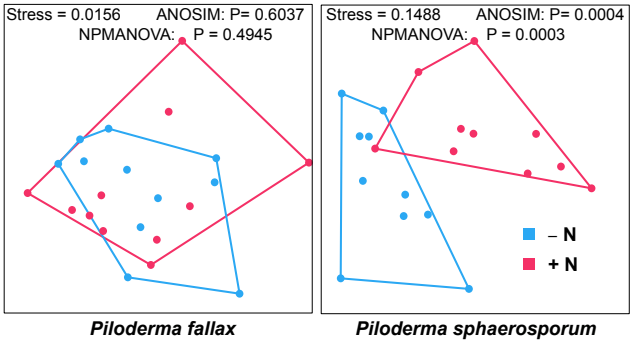


Figure 14. Nonmetric MultiDimensional Scaling ordinations showing the effects of short-term addition of N on community structure of bacteria associated with root tips of *Pinus sylvestris* plants colonised by the ectomycorrhizal fungi *Piloderma fallax* and *P. sphaerosporum*. Plants were grown in unfertilised E horizon soil (-N) from a boreal forest at Jädraås, Sweden or unfertilised E horizon soil subjected to a short-term N addition two weeks prior to harvesting (+N).

Suillus variegatus colonised roots in the O, E and B horizons, *P. fallax* colonised roots in the O and E horizons and *M. bicolor* colonised roots in the E and B horizons harboured statistically distinct bacterial communities irrespective of N treatment. *S. variegatus* associated bacterial microbiomes subjected to N addition in the O and E horizons were not significantly different when compared with the control treatment, but were significantly altered in the B horizon soil.

We identified a core microbiome consisting of about 94 genera that were commonly shared between the five different ectomycorrhizal fungi associated with root tips growing in E horizon in the absence of N addition (**Figure 15**). Approximately 14% of these genera were replaced following N addition. These included: *Enhydrobacter, Herminiimonas, Novosphingobium, Methylobacterium.* The bacterial genera uniquely associated with particular ectomycorrhizal fungi are 51 in *S. variegatus*, 18 genera in *P. fallax*, 32 genera in *P. sphaerosporum*, 26 in *M. variabilis* and 13 genera in *M. bicolor*. Between 83% to 100% of these genera were replaced following N addition, depending upon the ectomycorrhizal fungi.

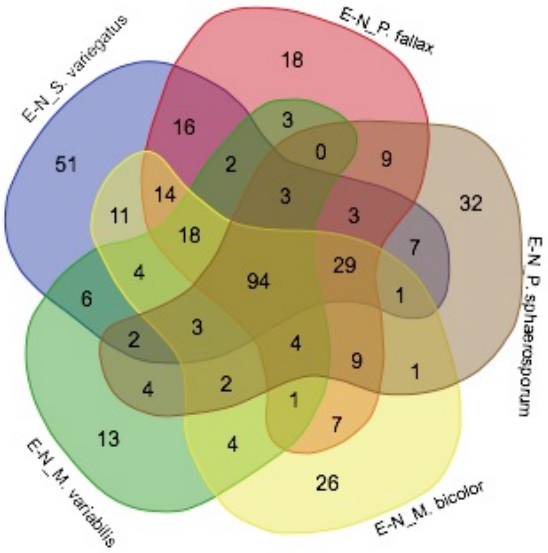


Figure 15. Five–way Venn diagram showing nunbers of bacterial genera uniquely colonising different ectomycorrhizal roots colonised by different combinations of ectomycorrhizal fungi.

5.3 Fungal and bacterial communities in soil and roots in a boreal forest podzol (Papers III and IV)

Symbiotic ectomycorrhizal fungi form diverse communities in boreal forests, mobilising nutrients from organic polymers with different degrees of recalcitrance and from mineral substrates that are weathered at different rates. These fungi may also sequester photosynthetically-derived carbon in the soil, but the different roles of individual taxa and their location within the soil profile are still poorly understood. The structure and activity of soil microbial communities are influenced by both atmospheric nitrogen deposition and applications of fertilizer but detailed knowledge of the community dynamics of these responses is still lacking. High throughput, massively parallel sequencing provides a new tool with which to investigate these effects. In the present study we investigated fungal and bacterial community structure in different horizons of a boreal forest at Lamborn, Sweden. In these two studies the bacteria associated with individual roots, colonised by different fungi, were not identified as in studies **I** and **II**, however the fungi and bacteria associated with pooled root samples from different soil horizons were identified from the same root samples.

5.4 Fungal communities in soil & roots in a boreal forest podzol (Paper III)

Overall differences in the structure and diversity of fungal communities colonising *Pinus sylvestris* root tips and adjacent rhizosphere soil were analysed using non-metric multidimensional scaling (NMDS) and rarefaction analysis (**Figure 16a,b**). The analyses show that there were significant differences in both structure and diversity of the communities found on roots and in adjacent soil. Diversity of fungi appeared to be higher in soil than on the ectomycorrhizal roots, presumably including additional saprotrophic species

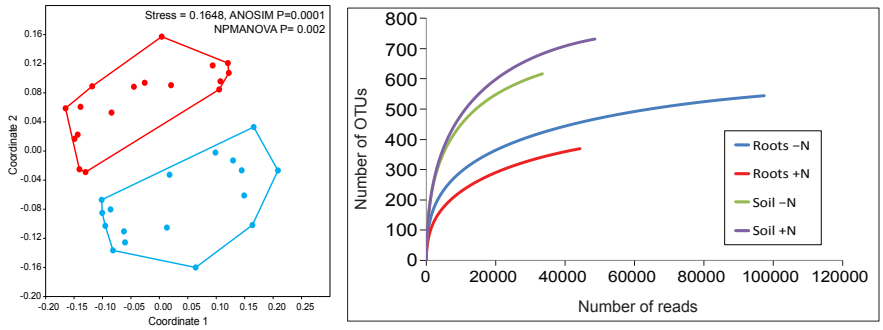


Figure 16. (a) Non-metric multidimensional scaling ordination of fungal community structure associated with *Pinus sylvestris* roots and adjacent soil in different horizons of a podzol at Lamborn, Sweden, (b) Rarefaction curves illustrating the total numbers of fungal OTUs associated with soil and *P. sylvestris* roots in a podzol at Lamborn, Sweden.

The composition of fungal communities was analysed in roots and adjacent rhizosphere soil in different podzol horizons using NMDS and ANOSIM significant values suggest that different soil horizons harbour different fungal communities **(Figure 17**). Differences in community structure between horizons are larger than those due to N fertilisation after 15 months.

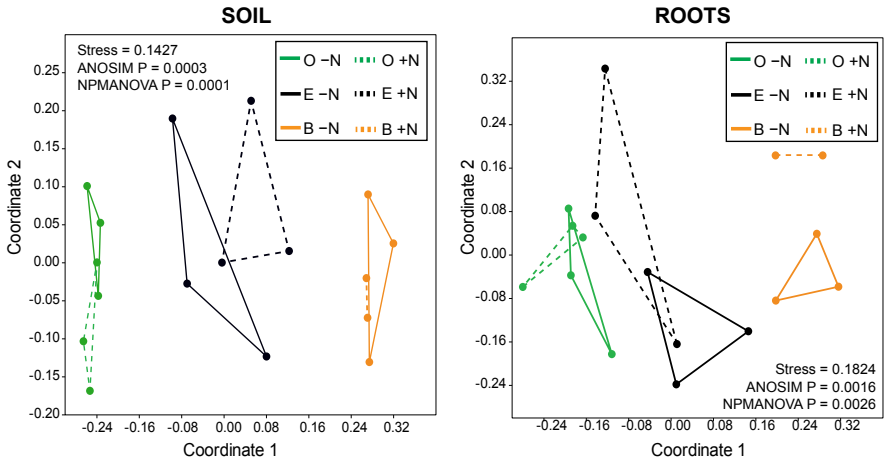
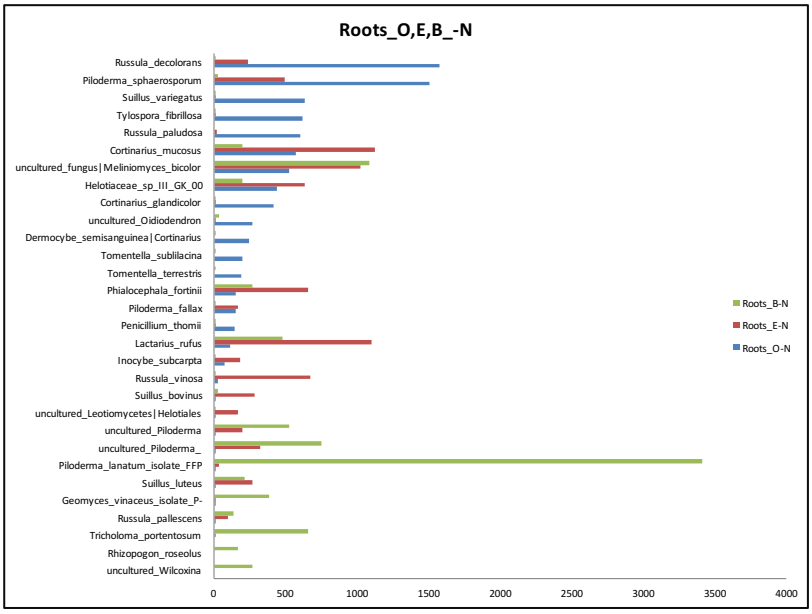


Figure 17. Non-metric multidimensional scaling ordinations of fungal community structure associated with soil and *Pinus sylvestris* ectomycorrhizal roots in different soil horizons (O, E, B) from a podzol at Lamborn, Sweden.

In roots from the O horizon *Russula decolorans* and *Piloderma sphaerosporum* were most abundant, accounting for 20% of the total fungal community. In total about 40 fungal OTUs occurred at an individual frequency above 1%, accounting for 58%, 59% and 74% of the total reads in the O, E and B horizons respectively. In roots *Russula decolorans*, *Suillus variegatus*, and *Piloderma sphaerosporum* occurred with high relative abundance in the O horizon, whereas *Meliniomyces bicolor* and *Russula paludosa* had higher relative abundance in the E horizon and *Piloderma lanatum*, *Tricholoma portentosum*, two unidentified *Piloderma* species, *Rhizopogon roseolus* and *R. bacillisporus* had higher relative abundance in the B horizon (**Figure 18a**).

However in soil (**Figure 18b**) *Russula decolorans, Piloderma sphaerosporum, Helotiales, Penicillium thomii, Cantherella_umbonata, Rhodotorula, Cystoderma amianthinum* and *Piloderma fallax* were more dominant in the O horizon. In the E horizon *Cortinarius*, uncultured *Piloderma*, *Lactarius rufus, Cortinarius vanduzerensis, Inocybe subcarpata,* and an uncultured fungus belonging to the *Saccharomycetales* were more dominant. In the B horizon *Meliniomyces bicolor, Tylospora,* an uncultured fungus belonging to the Agaricomycetales*, Oidiodendron chlamidosporicum, Piloderma lanatum, Umbelopsis dimorpha, Paratritiradium, Tricholoma portentosum,* an uncultured *Piloderma, Sagenomella,* and *Wilcoxina* were more dominant in the B horizon. There was no significant effect of nitrogen addition, either in the soil or the roots, but some taxa were reduced below detection limit. Individual comparisons and pairwise comparisons using NPMANOVA are presented in the manuscript.



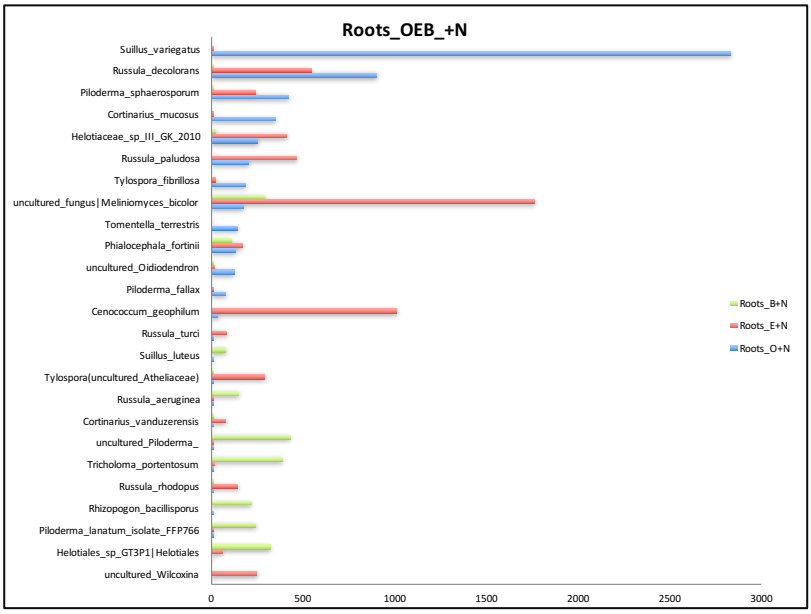
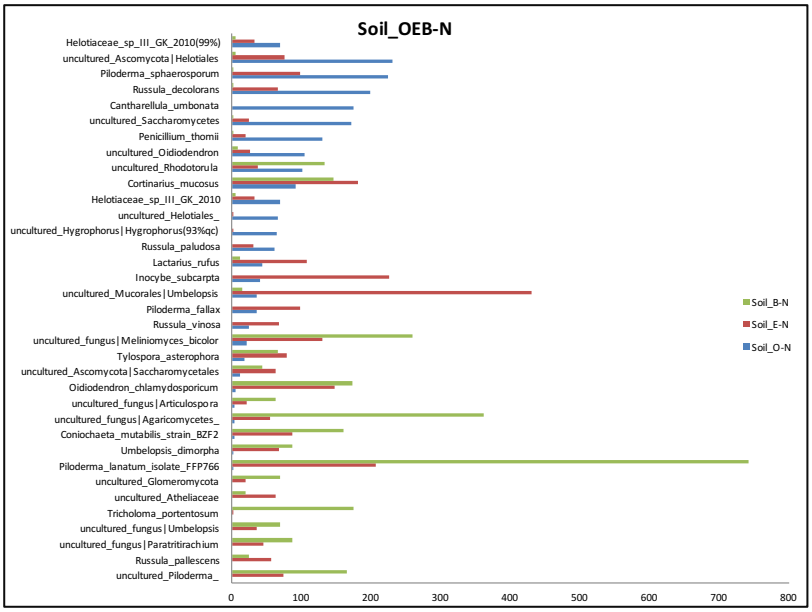


Figure 18a. Mean abundance (no. sequences) of different fungal taxa associated with *Pinus sylvestris* roots growing in different podzol horizons (O, E, B) in a boreal forest at Lamborn, Sweden. The soil was unfertilised (-N) or fertilised with 150 kg N ha-1 15 months prior to sampling (+N). The fungal taxa shown are those contributing more than 1% dissimilarity in pairwise SIMPER (similarity percentage analysis) comparisons between soil and roots.



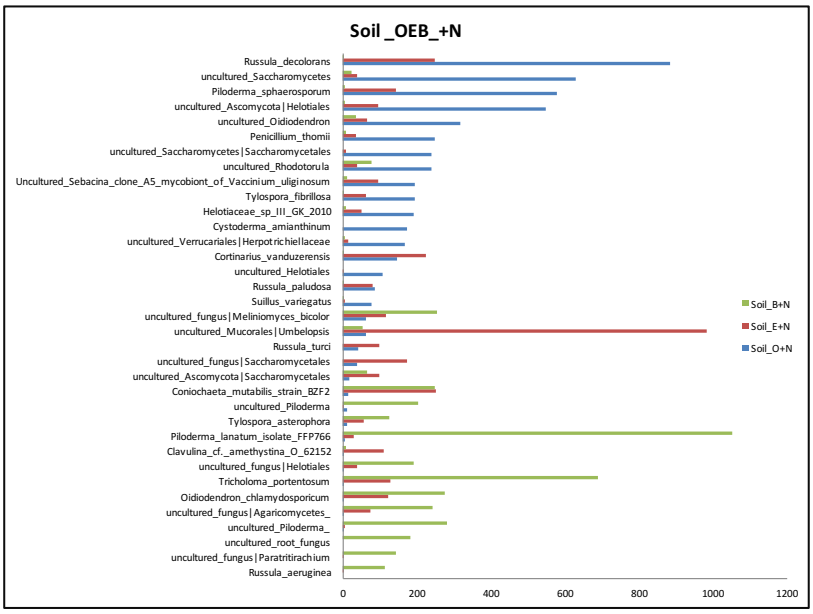


Figure 18b. Mean abundance (no. sequences) of different fungal taxa associated with soil from different podzol horizons (O, E, B) in a boreal forest at Lamborn, Sweden. The soil was unfertilised (-N) or fertilised with 150 kg N ha-1 15 months prior to sampling (+N). The fungal taxa shown are those contributing more than 1% dissimilarity in pairwise SIMPER (similarity percentage analysis) comparisons between soil and roots.

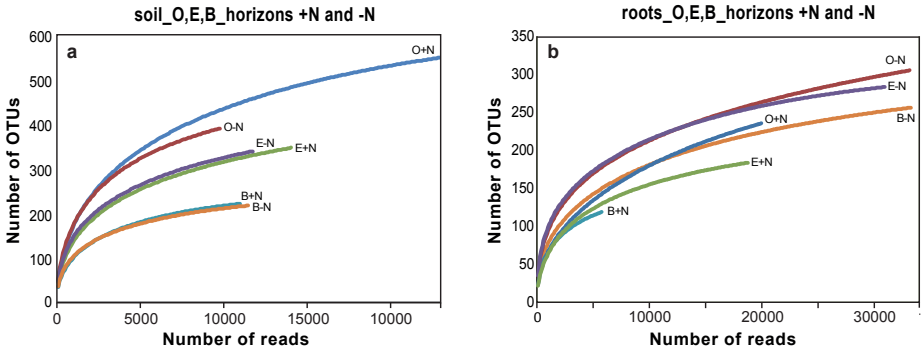


Figure19. Rarefaction curves illustrating the numbers of fungal OTUs associated with (a) soil and (b) *Pinus sylvestris* roots in different fertilised and unfertilised soil horizons from a boreal forest at Lamborn, Sweden.

Rarefaction analysis and the observed numbers of OTUs in the data set suggest that the soil has more fungal species than the roots. In total 807 OTUs in soil and 546 OTUs associated with roots were identified at the Lamborn field site (**Figure 19**). Deeper sequencing of both roots and soil from the same samples and NMDS analysis and significant ANOSIM values unsurprisingly suggested that significantly different fungal communities were colonising soil and mycorrhizal roots. On roots *Piloderma lanatum, Russula decolorans*, *Piloderma sphaerosporum, Meliniomyces bicolor, Cortinarius mucosa , Helotiales* spIII*, Lactarius rufus* and *Suillus variegatus* were more dominant than in the soil, whereas *Umbelopsis, Agaricomycetes, Saccharomycetes, Coniocheta mutabilis* and *Oidiodendron* were more dominant in the soil.
Rarefaction analysis (species richness), observed OTUs and Chao 1 diversity indices on roots shows that organic horizon harbours more species and more expected fungal diversity than the mineral horizons (O>E>B) and in the nitrogen addition treatment species richness and diversity is decreasing irrespective of the horizon and the effect is more pronounced in mineral horizons than in the O horizon (B>E>O). In soil the observed numbers of OTUs and species richness are similar to that for roots (O>E>B) with respect to horizons. Addition of nitrogen does not have significant effect in the mineral horizons but there is a significant effect in the O horizon. Chao 1 expected diversity indices suggest that when nitrogen was added there is significant increase in the O and B horizon communities and there is no significant effect in the E horizon communities.

5.5 Bacterial communities in soil & roots in a boreal forest podzol (Paper IV)

NMDS and rarefaction analyses of bacterial communities associated with soil and roots at Lamborn suggest that that these are statistically distinct, and that the bacterial diversity associated with soil is higher than that associated with roots – irrespective of N fertilisation (**Figure 20**).

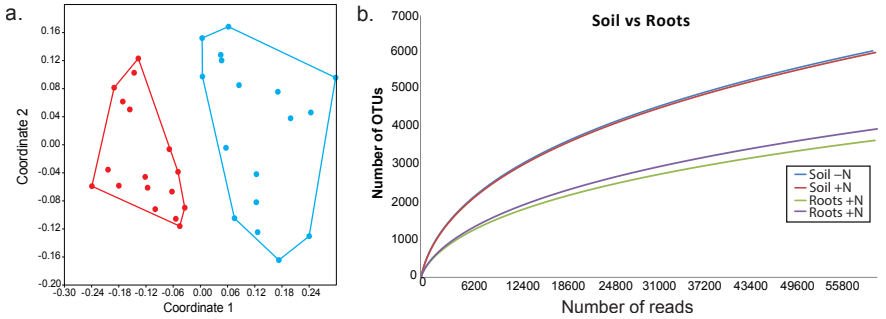


Figure 20. (a) Non-metric multidimensional scaling ordination of bacterial community structure associated with *Pinus sylvestris* roots and adjacent soil in different horizons of a podzol at Lamborn, Sweden. (b) Rarefaction curves illustrating the total numbers of bacterial OTUs associated with soil and *P. sylvestris* roots in a podzol at Lamborn, Sweden.

NMDS analysis of bacterial community structure in soil and on roots from different horizons suggest that differences between horizons are larger than those due to fertilisation (**Figure 21**).

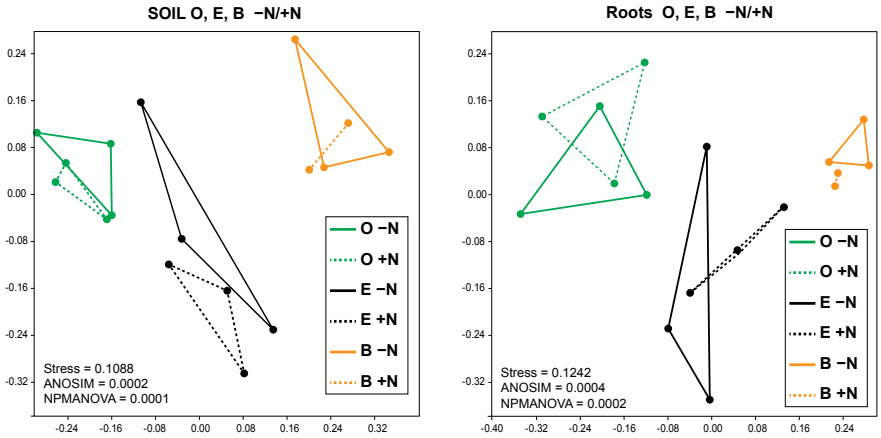


Figure 21. Non-metric multidimensional scaling ordinations of bacterial community structure associated with (a) rhizosphere soil and (b) *Pinus sylvestris* ectomycorrhizal roots in different soil horizons (O, E & B) from a podzol at Lamborn, Sweden.

Bacterial species richness and diversity was reduced in the N fertilised O horizon soil and the community composition also differed significantly from that in the unfertilised treatment, as found by Allison *et al.* (2007). Similar observations were made by Frey *et al.* (2014), they found a reduction in fungal biomass and activity following fertilisation compared to the unfertilised control plots. Jones *et al.* (2012) also reported that increased fertilisation resulted in the reduction of taxonomic richness on roots. Bacterial communities appear to be more sensitive to nitrogen addition than fungal communities (Krumins *et al.*, 2009). There was no short-term (2 w) effect of nitrogen additions on plant carbon allocation to soil microbiota but 60% reductions was observed after 1 y (Högberg *et al.*, 2010). Recently Blaško *et al.* (2013) have found a negative effect of nitrogen addition on both fungi and bacteria in soils but the authors used a PLFA based method for microbial community profiling that has been known to have poor taxonomic resolution. Fungal biomass (Mayor *et al.*, 2015) and ectomycorrzal species richness (Lilleskov *et al.*, 2002) declined dramatically after N inputs in two separate experiments. Soil enzyme activities and fungal community composition changed significantly following N fertilisation (Allison *et al.*, 2008).

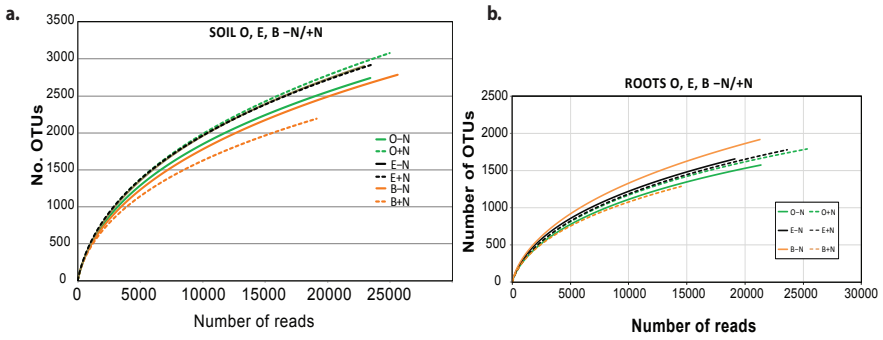


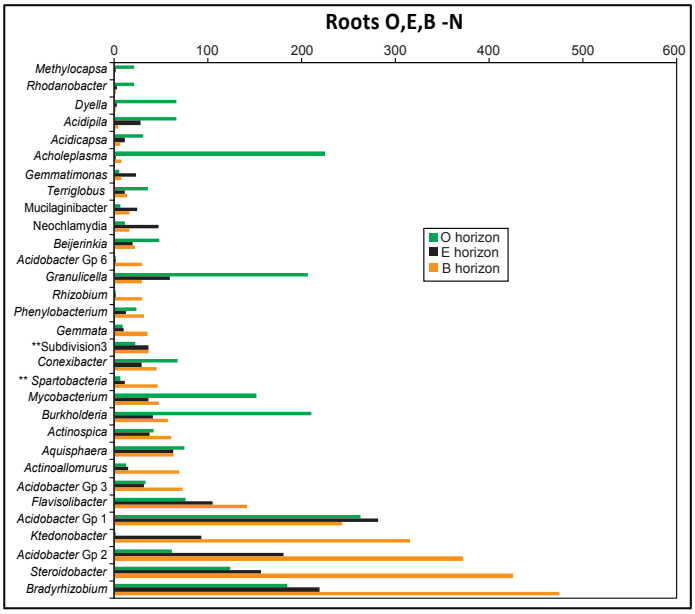
Figure 22. Rarefaction curves illustrating the numbers of bacterial OTUs associated with (a) soil and (b) *Pinus sylvestris* roots in different fertilised and unfertilised soil horizons from a boreal forest at Lamborn, Sweden.

Analysis of roots colonizing different soil horizons (**Figure 23a**) suggested that roots growing in O, E, and B-horizons harbour different bacterial communities. *Acidobacter Gp1, Aquisphaera were abundant in all the horizons. Acholeplasma*, *Granulicella*, *Mycobacterium, Burkhalderia, Dyella,* were more

abundant in the O horizon *Bradyrhizobium, Steroidobacter, Acidobacter GP2, Ktedonobacter* were more abundant in the B horizon. Addition of nitrogen does not have major differences but there were some differences in the mean abundance of the communities. *Bradyrhizobium ,Phenylobacterium*, *Rhodonobacter*, *Aquisphaera* increased in abundance in O-horizon roots but whereas *Acholeplasma, Acidobacterium* GP1 decreased in O horizon. *Actinospica*, *Conexibacter*, *Burkholderia*, *Mycobacterium* were increased in abundance in O, E horizons.but where as *Burkhalderia*, *Bradyrhizobium* and A*ctinoallomurus* were decreased in abudance in B-horizon. *Acidobacterium GP2 , Ktedonobacter* And *Steroidobacter* decreased in abundance in E,B horizon. *Dyella* increased in abundance in E horizon. Most of the above mentioned taxa on roots were previously reported to be intimately associated with Ectomycorrhizal root tips (Marupakula et al 2015, Nguyen and Bruns 2015, Izumi et al 2011).

Analysis of bacterial communities growing in different soil horizons (**Figure 23b**) shows that *Mycobacterium*, *Aquisphaera* were abundant in the O, E horizon and Acidobacterium Gp1, *Burkholderia*, *Phenylobacterium, Granulicella*, *Beijerinkia*, *Flavisolibacter* were more dominant in the O-horizon soil, Unidentifie*d spartobacteria* was more abundant in the E horizon. *Acidobacter Gp2*, *Bradyrhizobium* was abundant in all three horizons but is more dominant in the B horizon. *Acidobacterium* Gp6, *Gemmatimonas*, *Ktedonobacter* were more abundant in the B horizon. Addition of nitrogen does not have big differences but there were some differences like *Acidobacterium* Gp1, Gp2, *Gemmata* positivly affected in the mineral horizons and *Acidobacterium* Gp1 decreased abundance in the O horizon but no difference in *Acidobacterium* Gp2 in the O horizon. *Actinoallomurus* was increased in abundance O, E horizon, but decreased in abundance in the B-horizon (reduced to half). *Bradyrhizobiu*, *Steroidobacter* decreased abundance in O,E horizons and *Bradyrhizobium* increased in abundance in B horizon, *Mycobacterium, WPS 2*_genera_incertae_sedis increased in abundance in the O horizon but no big difference in the B horizon, *Ktedonobacter* reduced in abundance in the mineral horizons, *Aquisphaera* Increased in abundance in E horizon *Spartobacteria* increased in abundance in B horizon *Subdivision 3* Increased in abundance all horizons.

There was no significant overall effect of nitrogen addition on the bacterial community composition, and no large difference in the O and E horizons but in the B horizon an overall -ve effect was observed.



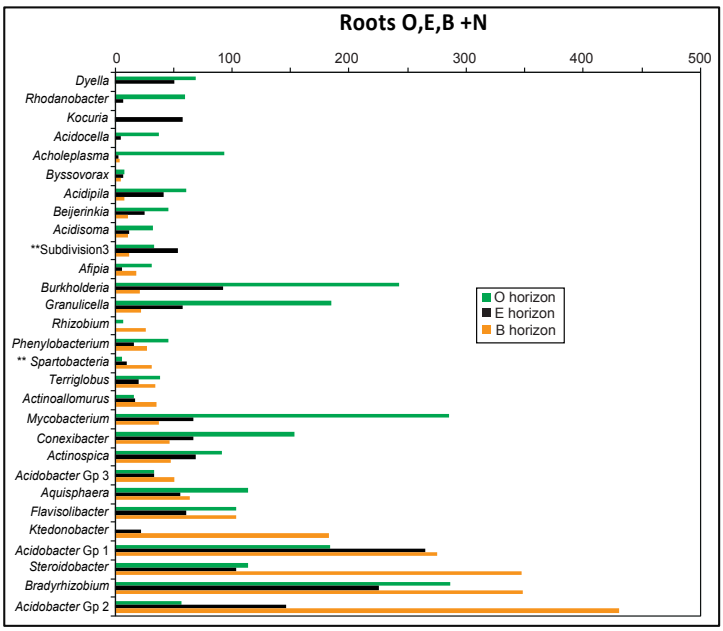
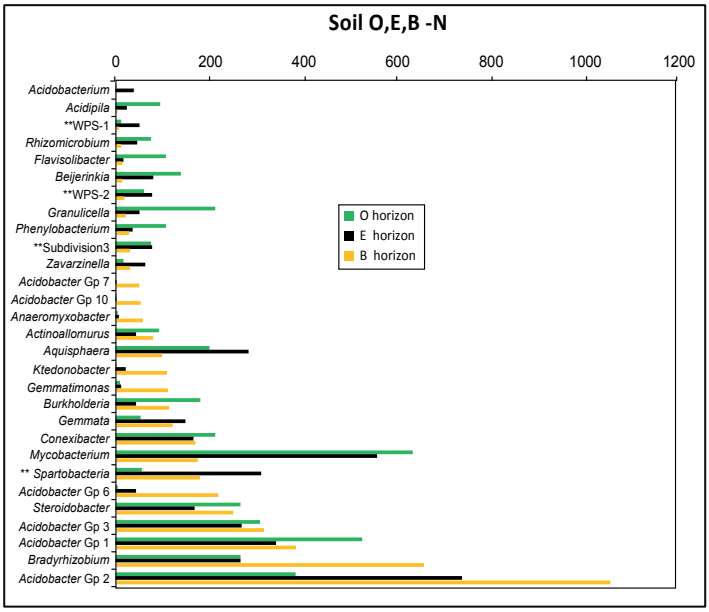


Figure 23a. Mean abundance (no. Sequences) of different bacterial genera associated with *Pinus sylvestris* roots growing in different podzol horizons (O, E, B) in a boreal forest at Lamborn, Sweden. The soil was unfertilised (-N) or fertilised with 150 kg N ha-1 15 months prior to sampling (+N). The bacterial genera shown are those contributing more than 1% dissimilarity in pairwise SIMPER (similarity percentage analysis) comparisons between soil and roots



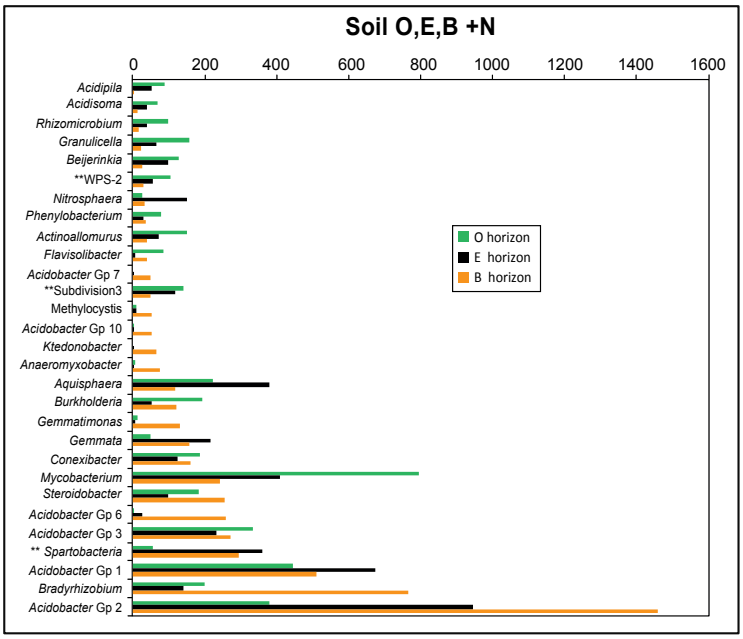


Figure 23b. Mean abundance (no. sequences) of different bacterial genera associated with soil from different podzol horizons (O, E, B) in a boreal forest at Lamborn, Sweden. The soil was unfertilised (-N) or fertilised with 150 kg N ha-1 15 months prior to sampling (+N). The bacterial genera shown are those contributing more than 1% dissimilarity in pairwise SIMPER (similarity percentage analysis) comparisons between soil and roots.

6 Conclusions and future perspectives

6.1 Conclusions

The main conclusions of the studies described in my thesis are as follows:

1. Analysis of single root tip bacterial microbiomes established that there was significant temporal variation in the structure of bacterial communities associated with ectomycorrhizal roots of *Pinus sylvestris* between 0 and 16 weeks, however the diversity of bacteria declined and significant differences were no longer observed at week 24.
2. Root tips colonised by different ectomycorrhizal fungi (*Russula* sp. 6 GJ-2013b*, Piloderma* spp., *Meliniomyces variabilis* and *Paxillus involutus*) harboured statistically distinct bacterial communities.
3. A core bacterial microbiome associated with ectomycorrhizal root tips was identified, consisting of 19 genera that were persistent, irrespective of temporal variation or the dominant fungi colonising root tips. Bacterial genera that exhibited specificity for any particular ectomycorrhizal fungus or were confined to a particular stage of root tip age, were also identified.
4. The results of study II extend those of study I and showed that the fungal and bacterial communities associated with root tips growing in the different soil horizons (O, E or B) were statistically different, regardless of N addition. Though the fungal communities were significantly affected by a short-term N addition in all horizons, the bacterial community composition was significantly affected only in the B horizon. *Suillus variegatus* was stimulated by N addition in the O and E horizons, whereas it decreased in abundance in the B horizon, possibly due to the increased abundance of *Rhizopogon bacillisporus*.

5. Following a short term N addition, bacterial species richness and diversity significantly increased in O horizon, decreased in B horizon and did not change in E horizon root tips colonised by ectomycorrhizal fungi.
6. Roots colonised by *Suillus variegatus* from O, E or B soil horizons also harboured distinct bacterial communities. Furthermore roots colonised by different fungal species within the same genera (*Suillus bovinus*/*S. variegatus, Piloderma fallax*/*P. sphaerosporum* and *Meliniomyces bicolor*/*M. variabilis*) also harboured significantly distinct bacterial communities. The intra-generic differences disappeared following the addition of N.
7. Bacterial communities associated with individual ectomycorrhizal fungi responded differently to N addition in different soil horizons. In the O and E horizons, bacterial communities associated with *P. fallax* and *S. variegatus* colonised roots were not affected by N additions. Similarly, N addition had no effect on bacterial communities associated with *Meliniomyces bicolor* or *M. variabilis* in the E horizon. However bacterial communities associated with *Piloderma sphaerosporum* colonised roots in the E horizon and *Suillus variegatus* colonised roots in the B horizon were both affected by N addition.
8. The field experiment described in study III showed that the fungal communities colonising roots and in the adjacent soil were statistically distinct, irrespective of the soil horizon. This suggests that simple screening of fungal communities in soil samples may not adequately reflect the true community composition of symbiotic fungi colonising tree root systems. As expected the diversity of fungi sampled from soil was higher than that sampled from roots since it also included saprotrophs colonising woody debris but not roots.
9. The fungal communities associated with soil from different horizons were statistically different from each other, as were the communities associated with the mycorrhizal roots growing within those soil horizons.
10. The effect of N fertilisation on fungal community structure within the individual soil horizons, both in soil and associated with roots, was not statistically significant, however there were significant effects on fungal diversity. Fungal species diversity, both in soil and associated with roots, was highest in the O horizon, decreasing successively with depth in the E and B horizons (O>E>B). Fungal species richness and diversity on roots was negatively affected by the N fertilisation in all horizons. The negative effect of fertilization on fungal diversity in soil was significant in the O horizon but not in the E and B horizons.

11. As with the fungal results in paper III, the bacterial communities associated with roots and with adjacent soil were statistically distinct in structure both from an overall point of view, as well as within individual soil horizons.
12. Bacterial communities associated with roots in each horizon were statistically distinct from each other, as were the communities associated with the adjacent soil.
13. No significant effect of the N fertilisation could be detected on bacterial community structure on roots or in soil. Bacterial diversity associated with roots was highest in the unfertilised B horizon and lowest in the N fertilised B horizon

6.2 General Conclusions

Root tips are the fundamental units of forest trees where nutrient exchange takes place between ectomycorrhizal fungi and plant roots and the different ectomycorrhizal fungi and bacteria colonising different root tips interact in different ways with the roots. This is thus an important niche in which to understand the microbiome structure and how it responds to environmental perturbations and ultimately affects the plant host. Previously many studies tried to answer this question and produced conflicting conclusions since most of the studies were done using pooled root tip material characterised using low resolution identification methods, in some cases with lack of sufficient replication, and often with high levels of environmental variation. We have tried to overcome the above problems by growing trees in microcosms providing a controlled environment and by analysing single root tips colonised by the dominant ectomycorrhizal fungi using high throughput sequencing. We have shown that different ectomycorrhizal fungal genera select distinct bacterial communities not only in the mor soil layer but also in different mineral horizons of a boreal podzol. We were also able to show that even root tips colonised by closely related ectomycorrhizal species of same genus select distinct bacterial microbiomes. This system allowed us to investigate the effects of short term nitrogen addition on microbe-microbe interactions and we found that ectomycorrhizal selection of bacteria is weakened during nitrogen addition removing the significant differences between species of the same genus.

Complementary field experiments involving N fertilisation enabled us to examine the effects of N on fungi present on roots or in soil in the O, E and B horizons of a boreal forest podzol. Effects of N fertilisation on diversity and community structure of fungi in soil were generally weaker than those on fungi colonising the roots. This could be explained by re-allocation of plant

assimilates away from roots towards the tree canopy during fertilisation. Fungal responses to N differed between fungi and were often context-specific, depending upon the soil horizon. N increased fungal diversity slightly in the O horizon soil but decreased it in the roots, particularly in the B horizon. This maybe due to reallocation of plant assimilates away from the roots during fertilisation. Fungal community structure varied significantly between horizons and within each soil horizon the community structure of fungi colonising ectomycorrhizal roots was significantly different from that associated with the soil, and responded differently to N fertilisation, suggesting that analyses of both soil and roots are necessary for accurate monitoring of environmental perturbations. Most of the time ectomycorrhizal fungal richness was decreased and soil fungi (saprotrophs) were increased. Such clear differences were not observed in the bacteria. The reason for this might be their vast diversity and the inability of the sequencing method to sequence the whole 16S sequence or be due to functional redundancy found in the bacterial genera. Bacterial species richness and diversity indices suggest that the bacterial diversity and species richness increases on the roots with depth and whereas fungi on the roots decrease with depth. Bacterial species richness is more in the E horizons then O and B-horizon in soil. Nitrogen addition on roots had a significant negative effect on the bacterial species in B horizon and no effect in the E horizon and significant positive effect in the O horizon and in the soil we also find the same patterns whereas fungi in soil behaved differently to those colonising roots. In roots there is a significant decrease in all the horizons whereas in soil only those in the O horizon were positively affected with no difference in the E and B horizons. Taking community composition and diversity indices together we can suggest that B horizon communities are drastically affected by nitrogen fertilization and looking at only soil will not be enough to understand the ecosystem process completely. One should look both soil and roots and bacteria and fungi together at the same site. Further experiments using transcriptomics and proteomics, combined with stable isotope probing using ^{13}C and ^{15}N will provide a better understanding of the functional consequences of these changes in community structure and diversity.

6.3 Future perspectives:

In the experiments described in this thesis we have characterised the bacterial microbiomes associated with *P. sylvestris* roots colonised by different ectomycorrhizal fungi, as well as the temporal variation in community structure. Variation in the structure and diversity of fungal and bacterial communities in different soil horizons and in different roots growing in these

horizons, as well as the effects of short-term and longer-term N fertilisation, have also been investigated using high throughput pyrosequencing.

The studies provide broad support for the idea that distinct communities of bacteria may exist in association with roots colonised by different fungi, and that the structure of these communities may vary with time. The use of single-root tip microbiomes, sampling from microcosms at defined time-points and high throughput sequencing of both fungi and bacteria helped to improve taxonomic resolution, minimise environmental variation and avoids the problems arising from pooled root material that is only morphologically characterised. The experiments provide new information about the distribution of fungi and bacteria between different organic and mineral horizons in boreal forest podzols and their responses to short and longer-term additions of N. Significant vertical stratification exists in fungal and bacterial community structure and different fungi show different responses to N addition. Responses to N addition are highly context dependent and influenced both by fungal identity and location within the soil profile. Improved information about the functional mechanisms underlying the observed differences needs to be obtained using complementary experimental approaches. Functional aspects of the interactions between different fungi and bacteria can be identified using a range of methods, including stable isotope probing (SIP), transcriptomic and proteomic profiling. Improved spatial resolution of sampling needs to be considered including sampling of bacteria associated with rhizomorphs and young, less well differentiated hyphae at the mycelial front of different ectomycorrhizal mycelia. Stable isotope probing of mycelial interactions with different substrates (and associated bacteria) will reveal more about the functional interactions involved in colonisation of organic substrates with different degrees of recalcitrance and different mineral substrates subject to biogenic weathering. Such studies are currently in progress in our laboratory (**Figures 24 & 25**).

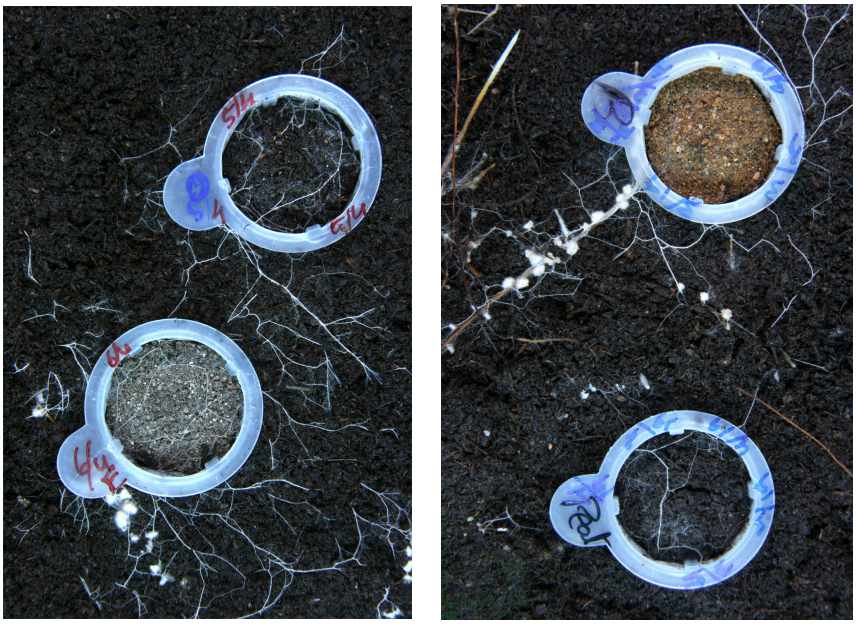


Figure 19. Close-up of laboratory microcosms allowing different ectomycorrhizal fungi to colonise patches containing different mineral and organic substrates.

These experiments will provide more information on spatial patterns of C allocation in different substrates/horizons and different mycorrhizal symbionts. Ultimately, identification of the different C compounds and enzymes produced by different mycorrhizal hyphae (and associated bacteria) will be necessary to understand their functional interactions with different substrates.

Recently much progress has been made in understanding the influence of different microorganisms and their interactions on patterns of C sequestration and nutrient release. Further progress will be made with new metatranscriptomic and phylogenomic studies allowing better understanding of mycelial homeostasis and responses to different types of disturbance in different environments. This will include continued identification of the major metabolic activities occurring in different podzol horizons (mRNA transcription profile and protein expression profile in different horizons).

Figure 20. Laboratory microcosm for ^{13}C-RNA stable isotope probing (SIP) studies. $^{13}CO_2$ is fed to the central plant and patterns of carbon allocation to different fungi and bacteria colonising different substrates are followed by ^{13}C-RNA SIP and 454 pyrosequencing.

6.4 List of illustrations

type are shown. The overlap of the genera constituting these cores and their distribution between different ectomycorrhizal types is shown in a second Venn diagram. The bacterial genera colonising at all time points and all mycorrhizal types are shown on the left. The unique bacterial assemblages consistently colonising at all time points, but exclusively for individual mycorrhizal types, are also shown.

Figure 9. (a) Nonmetric MultiDimensional Scaling ordinations showing differences in community structure of fungi colonising *Pinus sylvestris* roots growing in soil from different podzol horizons (O, E, B) from a boreal forest at Jädraås, Sweden. The soil was unfertilised (-N) or fertilised with urea as an N source (+N), **(b)** differences in community structure of fungi colonising roots between -N and +N treatments within each soil horizon, **(c)** pie charts showing relative abundance on different fungal taxa in the system described above.

Figure 10. Rarefaction curves showing the numbers of bacterial OTUs associated with ectomycorrhizal root tips of *Pinus sylvestris* plants growing in fertilised and unfertilised soil from different podzol horizons (O, E, B) from a boreal forest at Jädraås, Sweden. The soil was unfertilised (-N) or fertilised with urea as an N source (+N).

Figure 11. (a) Nonmetric MultiDimensional Scaling ordinations showing differences in community structure of bacteria associated with ectomycorrhizal root tips of *Pinus sylvestris* plants growing in soil from different podzol horizons (O, E, B) from a boreal forest at Jädraås, Sweden. The soil was unfertilised (-N) or fertilised with urea as an N source (+N), **(b)** differences in community structure of bacteria associated with ectomycorrhizal root tips of *P. sylvestris* plants growing in -N and +N treatments within each soil horizon.

Figure 12. (a) Nonmetric MultiDimensional Scaling ordinations showing differences in community structure of bacteria associated with root tips of *Pinus sylvestris* plants colonised by five different ectomycorrhizal fungi and growing in the E horizon of a podzol from a boreal forest at Jädraås, Sweden. Patterns of bacterial community structure are shown for unfertilised (-N) soil as well as soil exposed to a short term N addition of urea (+N).

Fig 12. (b) Mean abundance (no. sequences) of different bacterial genera associated with *Pinus sylvestris* root tips colonised by five different ectomycorrhizal fungi and growing in E horizon soil of a podzol from a boreal forest at Jädraås, Sweden. The bacterial genera shown are those contributing more than 1% dissimilarity in pairwise similarity percentage analysis (SIMPER) comparisons between different horizons. The soil was unfertilised (-N) or fertilised with urea as an N source (+N).

Figure 13. Non-metric multidimensional scaling ordinations of bacterial community structure associated with *Pinus sylvestris* roots colonized by three different pairs of ectmycorrhizal fungi growing in E horizon soil from a podzol at Jädraås, Sweden.

Figure 14. Nonmetric MultiDimensional Scaling ordinations showing the effects of short-term addition of N on community structure of bacteria associated with root tips of *Pinus sylvestris* plants colonised by the ectomycorrhizal fungi *Piloderma fallax* and *P. sphaerosporum*. Plants were grown in unfertilised E horizon soil (-N) from a boreal forest at Jädraås, Sweden or unfertilised E horizon soil subjected to a short-term N addition two weeks prior to harvesting (+N).

Figure 15. Five-way Venn diagram showing numbers of bacterial genera uniquely associated with different ectomycorrhizal roots colonised by different combinations of ectomycorrhizal fungi and growing in unfertilised E horizon soil (-N) from a boreal forest at Jädraås, Sweden.

Figure 16. (a) Non-metric multidimensional scaling ordination of bacterial community structure associated with *Pinus sylvestris* roots and adjacent soil in different horizons of a podzol at Lamborn, Sweden, **(b)** Rarefaction curves illustrating the total numbers of bacterial OTUs associated with soil and *P. sylvestris* roots in a podzol at Lamborn, Sweden.

Figure 17. Non-metric multidimensional scaling ordinations of bacterial community structure associated with a) soil and b) *Pinus sylvestris* ectomycorrhizal roots in different soil horizons (O, E, B) from a podzol at Lamborn, Sweden.

Figure 18. (a) Mean abundance (no. sequences) of different fungal taxa associated with *Pinus sylvestris* roots growing in different podzol horizons (O, E, B) in a boreal forest at Lamborn, Sweden. The soil was unfertilised (-N) or fertilised with 150 kg N ha^{-1} 15 months prior to sampling (+N). The fungal taxa shown are those contributing more than 1% dissimilarity in pairwise SIMPER (similarity percentage analysis) comparisons between soil and roots.

Figure 18. (b) Mean abundance (no. sequences) of different fungal taxa associated with soil from different podzol horizons (O, E, B) in a boreal forest at Lamborn, Sweden. The soil was unfertilised (-N) or fertilised with 150 kg N ha^{-1} 15 months prior to sampling (+N). The fungal taxa shown are those contributing more than 1% dissimilarity in pairwise SIMPER (similarity percentage analysis) comparisons between soil and roots.

Figure 19. Rarefaction curves illustrating the numbers of fungal OTUs associated with **(a)** soil and **(b)** *Pinus sylvestris* roots in different fertilised and unfertilised soil horizons from a boreal forest at Lamborn, Sweden.

Figure 20. (a) Non-metric multidimensional scaling ordination of bacterial community structure associated with *Pinus sylvestris* roots and adjacent soil in different horizons of a podzol at Lamborn, Sweden, **(b)** Rarefaction curves

References

Aerts, R. (1995). The advantages of being evergreen. *Trends in Ecology and Evolution* 10, 402–407.

Ahonen-Jonnarth, U., Göransson, A. & Finlay, R.D. (2000). Production of organic acids by mycorrhizal and non-mycorrhizal *Pinus sylvestris* L. seedlings exposed to elevated concentrations of aluminium and heavy metals. *New Phytologist* 146, 557–567.

Ahonen-Jonnarth, U., Van Hees, P.A.W., Lundström, U.S. & Finlay, R.D. (2003). Growth and nutrient uptake of ectomycorrhizal *Pinus sylvestris* seedlings in a natural substrate treated with elevated Al concentrations. *Tree Physiology 23*, 157-167.

Akselsson, C., Westling, O., Sverdrup, H., Holmqvist, J., Thelin, G. & Uggla, E. (2007). Impact of harvest intensity on long-term base cation budgets in Swedish forest soils. *Water Air & Soil Pollution: Focus* 7, 201–210.

Allison, S.D., Czimczik, C.I. & Treseder, K.K. (2008). Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. *Global Change Biology* 14, 1156-1168.

Allison, S.D., Hanson, C.A. & Treseder, K.K. (2007). Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. *Soil Biology & Biochemistry* 39, 1878-1887.

Altschul, S.F., Gish, W., Miller, W., Myers, W. & Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology* 215, 403-410.

Antony-Babu, S., Deveau, A., Van Nostrand, J.D., Zhou, J., Le Tacon, F., Robin C., Frey-Klett, P. & Uroz, S. (2014). Black truffle-associated bacterial communities during the development and maturation of *Tuber melanosporum* ascocarps and putative functional roles. *Environmental Microbiology* 16, 2831–2847.

Aspray, T.J., Jones, E. E., Davies, M. W., Shipman, M. & Bending, G.D. (2013). Increased hyphal branching and growth of the ectomycorrhizal fungus *Lactarius rufus* by the helper bacterium *Paenibacillus* sp. *Mycorrhiza* 23, 403-410.

Averill, C., Turner, B.L. & Finzi, A.C. (2014). Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature* 505, 543-545.

Avis, P.G., McLaughlin, D.J., Dentinger, B.C. & Reich, P.B. (2003). Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of Russula spp. in a temperate oak savanna. *New Phytologist* 160, 239-253.

Bahr, A., Ellstrom, M., Akselsson, C., Ekblad, A., Mikusinska, A. & Wallander, H. (2013). Growth of ectomycorrhizal fungal mycelium along a Norway spruce forest nitrogen deposition gradient and its effect on nitrogen leakage. *Soil Biology & Biochemistry* 59, 38-48

Baldrian, P., Kolaiřík, M., Štursová, M., Kopecký, J., Valášková, V., Větrovský, T., Žifčáková, L., Šnajdr, J., Rídl, J., Vlček, Č. & Voříšková, J. (2012). Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME Journal* 6, 248-258.

Bates, S.T., Cropsey, G.W.G., Caporaso, J.G., Knight, R. & Fierer, N. (2011). Bacterial communities associated with the lichen symbiosis. *Applied &Environmental Microbiology* 77, 1309-1314.

Berch, S.M., Brockley, R.P., Battigelli, J.P., Hagerman, S. & Holl, B. (2006). Impacts of repeated fertilization on components of the soil biota under a young lodgepole pine stand in the interior of British Columbia. *Canadian Journal of Forest Research* 36, 1415-1426.

Bergh, J., Linder, S., Lundmark, T. & Elfving, B. (1999). The effect of water and nutrient availability on the productivity of Norway spruce in northern and southern Sweden. *Forest Ecology &Management* 119, 51-62

Bertaux, J., Schmid, M., Prevost-Boure, N.C., Churin, J.L., Hartmann, A., Garbaye, J. & Frey-Klett, P. (2003). *In situ* identification of intracellular bacteria related to *Paenibacillus* spp. in the mycelium of the ectomycorrhizal fungus *Laccaria bicolor* S238N. *Applied & Environmental Microbiology* 69, 4243-4248.

Bertaux, J., Schmid, M., Hutzler, P., Hartmann, A., Garbaye, J. & Frey-Klett P. (2005). Occurrence and distribution of endobacteria in the plant-associated mycelium of the ectomycorrhizal fungus *Laccaria bicolor* S238N. *Environmental Microbiology* **7**: 1786-1795.

Blaško, R., Högberg, P., Bach, L.H. & Högberg, M.N. (2013). Relations among soil microbial community composition, nitrogen turnover, and tree growth in N-loaded and previously N-loaded boreal spruce forest. *Forest Ecology & Management* 302, 319-328.

de Boer, W., Folman, L.B., Summerbell, R.C. & Boddy, L. (2005) Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews* **29:** 795–811.

Boersma, F.G.H., Warmink, J.A., Andreote, F.A. & van Elsas, J.D. (2009). Selection of Sphingomonadaceae at the base of *Laccaria proxima* and *Russula exalbicans* fruiting bodies. *Applied & Environmental Microbiology* 75, 1979–1989.

Bonfante, P. & Anca, I.A. (2009). Plants, mycorrhizal fungi and bacteria: a network of interactions. *Annual Review of Microbiolology* **63**, 363-383.

Bradford, M.A. (2014). Good dirt with good friends. *Nature* 505, 486-487.

Bradshaw, C.J.A. & Warkentin, I.G. (2015). Global estimates of boreal forest carbon stocks and flux. *Global & Planetary Change* 128, 24–30.

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren Van Themaat, E., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F.O., Amann, R., Eickhorst, T. & Schulze-Lefert, P. (2012). Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* 487, 91-95.

Bulgarelli D., Schlaeppi K., Spaepen S., Ver Loren van Themaat E., & Schulze-Lefert P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology*. 64,807–838.

Burke, D.J., Dunham, S.M. & Kretzer, A.M. (2008). Molecular analysis of bacterial communities associated with the roots of Douglas fir (*Pseudotsuga menziesii*) colonized by different ectomycorrhizal fungi. *FEMS Microbiology Ecology* 65, 299–309.

Burton, P.J., Bergeron, Y., Bogdanski, B.E.C., Juday, G.P., Kuuluvainen, T., McAfee, B.J., Ogden, A., Teplyakov, V.K., Alfaro, R.I., Francis, D.A., Gauthier, S. & Hantula, J. 2010. Sustainability of boreal forests and forestry in a changing environment. In Forests and society responding to global drivers of change. Edited by G. Mery., P. Katila, G., Galloway, R.I., Alfaro, M., Kanninen, M., Lobovikov & J. Varjo. International Union of Forest Research Organizations, Vienna, Austria, pp. 249–282.

Calvaruso, C., Turpault, M. P., Leclerc, E. & Frey-Klett, P. (2007). Impact of ectomycorrhizosphere on the functional diversity of soil bacterial and fungal communities from a forest stand in relation to nutrient mobilization processes. *Microbial Ecology* 54, 567-577.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N. & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences* 108 4516-4522.

Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay R.D., Wardle, D.A. & Lindahl, B.D. (2013). Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science* 339, 1615-1618.

Clemmensen, K.E., Finlay, R.D., Dahlberg, A., Stenlid, J., Wardle, D.A. & Lindahl, B.D. (2015). Functional shifts in mycorrhizal fungal communities regulate carbon sequestration in boreal forests. *New Phytologist* 205, 1525-1536.

Coince, A., Caël, O., Bach, C., Lengellé, J., Cruaud, C., Gavory, F., Morin, E., Murat, C., Marçais, B. & Buée, M. (2013). Below-ground fine-scale distribution and soil versus fine root detection of fungal and soil oomycete communities in a French beech forest. *Fungal Ecology* 6, 223-235.

Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A., Kuske, C.R. & Tiedje, J.M. (2014). Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Research* **42** (D1), D633-D642.

Committee, I. (2014). Importance and relationship of boreal forests to climate. Retrieved from *http://www.eoearth.org/view/article/153787*

Cox, F., Barsoum, N., Lilleskov, E.A., & Bidartondo, M.I. (2010). Nitrogen availability is a primary determinant of conifer mycorrhizas across complex environmental gradients. *Ecology Letters* 13, 1103-1113

Demoling, F., Nilsson, L.O., & Baath, E. (2008). Bacterial and fungal response to nitrogen fertilization in three coniferous forest soils. *Soil Biology & Biochemistry* 40, 370-379.

Deveau, A., Palin, B., Delaruelle, C., Peter, M., Kohler, A., Pierrat, J. C., Sarniguet, A., Garbaye, J., Martin, F., & Frey-Klett, P. (2007). The mycorrhiza helper *Pseudomonas fluorescens* BBc6R8 has a specific priming effect on the growth, morphology and gene expression of the ectomycorrhizal fungus *Laccaria bicolor* S238N. *New Phytologist* 175, 743–755.

Dickie, I.A., Xu, B. & Koide, R.T. (2002). Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. *New Phytologist* 156, 527-535.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C. & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**, 2194-2200.

Eilers, K.G., Debenport, S., Anderson, S., & Fierer, N. (2012). Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. *Soil Biology &Biochemistry* 50, 58-65.

Ekblad, A., Wallander, H., Godbold, D.L., Cruz, C., Johnson, D., Baldrian, P., Björk, R.G., Epron, D., Kieliszewska-Rokicka, R., Kjøller, R., Kraigher, H., Matzner, E., Neumann, J. & Plassard, C. (2013). The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling. *Plant & Soil* 366**,** 1–27.

Esseen, P. A., Ehnström, B., Ericson, L. & Sjöberg, K. (1997). Boreal Ecosystems and Landscapes: Structures, Processes and Conservation of Biodiversity. *Ecological Bulletins* 46, 16-47.

Estrada-de Los Santos, P., Bustillos-Cristales, R. & Caballero-Mellado, J. (2001). *Burkholderia*, a genus rich in plant-associated nitrogen fixers with wide environmental and geographic distribution. *Applied & Environmental Microbiology* 67, 2790–2798.

Feng, X.J., Simpson, A.J., Schlesinger, W.H., & Simpson, M.J. (2010). Altered microbial community structure and organic matter composition under elevated CO_2 and N fertilization in the duke forest. *Global Change Biology* 16, 2104-2116.

Finlay, R.D. (2008). Ecological aspects of mycorrhizal symbiosis with special emphasis on the functional diversity of interactions involving the extraradical mycelium. *Journal of Experimental Botany* 59, 1115-1126.

Finlay, R., Wallander, H., Smits, M., Holmström, S., van Hees, P., Lian, B. & Rosling, A. (2009). The role of fungi in biogenic weathering in boreal forest soils. *Fungal Biology Reviews* 23, 101-106.

Fransson, PMA., Taylor, AFS. & Finlay, RD. (2000). Effects of continuous optimal fertilisation on belowground ectomycorrhizal community structure in a Norway spruce forest. *Tree Physiology* 20, 599-606.

Fransson, P.A. & Rosling, A. (2014). Fungal and bacterial community responses to *Suillus variegtus* extraradical mycelia and soil profile in Scots pine microcosms. *Plant &Soil* 385, 255-272.

Frey-Klett, P., Chavatte, M., Clausse, M.L., Courrier, S., Le Roux , C., Raaijmakers, J., Martinotti, M.G., Pierrat, J.C. & Garbaye, J. (2005). Ectomycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent pseudomonads. *New Phytologist* 165, 317-28.

Frey-Klett, J., Garbaye, J. & Tarkka, M. (2007). The mycorrhiza helper bacteria revisited. *New Phytologist* 176, 22-36.

Frey, S.D., Ollinger, S., Nadelhoffer, K., Bowden, R., Brzostek, E., Burton, A., Caldwell, B. A., Crow, S., Goodale, C. L., Grandy, A. S., Finzi, A., Kramer, M.G., Lajtha, K., LeMoine, J., Martin, M., McDowell, W. H., Minocha, R. l., Sadowsky, J. J., Templer, P. H. & Wickings, K. (2014). Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests. *Biogeochemistry* 121, 305-316.

Gadgil, R.L. & Gadgil, P.D. (1971). Mycorrhiza and litter decomposition. *Nature* 233, 133.

Gadgil, R.L. & Gadgil, P.D. (1975). Suppression of litter decomposition by mycorrhizal roots of *Pinus radiata*. *New Zealand Journal of Forestry Science* 5, 35–41.

Garbaye J. (1994). Helper bacteria: a new dimension to the mycorrhizal symbiosis. *New Phytologist* 128, 197-210.

Gardes, M. & Bruns, T.D. (1993). ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2, 113-118.

Gazzè, S.A., Saccone, L., Vala Ragnarsdottir, K., Smits, M.M., Duran, A.L., Leake, J.R., Banwart, S.A. & McMaster, T.J. (2012). Nanoscale channels on ectomycorrhizal colonized chlorite: Evidence for plant-driven fungal dissolution. *Journal of Geophysical Research: Biogeosciences* 117, G00N09, 1-8, doi:10.1029/2012JG002016

Genney, D.R., Anderson, I.C. & Alexander, I.J. (2006). Fine-scale distribution of pine ectomycorrhizas and their extramatrical mycelium. *New Phytologist* 170, 381-390.

Godbold, D.L., Hoosbeek, M.R., Lukac, M., Cotrufo, M.F., Janssens, I.A., Ceulemans, R., Polle, A., Velthorst, E.J., Scarascia-Mugnozza, G., DeAngelis, P., Miglietta, F., Peressotti, A. (2006). Mycorrhizal hyphal turnover as a dominant process for carbon input into soil organic matter. *Plant & Soil* 281, 15–24

Hartmann, M., Lee, S., Hallam, S.J. & Mohn, W.W. (2009). Bacterial, archaeal and eukaryal community structures throughout soil horizons of harvested and naturally disturbed forest stands. *Environmental Microbiology* 11, 3045-3062.

Hasselquist, N.J. & Hogberg, P. (2014). Dosage and duration effects of nitrogen additions on ectomycorrhizal sporocarp production and functioning: an example from two N-limited boreal forests. *Ecology & Evolution* 4, 3015-3026.

Hawksworth, D.L. (1991). The Fungal Dimension of Biodiversity - Magnitude, Significance, and Conservation. *Mycological Research* 95, 641-655.

Heimann, M. & Reichstein, M. (2008). Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* 451, 289-292.

Heinemeyer, A., Hartley, I.P., Evans, S.P., De La Fuente, JaC. & Ineson, P. (2007). Forest soil CO_2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. *Global Change Biology* 13, 1786e1797..

Heinonsalo, J. & Sen, R. (2007). Scots pine ectomycorrhizal fungal inoculum potential and dynamics in podzol-specific humus, eluvial and illuvial horizons one and four growth seasons after forest clear-cut logging. *Canadian Journal of Forest Research.* 37, 404-414.

Heinonsalo, J., Jørgensen, K.S. & Sen, R. (2001). Microcosm-based analyses of Scots pine seedling growth, ectomycorrhizal fungal community structure and bacterial carbon utilization profiles in boreal forest humus and underlying illuvial mineral horizons. *FEMS Microbiology Ecology* 36, 73-84.

Högberg, M.N., Briones, M.J.I., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., Thornton, B., Hurry, V., Linder, S., Näsholm, T. & Högberg, P. (2010). Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. *New Phytologist* 187, 485-493.

Högberg, P., Johannisson, C., Yarwood, S., Callesen, I., Nasholm, T., Myrold, D.D., & Hogberg, M.N. (2011). Recovery of ectomycorrhiza after 'nitrogen saturation' of a conifer forest. *New Phytologist* 189, 515-525.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Hogberg, M.N. (2001). Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* 411, 789-792.

Hogberg, M.N., Yarwood, S.A. & Myrold, D.D. (2014). Fungal but not bacterial soil communities recover after termination of decadal nitrogen additions to boreal forest. *Soil Biology & Biochemistry* 72, 35-43.

Ihrmark, K., Bödeker, I., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E. & Lindahl, B.D. (2012). New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology* 82, 666–677.

Izumi, H. & Finlay, R.D. (2011). Selective association of bacterial and ascomycete communities with ectomycorrhizas in a Swedish subarctic birch forest. *Environmental Microbiology* 13, 819-830.

Izumi, H., Anderson, I.C., Alexander, I.J., Killham, K., & Moore, E.R. (2006). Endobacteria in some ectomycorrhiza of Scots pine (*Pinus sylvestris*). *FEMS Microbiology Ecology* 56, 34-43.

Izumi, H., Cairney, J.W.G., Killham, K., Moore, E.R.B., Alexander, I.J. & Anderson, I.C. (2007a). Bacteria associated with ectomycorrhizas of slash pine (*Pinus elliottii*) in south-eastern Queensland Australia. *FEMS Microbiology Letters* 282, 196-204.

Izumi, H., Cairney, J.W., Killham, K., Moore, E., Alexander, I.J. & Anderson, I. C. (2008). Bacteria associated with ectomycorrhizas of slash pine (*Pinus elliottii*) in south-eastern Queensland, Australia. *FEMS Microbiology Letters* 282, 196–204.

Izumi, H., Elfstrand, M. & Fransson, P. (2013). *Suillus* mycelia under elevated atmospheric CO_2 support increased bacterial communities and scarce nifH gene activity in contrast to *Hebeloma* mycelia. *Mycorrhiza* 23, 155–165.

Izumi, H., Moore, E.R.B., Killham, K., Alexander, I.J. & Anderson, I.C. (2007b). Characterisation of endobacterial communities in ectomycorrhizas by DNA- and RNA-based molecular methods. *Soil Biology & Biochemistry* 39, 891–899.

Jacobson, S. & Pettersson, F. (2010). An assessment of different fertilization regimes in three boreal coniferous stands. *Silva Fennica* 44, 815-827.

Johansson, E., Fransson, P.M.A., Finlay, R.D. & van Hees, P.A.W. (2008a). Quantitative analysis of exudates from soil living basidiomycetes in pure culture as a response to metal (Pb, Cd and As) stress. *Soil Biology & Biochemistry* 40, 2225– 2236.

Johansson, E., Fransson, P.M.A., Finlay, R.D. & van Hees, P.A.W. (2008b). Quantitative analysis of root and ectomycorrhizal exudates as a response to heavy metal (Pb, Cd and As) stress. *Plant & Soil* 313, 39–54.

Johansson, E., Fransson, P.M.A., Finlay, R.D., and van Hees, P.A.W. (2009). Quantitative analysis of soluble exudates produced by ectomycorrhizal roots as a response to ambient and elevated CO_2. *Soil Biology & Biochemistry* 41, 1111–1116.

Jones, M.D., Phillips, L.A., Treu, R., Ward, V. & Berch, S.M. (2012). Functional responses of ectomycorrhizal fungal communities to long-term fertilization of lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. latifolia Engelm.) stands in central British Columbia. *Applied Soil Ecology* 60, 29-40

Jones, D.L., Nguyen, C. & Finlay, R.D. (2009). Carbon flow in the rhizosphere: carbon trading at the soil-root interface. *Plant & Soil* 321, 5-33.

Jongmans, A.G., van Breemen, N., Lundström, U., Finlay, R.D, van Hees, P.A.W., Giesler, R., Melkerud, P.A., Olsson, M., Srinivasan, M. & Unestam, T. (1997). Rock-eating fungi: a true case of mineral plant nutrition? *Nature* 389, 682–683.

Kaiser, C., Fuchslueger, L., Koranda, M., Gorfer, M., Stange, C.F., Kitzler, B., Rasche, F., Strauss, J., Sessitsch, A., Zechmeister-Boltenstern, S. & Richter, A. (2011). Plants control the seasonal dynamics of microbial N cycling in a beech forest soil by belowground C allocation. *Ecology* 92, 1036-1051.

Kataoka, R., Taniguchi, T., Ooshima, H. & Futai, K. (2008). Comparison of bacterial communities established on the mycorrhizae formed on *Pinus thunbergii* root tips by eight species of fungi. *Plant & Soil* 304, 267–275.

Keller, S., Schneider, K. & Sussmuth, R.D. (2006). Structure elucidation of auxofuran, a metabolite involved in stimulating growth of fly agaric, produced by the mycorrhiza helper bacterium Streptomyces AcH 505. *The Journal of Antibiotics* 59, 801-803.

Kjøller, R., Nilsson, L.O., Hansen, K., Schmidt, I.K., Vesterdal, L. & Gundersen, P. (2012). Dramatic changes in ectomycorrhizal community composition, root tip abundance and mycelial production along a stand-scale nitrogen deposition gradient. *New Phytologist* 194, 278-286.

Klaminder, J., Lucas, R.W., Futter, M.N., Bishop, K.H., Köhler, S.J., Egnell, G. & Laudon, H. (2011). Silicate mineral weathering rate estimates: Are they precise enough to be useful when predicting the recovery of nutrient pools after harvesting? *Forest Ecology & Management* 261, 1-9.

Koyama, A., Wallenstein, M.D., Simpson, R.T. & Moore, J.C. (2014). Soil bacterial community composition altered by increased nutrient availability in Arctic tundra soils. *Frontiers in Microbiology* 5.

Krumins, J.A., Dighton, J., Gray, D., Franklin, R. B., Morin, P.J. & Roberts, M.S. (2009). Soil microbial community response to nitrogen enrichment in two scrub oak forests. *Forest Ecology & Management* 258, 1383-1390.

Labbé, J.L., Weston, D.J., Dunkirk, N., Pelletier, D.A., & Tuskan, G.A. (2014). Newly identified helper bacteria stimulate ectomycorrhizal formation in *Populus*. *Frontiers in Plant Science* 5, 579.

Landeweert, R., Hofflund, E., Finlay, R.D. & van Breemen, N. (2001). Linking plants to rocks: Ectomycorrhizal fungi mobilize nutrients from minerals. *Trends in Ecology & Evolution* 16, 248–254.

LeBauer, D.S., & Treseder, K.K. (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89, 371-379.

Lepleux, C., Turpault, M.P., Oger, P., Frey-Klett, P. & Uroz, S. (2012). Correlation of the abundance of betaproteobacteria on mineral surfaces with mineral weathering in forest soils. *Applied and Environmental Microbiology* 78: 7114–7119.

Lilleskov, E.A., Fahey, T.J., Horton, T. R. & Lovett, G.M. (2002). Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. *Ecology* 83, 104-115.

Lilleskov, E.A., Hobbie, E.A., & Horton, T.R. (2011). Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. *Fungal Ecology* 4, 174-183.

Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Högberg, P., Stenlid, J. & Finlay, R.D. (2007). Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytologist* 173, 611-620.

Lindahl, B., Taylor, A.F.S. & Finlay, R.D. (2002). Defining nutritional constraints on carbon cycling – towards a less "phytocentric" perspective. *Plant & Soil* 242, 123-135.

Lindahl, B.D., de Boer, W. & Finlay, R.D. (2010). Disruption of root carbon transport into forest humus stimulates fungal opportunists at the expense of mycorrhizal fungi. *The ISME Journal* 4, 872-881.

Lindahl, B.D., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R., Koljalg, U., Pennanen, T., Rosendahl, S., Stenlid, J. & Kauserud, H. (2013). Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. *New Phytologist* 199, 288–299.

Lopez-Mondejar, R., Voriskova, J., Vetrovsky, T., & Baldrian, P. (2015). The bacterial community inhabiting temperate deciduous forests is vertically stratified and undergoes seasonal dynamics. *Soil Biology & Biochemistry* 87, 43-50.

Lundström, U.S., van Breemen, N. & Bain, D. (2000). The podzolization process. A review. *Geoderma* 94, 91-107.

Marupakula, S., Mahmood, S. & Finlay, R.D. (2015). Analysis of single root-tip microbiomes suggests that distinctive bacterial communities are selected by *Pinus sylvestris* roots colonised by different ectomycorrhizal fungi. *Environmental Microbiology* (In Press)**.** *Environmental Microbiology* doi: 10.1111/1462-2920.13102

Mayor, J.R., Mack, M.C. & Schuur, E.A.G. (2015). Decoupled stoichiometric, isotopic, and fungal responses of an ectomycorrhizal black spruce forest to nitrogen and phosphorus additions. *Soil Biology & Biochemistry* 88, 247-256.

McGuire, K.L., Allison, S.D., Fierer, N. & Treseder, K.K. (2013). Ectomycorrhizal-Dominated Boreal and Tropical Forests Have Distinct Fungal Communities, but Analogous Spatial Patterns across Soil Horizons. *PLoS ONE* 8,68278

Nallanchakravarthula, S., Mahmood, S., Alström, S. & Finlay, R.D. (2014). Influence of soil type, cultivar and *Verticillium dahliae* on the structure of the root and rhizosphere fungal microbiome of strawberry. *PLoS ONE* 9,111455.

Nawrocki, E.P. & Eddy, S.R. (2013). Infernal 1.1: 100-fold faster RNA homology searches. *Bioinformatics* 29, 2933-3935.

Nazir, R., Warmink, J.A., Boersma, H. & van Elsas, J.D. (2010). Mechanisms that promote bacterial fitness in fungal-affected soil microhabitats. *FEMS Microbiology Ecology* 21, 169-185.

Nazir, R., Zhang, M., de Boer W. & van Elsas, J. D. (2012). The capacity to comigrate with *Lyophyllum* sp. strain Karsten through different soils is spread among several phylogenetic groups within the genus *Burkholderia. Soil Biology & Biochemistry* **50**: 221-233.

Nazir, R., Tazetdinova, D.I. & van Elsas, J.D. (2014). *Burkholderia terrae* BS001 migrates proficiently with diverse fungal hosts through soil and provides protection from antifungal agents. *Frontiers in Microbiology* **5**: Article 598.

Nguyen, N.H. & Bruns, T.D. (2015). The microbiome of *Pinus muricata* ectomycorrhizae: community assemblages, fungal species effects, and *Burkholderia* as important bacteria in multi partnered symbioses. *Microbial Ecology* 69, 914-921.

Nilsson, M.C. & Wardle, D.A. (2005). Understory vegetation as a forest ecosystem driver: evidence from the northern Swedish boreal forest. *Frontiers in Ecology & the Environment* 3, 421-428.

Nilsson, L.O. & Wallander, H. (2003). Production of external mycelium by ectomycorrhizal fungi in a Norway spruce forest was reduced in response to nitrogen fertilization. *New Phytologist* 158, 409-416.

O'Brien, H.E., Parrent, J.L., Jackson, J.A., Moncalvo, J.M. & Vilgalys, R. (2005). Fungal community analysis by large-scale sequencing of environmental samples. *Applied & Environmental Microbiology* 71, 5544-5550.

Ostonen, I., Helmisaari, H.S., Borken, W., Tedersoo, L., Kukumagi, M. & Bahram, M. Lindroos, A.J., Nöjd, P., Uri, V., Merilä, P., Asi, E. & Lõhmus, K. (2011) Fine root foraging strategies in Norway spruce forests across a European climate gradient. *Global Change Biology* **17**: 3620-3632.

Pan, Y.D., Birdsey, R.A., Fang, J.Y., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S.W., McGuire, AD., Piao, S., Rautiainen, A., Sitch, S. Hayes, D. (2011). A large and persistent carbon sink in the world's forests. *Science* 333, 988–993.

Parrent, J.L. & Vilgalys, R. (2007). Biomass and compositional responses of ectomycorrhizal fungal hyphae to elevated CO2 and nitrogen fertilization. *New Phytologist* 176, 164-174.

Paul, L. R., Chapman, B. K. & Chanway, C. P. (2007). Nitrogen fixation associated with *Suillus tomentosus* tuberculate ectomycorrhizae on *Pinus contorta* var. *latifolia. Annals of botany* **99**: 1101–1109.

Perez-Moreno, J. & Read, D.J. (2000). Mobilization and transfer of nutrients from litter to tree seedlings via the vegetative mycelium of ectomycorrhizal plants. *New Phytol* **145**: 301– 309.

Persson T. (ed.) (1980). Structure and function of northern coniferous forests an ecosystem study. *Ecological Bulletins* 32. Stockholm, Sweden

Quirk, J., Beerling, D.J., Banwart, S.A., Kakonyi, G., Romero-Gonzalez, M.E. & Leake J.R. (2012). Evolution of trees and mycorrhizal fungi intensifies silicate mineral weathering. *Biology Letters* 8, 1006–1011.

Ramette, A. (2007). Multivariate analyses in microbial ecology. *FEMS Microbiology Ecology* 62, 142-160.

Read, D.J., Leake, J.R. & Perez-Moreno, J. (2004). Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Canadian Journal of Botany* 82, 1243-1263

Read, D.J. & Perez-Moreno, J. (2003). Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist* 157, 475-492.

Riedlinger., J., Schrey., SD., Tarkka., MT., Hampp., R., Kapur., M., Fiedler., H-P., (2006). Auxofuran, a novel metabolite that stimulates the growth of fly agaric, is produced by the

mycorrhiza helper bacterium *Streptomyces* strain AcH 505. *Applied & Environmental Microbiology* 72, 3550-3557.

Rosling, A., Landeweert, R., Lindahl, B.D., Larsson, KH., Kuyper, T.W.,Taylor, A.F.S., & Finlay, R.D. (2003). Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. *New Phytologist.* 159, 775-783.

Rosling, A., Lindahl, B.D., & Finlay, R.D. (2004). Carbon allocation to ectomycorrhizal roots and mycelium colonising different mineral substrates. *New Phytologist* 162, 795-802

Saccone, L., Gazzè, S., Duran, A., Leake, J., Banwart, S., Ragnarsdóttir, K., Smits, M. & McMaster, T. (2011). High resolution characterization of ectomycorrhizal fungal-mineral interactions in axenic microcosm experiments. *Biogeochemistry* 111, 411–425.

Sarand, I., Haario, H., Jørgensen, K.S. & Romantschuk, M. (2000). Effect of inoculation of a TOL plasmid containing mycorrhizosphere bacterium on development of Scots pine seedlings, their mycorrhizosphere and the microbial flora in m-toluate-amended soil. *FEMS Microbiology Ecology* 31, 127-141.

Sarand, I., Timonen, S., Koivula, T., Peltola, R., Haahtela, K., Sen, R. & Romantschuk, M. (1999). Tolerance and biodegradation of *m*-toluate by Scots pine, a mycorrhizal fungus and fluorescent pseudomonads individually and under associative conditions. *Journal of Applied Microbiology* 86: 817-826.

Sarand, I., Timonen, S., Nurmiaho Lassil, E.L., Koivula, T., Haahtela, K., Romantschuk, M. & Sen, R. (1998). Microbial biofilms and catabolic plasmid harbouring degradative fluorescent pseudomonads in Scots pine mycorrhizospheres developed on petroleum contaminated soil. *FEMS Microbiology Ecology* 27: 115-126.

Schrey, S. D., Erkenbrack, E., Früh, E., Fengler, S., Hommel, K., Horlacher, N., Schulz, D., Ecke, M., Kulik, A., Fiedler, H.P., Hampp, R., & Tarkka,M. T., (2012). Production of fungal and bacterial growth modulating secondary metabolites is widespread among mycorrhiza-associated streptomycetes. *BMC Microbiology* 12: 164.

Shade, A. & Handelsman, J. (2012). Beyond the Venn diagram: the hunt for a core microbiome. *Environmental Microbiology* 14, 4–12

Shahin, O., Martin St Paul, N., Rambal, S., Joffre, R. & Richard, F. (2013). Ectomycorrhizal fungal diversity in Quercus ilex Mediterranean woodlands: variation among sites and over soil depth profiles in hyphal exploration types, species richness and community composition. *Symbiosis* 61,1–12

Smits, M.M., Bonneville, S., Benning, L.G., Banwart, S.A., & Leake, J.R. (2012). Plant-driven weathering of apatite - the role of an ectomycorrhizal fungus. *Geobiology* 10, 445-456.

Steenhoudt, O. & Vanderleyden, J. (2000). Azospirillum, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. *FEMS Microbiology Reviews* 24, 487–506.

Sterkenburg, E., Bahr, A., Brandström-Durling, M., Clemmensen, K.E. & Lindahl, B.D. (2015). Changes in fungal communities along a boreal forest soil fertility gradient. *New Phytologist* 207, 1145-1158**.**

Sun, Y.P., Unestam, T., Lucas, S.D., Johanson, K.J., Kenne, L. & Finlay, R.D. (1999). Exudation-reabsorption in mycorrhizal fungi, the dynamic interface for interaction with soil and other microorganisms. *Mycorrhiza* 9, 137-144.

Taylor, L.L., Leake, J.R., Quirk, J., Hardy, K., Banwart, S.A. & Beerling, D.J. (2009). Biological weathering and the long-term carbon cycle: integrating mycorrhizal evolution and function into the current paradigm, *Geobiology* 7, 171–191.

Taylor, L.L., Banwart, S.A., Valdes, P.J., Leake, J.R. & Beerling, D.J. (2012). Evaluating the effects of terrestrial ecosystems, climate and carbon dioxide on weathering over geological time: a global-scale process-based approach. *Philosophical Transactions of the Royal Society B* 367, 565–582.

Taggart, R.E. & Cross, AT. (2009). Global greenhouse to icehouse and back again: The origin and future of the Boreal Forest Biome. *Global & Planetary Change* 95, 115-121.

Tamm, C.O. (1991). Denitrification in Forest Ecosystems - Preface. *Forest Ecology & Management* 44; 1-3.

Tanaka, M. & Nara, K. (2009). Phylogenetic diversity of non-nodulating *Rhizobium* associated with pine ectomycorrhizae. *FEMS Microbiology Ecology* 69, 329-343.

Tarkka, M.T. & Frey-Klett, P. (2008). Mycorrhizal Helper Bacteria. In Mycorrhiza. State of the Art, Genetics and Molecular Biology, Eco-Function, Biotechnology, *Eco-Physiology,* Varma, A. (ed). Berlin/Heidelberg, Springer, pp. 113-132.

Tedersoo, L., Koljalg, U., Hallenberg, N. & Larsson, K.H. (2003). Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. *New Phytologist* 159, 153-165.

Toljander, J.F., Paul, L., Lindahl, B.D., Elfstrand, M. & Finlay, R.D. (2007). Influence of AM fungal exudates on bacterial community structure. *FEMS Microbiology Ecology* 61, 295–304.

Torsvik, V., Sorheim, R. & Goksoyr, J. (1996). Total bacterial diversity in soil and sediment communities - A review. *Journal of Industrial Microbiology* 17, 170-178.

Turlapati, S.A., Minocha, R., Bhiravarasa, P.S., Tisa, L.S., Thomas, W.K. & Minocha, S.C. (2012). Chronic N-amended soils exhibit an altered bacterial community structure in Harvard Forest, MA, USA. *FEMS Microbiology Ecology* 83, 478-493.

Uroz, S., Buée, M., Murat, C., Frey-Klett, P. & Martin, F. (2010). Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. *Environmental Microbiology Reports* 2, 281–288.

Uroz, S., Calvaruso, C., Turpault, M.P. & Frey-Klett, P. (2009). Mineral weathering by bacteria: ecology, actors and mechanisms. *Trends in Microbiology* 17, 378–387.

Uroz, S., Calvaruso, C., Turpault, M.P., Pierrat, J.C., Mustin, C. & Frey-Klett, P. (2007). Effect of the mycorrhizosphere on the genotypic and metabolic diversity of the soil bacterial communities involved in mineral weathering in a forest soil. *Applied and Environmental Microbiology* 73, 3019–3027.

Uroz, S., Courty, P E., Pierrat, J.C., Peter, M., Buée, M., Turpault, M.P., Garbaye, J. & Frey-Klett, P. (2013). Functional profiling and distribution of the forest soil bacterial communities along the soil mycorrhizosphere continuum. *Microbial Ecology* 66, 404-15.

Uroz, S., Ioannidis, P., Lengelle, J., Cébron, A., Morin, E., Buée, M. & Martin, F. (2013). Functional assays and metagenomic analyses reveal differences between the microbial communities inhabiting the soil horizons of a Norway Spruce Plantation. *PLoS ONE* 8,

Uroz, S., Oger, P., Lepleux, C., Collignon, C., Frey-Klett, P. & Turpault, M.P. (2011b) Bacterial weathering and its contribution to nutrient cycling in temperate forest ecosystems. *Research in Microbiology* 162**,** 820–831.

Uroz, S., Oger, P., Morin E. & Frey-Klett, P. (2012). Distinct ectomycorrhizospheres share similar bacterial communities as revealed by pyrosequencing-based analysis of 16S rRNA genes. *Applied & Environmental Microbiology* 78, 3020-3024.

Uroz, S., Turpault, M.P., van Scholl, L., Palin, B. & Frey- Klett, P. (2011a). Long term impact of mineral amendment on the distribution of mineral weathering associated bacterial communities from the beech *Scleroderma citrinum* ectomycorrhizosphere. *Soil Biology & Biochemistry* 43: 2275-2282.

van Breemen, N., Finlay, R.D., Lundström, U., Jongmans, A.G., Giesler, R. & Melkerud, P.A. (2000). Rock-eating mycorrhizal fungi: A true case of mineral plant nutrition? *Biogeochemistry* 49, 53-67.

van Hees, P.A.W., Jones, D.L., Finlay, R.D., Godbold, D.L. & Lundstrom, U.S. (2005) The carbon we do not see – the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. *Soil Biology & Biochemistry* 37: 1–13.

van Hees, P.A.W., Rosling, A., Essén, S., Godbold, D.L., Jones, D.L. & Finlay, R.D. (2006a). Oxalate and ferricrocin exudation by the extramatrical mycelium of an ectomycorrhizal fungus in symbiosis with Pinus sylvestris. *New Phytologist* 169, 367–377.

van Hees, P.A.W., Rosling, A., Lundstrom, U.S., & Finlay, R.D. (2006b). The biogeochemical impact of ectomycorrhizal conifers on major soil elements (Al, Fe, K and Si). *Geoderma* 136, 364–377

Vik, U., Logares, R., Blaalid, R., Halvorsen, R., Carlsen, T., Bakke, I., Kolstø, A.B., Økstad, O.A. & Kauserud, H. (2013). Different bacterial communities in ectomycorrhizae and surrounding soil. *Scientific Reports* 3, 3471.

Vitousek, P.M. & Howarth, R.W. (1991). Nitrogen Limitation on Land and in the Sea - How Can It Occur. *Biogeochemistry* 13, 87-115

Voříšková, J., Brabcová, V., Cajthaml, T. & Baldrian, P. (2014). Seasonal dynamics of fungal communities in a temperate oak forest soil. *New Phytologist* 201**,** 269-278.

Wallander, H., Ekblad, A. & Bergh, J. (2011). Growth and carbon sequestration by ectomycorrhizal fungi in intensively fertilized Norway spruce forests. *Forest Ecology & Management* 262, 999-1007

Wang, Q., Garrity, G.M., Tiedje, J.M. & Cole, J.R. (2007). Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* 73, 5261-5267.

Warmink, J.A., Nazir, R. & van Elsas, J.D. (2009). Universal and species-specific bacterial 'fungiphiles' in the mycospheres of different basidiomycetous fungi. *Environmental Microbiology* 11, 300–312.

Warmink, J.A., Nazir, R., Corten, B. & van Elsas, J.D. (2011). Hitchhikers on the fungal highway: The helper effect for bacterial migration via fungal hyphae. *Soil Biology & Biochemistry* 43, 760–765.

Warmink, J.A. & van Elsas, J.D. 2008. Selection of bacterial populations in the mycosphere of *Laccaria proxima*: Is type III secretion involved? *ISME J.* 2: 887–900.

White, TJ., Bruns, T.D., Lee, S.B. & Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), *PCR Protocols - a Guide to Methods and Applications*. Academic Press, San Diego, CA, pp. 315-322.

Yarwood, S.A., Myrold, D.D. & Högberg, M.N. (2009). Termination of belowground C allocation by trees alters soil fungal and bacterial communities in a boreal forest. FEMS Microbiology Ecology 70, 151-162.

Acknowledgements

First of all I would like to thank my parents **Chandrakala** and **Ramaswamy** who constantly supported, encouraged and motivated me throughout my career to pursue higher studies. They always wanted me to achieve greater heights in my life. My brothers Srikanth, Vikram, Vinod and my aunt Edamma, whose love for me is in unswerving. My little cute angel **Vidhatri**, changed my life completely, she became the source of my inspiration. I would like to say big thanks to the love of my life **Swathi**, who was always understanding and supportive throughout my PhD studies. I am grateful to all my relatives for their affection and love. I love you all

My PhD Journey was a rather an accident, I came to do my PhD in biofilms and ended up doing research on ectomycorrhizal and bacterial interactions. Even my understanding of fungi and plant was minimal but with the help and motivation inculcated by my supervisors, I took up the challenge and managed to complete the marathon. I am fortunate to have supervisors, who are very understanding and supportive even when experiments fail one after another. I would like to thank my supervisors for being generous and for their great help in planning experiments and fruitful discussions. At times I was depressed with negative results and missing my deadlines, Roger would never criticize, instead he used to say `this is not the end of world and he used to treat us as his own children and let us commit mistakes and help learn from our mistakes. I admire Roger´s simplicity, easy-going approach, thought provoking discussions and his helping nature.

I admire Shahid for his perseverance in doing science, he never gets tired and is always ready for discussion or any help with the experiments. I really venerate his penchant for learning, his way of designing experiments. His philosophy of life is very interesting to me, he used to say that if a student fails it's not his mistake but it's the teacher's mistake and the student is like a white paper and its up to a teacher what to write on it.

I would like to thank my former supervisors Salme timmusk for introducing me to the mykopat family and Anton Zavialov for giving me an opportunity to work in his lab and teaching molecular and protein chemistry techniques. I would like to thank all the teachers who had taught me to become what I am today.

I would also like to thank my group members Hironari, Juan, Helen, Srivathsa, Saroswathi, Zaenab and Tina. Sadhana Alström for your advice and moral support

I would like to gratefully acknowledge my colleagues and all the PhD students for their help and advice during my stay in the department. Computer administrator leslie Paul for all the help and support provided all these years to me, Secretaries Karin and Erica you have been really helpful for me all these years, Thank you all. Maria and Rena for casual chat, Anders for his help in literature search, and Marianne for her critical review.

A special thanks to Srivathsa, Mukesh, Prakash, Rajeev for wonderful times we had together, those were some of the great moments during my PhD studies.

I would like to thank two most important people Mukunda and Ramesh Raju for their great help, moral support and advice in my decisions, special thanks to Ganesh for helping me in critical times of my career, Ravi, Sudhakar, Srivathsa, Laura for the memorable days in Sweden.

I would also like to thank all my friends in Uppsala who had made my days in Sweden, Rashad, Gopi, Avinash, Prasad, Chandu, Gangadhar, Madhu, Raghuveer, Sunil, Kiran K, Kiran J, Amar, Shashi, Sethu, Srinu, Charan, Sunil, Geetha, Vijaya, Sushma, Madhuri K, Madhuri Raghuveer, Saritha, Gayathri, Bhavya Archana, Indu, Sowmya, Anusha, and Sony, Preethi.

Finally I want to thank all my friends in India for all the love and support you gave me. Special thanks to my best friends in India Subbu, Pranay, Rajesh, Madhu, Sunil, Parvi and Bhaskar for always being there for me.