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Who comes first?

Implications of the plant-microbiome-soil continuum feedback on plant performance

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Cover: Scots pine seedling establishing in a thin organic layer on top of a rock
(photo: David Castro)

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Who comes first? Implications of the plant-microbiome-soil continuum feedback on plant performance

Abstract

Plants are sessile organisms that rely on their ability to explore the soil to access the nutrients and water they need to survive. Plants have co-evolved with certain groups of bacteria and fungi that provide nutrients and water and enhance tolerance to abiotic and biotic stressors in exchange for Carbon (C). This symbiotic interaction is central to plant establishment and survival in harsh environments, where edaphic properties exert selective pressures on plant growth and modulate the composition of the soil microbiome community, with potential detrimental effects on ecosystem composition. Globally, more than 50% of the biodiversity hotspots are in soils with particular characteristics, thus, edaphic properties are considered as second in importance after climatic variables. In this thesis, the aim was to study the impact of the soil properties on plant establishment and performance and how this affects the ability of the plant to recruit a microbial community to their roots, with a focus on the ectomycorrhizal fungi (EcM). The results presented in this thesis suggest that both edaphic properties and soil microbiome modulates plant establishment and growth. In addition, changing edaphic properties induce changes in plant metabolism that have direct impact on the root-associated community. These changes redefine plants' C economy toward less demanding symbionts, having direct impact on the soil organic matter (SOM) dynamics. My results provide new insights on how anthropogenic-induced changes in the soil can have a strong impact on the soil ecology, which can in consequence, have a major impact in the forest biodiversity.

Keywords: Plant-microbiome interaction, Scots pine, Norway spruce, fungal community, nitrogen fertilization, forest management, boreal forest.

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Vem kommer först? Implikationer av samspel och återkoppling mellan växter, markegenskaper och mikrobiom för plantors tillväxt

Abstrakt

Växter är fastsittande organismer som är beroende av förmågan att utforska jorden för att få vatten och näringsämnen som de behöver för att överleva. Växter har utvecklats i samevolution med vissa grupper av bakterier och svampar som både ger tillgång till näringsämnen och vatten och kan förbättra växters tolerans mot abiotiska och biotiska stressfaktorer, i utbyte mot kol. Denna symbiotiska interaktion är central för växters förmåga att växa och överleva i hårda miljöer, där marken utövar selektivt tryck både på plantors tillväxt och kompositionen av jordmikrobiomet, vilket kan ha skadliga effekter på ekosystemens sammansättning. Globalt befinner sig mer än 50% av alla biodiversitets hotspots i områden med speciella markegenskaper, vilket gör jordmånsförhållanden nästan lika viktigt som klimat. Denna avhandling hade som mål att studera inflytandet av markegenskaper på prestanda och etablering av växter, och hur detta påverkar växternas förmåga att rekrytera mikroorganismer till rötterna, detta med fokus på ektomykorrhizasvampar. Resultaten i denna avhandling talar för att både jordmånsegenskaper och markens mikrobiom modulerar etablering och tillväxt av plantor. Dessutom leder en förändring i markegenskaper till en förändring i växtens metaboliska processer, vilket också har en direkt påverkan på de mikrobiella samhällena i rötterna. Dessa förändringar skiftar växternas kolekonomi till mindre krävande symbionter, vilket också har direkta påverkningar på dynamikerna som styr markens organiska sammansättning. Mina resultat ger nya insikter om hur antropogena förändringar i marken kan ha starka effekter på markekologin, vilket i sin tur kan ha vidräckande konsekvenser för biodiversitet i skogen.

Nyckelord: Växt-mikrob interaktioner, tall, gran, svampsamhällen, kvävegödsling, skogsbruk, boreal skog.

Dedication

To Johanna

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. **Castro D**., Concha C., Ibáñez C., Jamett F. & Hurry V.* (2022). Soil microbiome influences on seedling establishment and growth of *Prosopis chilensis* and *Prosopis tamarugo* from Northern Chile. (manuscript)

II. Schneider A.N.‡, **Castro D**.‡, Holmlund M., Näsholm T., Hurry V. & Street N.R.* (2022). Organic N addition improves root growth without changing fungal communities in outplanted conifer seedlings. (manuscript)

III. **Castro D**.‡, Schneider A.N.‡, Holmlund M., Näsholm T., Street N.R. & Hurry V.* (2021). Effects of Early, Small-Scale Nitrogen Addition on Germination and Early Growth of Scots Pine (Pinus sylvestris) Seedlings and on the Recruitment of the Root-Associated Fungal Community. Forests, 12 (11), 1589.

IV. **Castro D**.‡, Campbell C.D.‡, Ishida T., Henriksson N., Serrano A., Stangl Z.R., Blackburn M., Street N.R., Näsholm T. & Hurry V.* (2022). Effect of increasing N-status of Norway spruce by direct addition of N to the transpiration stream on the root-associated fungal community composition and function. (manuscript)

V. Law S.R., Serrano A. Daguerre Y., Sundh J., Schneider A.N., Stangl Z.R., **Castro D**., Grabherr M., Näsholm T., Street N.R.* &

Hurry V.* (2022). Metatranscriptomics captures dynamic shifts in mycorrhizal coordination in boreal forests. (submitted)

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*Corresponding author. ‡ These authors contributed equally to this work.

Other contribution of David Castro not included in this thesis:

Bonner M.T.L., **Castro D**, Schneider A.N., Sundström G., Hurry V., Street N.R. & Näsholm T. (2019). Why does nitrogen addition to forest soils inhibit decomposition? Soil Biology and Biochemistry, 137, 107570.

The contribution of David Castro to the papers included in this thesis was as follows:

I. Planning and performing experiment. Data preparation, analysis and visualization. Script programming. Manuscript writing and editing.

II. Planning and sampling. Data analysis and figure visualization. Script programming. Manuscript editing.

III. Planning and sampling. Data preparation, analysis and visualization. Script programming. Manuscript writing and editing.

IV. Data preparation, analysis and visualization. Script programming. Manuscript writing and editing.

V. Data preparation and analysis. Manuscript editing.

List of figures

Abbreviations

ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylate
Al	Aluminium
AM	Arbuscular mycorrhizas
AODC	Acridine orange direct count
C	Carbon
CFU	Colony-forming units
CMN	Common mycorrhizal network
CSSP	Common symbiotic signalling pathway
CTC	5-Cyano-2,3-ditolyl tetrazolium chloride
DA	Differentially abundant
DE	Differential expression
DOC	Dissolved organic C
EcM	Ectomycorrhizas
EdM	Endomycorrhizas
ErM	Ericoid mycorrhiza
Fe	Iron
G+C	Guanine plus cytosine
GA	Gibberellic acid

GH	Glycoside hydrolases
HCN	Hydrogen cyanide
iN	Inorganic Nitrogen
ITS	Internal transcribed spacer
KNO_3	Potassium nitrate
KOGs	Eukaryotic Orthologous Groups
LCOs	Lipochitooligosaccharides
MHB	Mycorrhizal helper bacteria
microCFU	Micro colony-forming units
N	Nitrogen
NGS	Next-generation sequencing
NH_4NO_3	Ammonium nitrate
OM	Orchid mycorrhiza
oN	Organic Nitrogen
P	Phosphorus
PGPB	Plant growth-promoting bacteria
PGPM	Plant growth-promoting microbes
PLFA	phospholipid-derived fatty acids
rDNA	Ribosomal DNA
rRNA	Ribosomal RNA
S	Sulfur
SOM	Soil organic matter
SOTU	Swarm operational taxonomic units
SWEET2	Sugars Will Eventually Be Exported Transporter
T-RFLP	Terminal restriction fragment length polymorphism

1. Introduction

Soils are complex habitats harbouring a wide diversity of microorganisms, from archaea to microarthropods, which play key roles in the biogeochemical cycles of the earth (Dubey *et al.*, 2019). At the same time, soils are the substrates where terrestrial plants, through their root system, are anchored (Bengough *et al.*, 2016; Kolb *et al.*, 2017) and obtain water and nutrients for growth (Motte & Beeckman, 2019; Motte *et al.*, 2019). It is in the soil where the root systems from different plant species interact with this diverse community of microorganism in an intricate network of competition, mutualism, parasitism and commensalism, with nutrient exchange playing a central role (Folse & Allison, 2012). These simultaneous ecological interactions belowground have a central influence on several key soil nutrients, including Phosphorus (P), Sulfur (S), Carbon (C) and Nitrogen (N), among others (Falkowski *et al.*, 2008; Fierer, 2017; Schloter *et al.*, 2018), increasing their availability in the soil. The nutrient content in the soil, together with soil moisture, soil organic matter (SOM), pH, particle aggregation, and other soil-related characteristics are known as edaphic properties, which are recognized as second in importance after climate for determining plant distribution (Hulshof & Spasojevic, 2020).

Due to a combination of geological and biological processes, soils are organized in different horizons, corresponding to layers with different depths and edaphic properties (Delgado & Gómez, 2016; Hartemink *et al*., 2020). For example, the O and A horizons (*i.e.*, upper soil horizons rich in organic matter) are home to most biological activity, where earthworms, microbes, plant litter, wood decay are important components for soil formation (see Minasny *et al*. (2008)). In the soils of European conifer forests, this stratification of the soil is mirrored by a vertical separation in the fungal community, based on functional groups. Thus, saprotrophic fungi are the

dominant group in the L horizon (*i.e.*, litter horizon), where plant litter and decaying wood accumulates, whereas mycorrhizal fungi are the dominant group in the H horizon (*i.e.*, humus horizon), where more decomposed humus accumulates (Rosling *et al*., 2003; Lindahl *et al*., 2007; Baldrian *et al*., 2012). A study comparing the Alaskan boreal forest and Guyanan tropical forests found similar spatial patterns in the microbial community in the soil horizons, where saprobes were the dominant group found in plant litter while mycorrhiza dominated within the first 20 cm of soil (McGuire *et al*., 2013). Such findings provide evidence of common responses in soil functional groups despite differences in climate, indicative of common traits in soil ecology.

In addition, some plant species, known as edaphic endemic, have, as the name suggests, a narrow distribution range limited only to specific edaphic properties such as extreme pH, ion content, or heavy metal (Rajakaruna, 2018; Burge, 2020). These plant species have adapted to local edaphic conditions that have been proposed to portray high risk for plant distribution, depending on the risk of potential changes in the edaphic properties due to anthropogenic activities and plant tolerance to stress (Corlett & Tomlinson, 2020). Given the close relationship between the soil properties and the organisms sustained by it, in recent years the plant-microbiome-soil continuum model has been proposed (Goss-Souza *et al*., 2020; Babin *et al*., 2021; Tsiknia *et al*., 2021). This model proposes an integrative view of plant and soil community studies, where plant and soil communities act as a functional unit, responding to the edaphic properties of the surrounding soil.

1.1 Effects of forestry management on seedling establishment and survival

1.1.1 Forest management in Sweden

Forest lands are one of the largest terrestrial biomes, accounting for *c.* 4 billion hectares (about 31% of the land surface) (FAO & UNEP, 2020). These lands, in addition to being of economic importance, provide important ecosystem services, for example improving air quality through pollutant removal, C sequestration though photosynthesis, or erosion prevention through improving edaphic properties (Foley *et al.*, 2005, 2011; Jenkins & Schaap, 2018). Within forest lands, *c.* 27% is located in the boreal climatic

zone (FAO & UNEP, 2020). Located between 45° to 70° northern latitude, the boreal forest is characterized by short growing seasons, long winters with below-zero temperatures and snow coverage that lasts several months (Gauthier *et al.*, 2015). Spanning Fenno-Scandinavia, Eurasia and North America, the boreal forest is characterized by low plant diversity, with a dominance of conifers such as *Picea* spp. and *Pinus* spp., and some angiosperms such as *Populus* spp. and *Betula* spp. (Gauthier *et al.*, 2015). In 2017, forested land in Sweden occupied 69% of the country. Representing an important component in the country's industry, it also provides a range of ecosystem services such as biomass production and cultural services (*e.g.*, recreation and cultural legacy for the Sami people) (Millenium Ecosystem Assessment, 2005; Nilsson & Wardle, 2005; Gauthier *et al.*, 2015; Vauhkonen & Ruotsalainen, 2017; Östlund & Norstedt, 2021).

At the beginning of the 20th century, Swedish authorities declared a national forest policy that included planned felling and planting to regenerate the large proportion of forest lost due to heavy felling during the 19th century (Helander, 2015). As a result of this change, Sweden is now one of the countries in the EU with the greatest forest cover, and is the second largest European exporter of paper, pulp, and sawn timber (Bjärstig & Keskitalo, 2013). Given the economic importance of the boreal forest for the Swedish economy, different forest management strategies are used to achieve higher stand biomass. For example, stand thinning to reduce competition (Cao *et al*., 2008; Trentini *et al*., 2020); mono- or polyculture stands, which have been shown to have different impacts on biomass depending on the edaphic properties (Felton *et al*., 2010, 2016). In addition, site preparation and the introduction of high-quality planting material are used to enhance plant establishment and seedling growth and to improve yield and value from the planted material (Oleskog & Sahlén, 2000; Wennström, 2001; Helander, 2015).

Site preparation corresponds to the physical preparation of the soil to improve establishment, which follows clear-cutting and precedes planting. In Sweden, soil scarification and mounding are the most commonly used methods of site preparation (Löf *et al*., 2012). These methods expose the underlying mineral soil, or create an elevated mineral-organic soil layer (*i.e.*, capped mound soil) that enhances seedling survival by increasing soil moisture, soil temperature and mineral content availability (Mäkitalo, 1999; Archibold *et al*., 2000; Löf *et al*., 2012). In addition, when used within stand

gaps, site preparation reduces belowground competition with larger trees for nutrients, enhancing seedling growth and biomass production (Wennström *et al*., 1999; Löf *et al*., 2012; Axelsson *et al*., 2014; Pasanen *et al*., 2016). However, site preparation can have negative effects on edaphic properties, such as increasing nutrient leaching into ground water (Dai *et al*., 2001; Piirainen *et al*., 2007), as well as soil erosion and runoff (Keenan & (Hamish) Kimmins, 1993; Mohr *et al*., 2013). In addition, site preparation induces local extinction of mosses and liverworts, and rapid germination of early successional herbs (Craig & Macdonald, 2009; Vanha-Majamaa *et al*., 2017), which can exert competitive pressure on economically important plant species (Fløistad *et al*., 2018). In these managed sites, either seeds or nursery-produced seedlings are used as planting material (Ceccon *et al*., 2016). The selection of the planting material to be used depends on the aim of the stand, site location and site edaphic properties.

Direct seeding

Direct seeding has been used for reforestation in many countries, such as Iceland (Pétursson & Sigurgeirsson, 2004), Russia (Ilintsev *et al.*, 2021), United Kingdom (Willoughby, 2004) and Scandinavia (*e.g.*, Denmark, Finland and Sweden) (Madsen & Jöf, 2005; Hyppönen & Hallikainen, 2011). The first references of direct seeding for reforestation come from the 14th century, becoming a viable option during the 19th century's sustainable reforestation programs (Grossnickle & Ivetić, 2017). In Sweden, direct seeding is recommended for northern sites, as higher germinant survival rates are achieved (Wennström *et al.*, 2007; Ersson, 2021). In addition, direct seeding is preferred for poor, sandy soils (Birkedal, 2006; Skogsstyrelsen, 2020; Ersson, 2021).

In addition to edaphic properties, the genetic background of the seed (*i.e.*, genetic qualities inherited from parental trees), seed size and seed coat all have an impact on seed germination and seedling establishment (Castro, 1999; Bruce *et al.*, 2007; Bai & Settles, 2015; Novikov *et al.*, 2019). Given the importance of high-quality planting material, the Swedish forest industry started seed orchard trials in around 1949, following the efforts in Denmark in 1934 (Lindgren *et al.*, 2007). Using selective crossing of plus-trees, while minimizing pollen contamination from self-fertilization or surrounding naturally established trees, seed orchard owners aim to produce genetically superior seeds (Funda *et al.*, 2015). Using these methods, by 2006, seed orchards contribute 78% and 49% of the seed used for outplanting Scots pine

and Norway spruce seedlings, respectively (Lindgren *et al.*, 2007). However, current orchard seed production is not sufficient to meet the needs of the Swedish forestry, and in 2019, *c.* 2230 kg of seeds were imported to Sweden from Europe and other countries (Skogsstyrelsen, 2020).

High-quality, orchard produced seeds are a valuable planting material, thus, it is mostly used for nursery seedling production, because direct seeding has a low success rate (Ceccon *et al.*, 2016; Grossnickle & Ivetić, 2017), as seeds often do not fulfil their minimal requirement of moisture, or the radicle does not find suitable soil in which to establish (Domevščik, 2022). For this reason, where direct seeding is used, it often relies on open pollinated stand seeds, shelterwood seeds, or other less valuable seed (Birkedal, 2006; Skogsstyrelsen, 2020). In addition, seed burring after seeding (Nilson & Hjältén, 2003) and site preparation are used by forest companies during direct seeding to increase seed germination success, as the first protects the seed from granivores (*e.g.*, birds and rodents) and the second improves soil qualities (Birkedal, 2006). Furthermore, the use of solid matrix priming products, which correspond to compounds that increase water absorption and moisture retention (*e.g.*, Bentonite, superabsorbent polymers, worm castings) (Madsen *et al.*, 2018) have been shown to enhance seed germination by increasing moisture and protecting the seed from osmotic stress during imbibition (Palma & Laurance, 2015; Wu *et al.*, 2019). Such matrix priming products, in addition, can be enriched with limiting nutrients to further improve seed germination. For example, it has been observed that N increases seed germination, as it has been suggested to accelerate ABA degradation (Holdsworth *et al.*, 2008), inducing seeds to more rapidly enter phase III (Holdsworth *et al.*, 2008; Zhang *et al.*, 2020), making them a new alternative for direct seeding, even with orchard produced seed, although further research should be performed before increasing the number of orchard seeds for direct seeding instead of using them for nursery grown seedling (Figure 1).

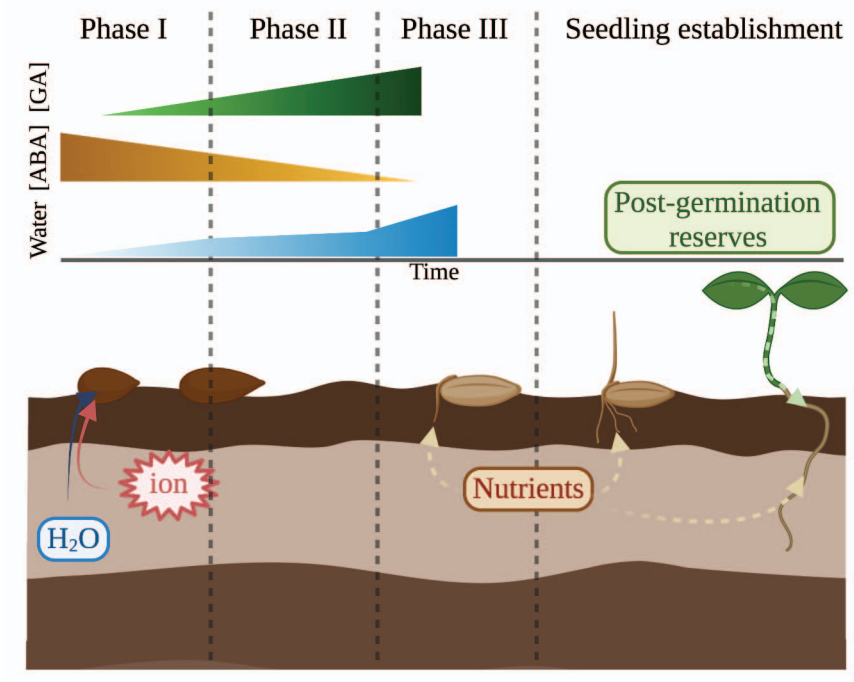

Figure 1. Overview of seedling seed germination and seedling establishment. During phase I, the low water potential of the seed induces quick water uptake reactivates cell metabolism. During phase II, increases in gibberellic acid (GA) content counteract abscisic acid (ABA) seed germination inhibitory function, water uptake stabilises, and embryo start to grow. In phase III, the high GA:ABA ratio led to radicle protrusion, marking the beginning of seedling establishment, where radicle/root systems explore the soil for nutrients while initially fed by the mobilized post-germination reserved. Based on Holdsworth *et al.* (2008), Weitbrecht *et al.* (2011), Rajjou *et al.* (2012) and Wang & Ruan (2016). Figure created with BioRender.com.

Nursery grown seedling planting

Annually, *c.* 400 million seedlings are planted in Sweden, mostly grown from orchard-produced seed in nurseries whose capacities ranges from less than 1 up to 100 million seedlings per year (Helander, 2015; Skogsstyrelsen, 2020). These seedlings start their growth in the nursery greenhouse under controlled growing conditions and are then moved outside to be grown on and for frost acclimation before being considered ready for planting (Skogsstyrelsen, 2020). Seedling outplanting in the Swedish forest usually takes place after scarification of a harvested site, where a specific location in the trenches is chosen for planting depending on climatic conditions, latitude and plant species (Helander, 2015; Skogsstyrelsen, 2020; Häggström *et al.*,

2021). For example, in wetter sites, capped mound soil is the best planting spot, whereas in drier sites, exposed mineral soil is preferred as it remains wet for longer (Skogsstyrelsen, 2020; Häggström *et al.*, 2021).

To ensure seedling outplanting success, a high growth rate of seedlings during nursery cultivation is imperative, as larger seedlings have higher survival potential and grow faster after outplanting (Skogsstyrelsen, 2020). In Sweden, nursery owners use inorganic N (iN) such as ammonium and nitrate or, more recently, organic N (oN), such as arGrow® (Gruffman *et al.*, 2012; Lim *et al.*, 2021), with the latter being shown to improve seedling morphology and outplanting performance (Rytter *et al.*, 2003; Gruffman *et al.*, 2012, 2014; Bahramov *et al.*, 2020). By using these high-quality nursery grown seedlings, outplanting generally results in high establishment success rates, reaching seedling survival rates of about 60% (Palma & Laurance, 2015), partially as a result of planting material having a well-developed root system, which allows the planted seedling to quickly explore the soil for water and nutrients (Davis & Jacobs, 2005; Rellán-Álvarez *et al.*, 2016; Izzo *et al.*, 2019) (Figure 1).

1.1.2 Water and nutrient uptake, and plant growth

Water uptake in the roots occurs through three possible pathways; apoplastic, symplastic and transcellular path, the last two known as the "cell-to-cell" pathway (Martínez-Ballesta *et al.*, 2006; Blum, 2011) (Figure 2a). Most of the water is believed to move through the apoplastic pathway in the root cortex, and once it reaches the Casparian strip, an impermeable deposit of suberin and lignin that limits apoplastic water transport, the cell-to-cell pathway becomes the dominant pathway for water transport towards the xylem (Steudle, 2000; Martínez-Ballesta *et al.*, 2006). Broadly, apoplastic water uptake occurs following the water potential gradient, as the apoplast represents low hydraulic resistance, controlled partially by the water loss through the leaves (Steudle, 2000; Martínez-Ballesta *et al.*, 2006). On the other hand, cell-to-cell water flow is partially controlled through a family of protein channels known as aquaporins that enhance water transport through the plasma membrane (Martínez-Ballesta *et al.*, 2006). Although different in nature, all three pathways of water uptake synergically control the water flow through the plant, partially regulating cell turgor and ultimately, solute flow through the vascular stream (Blum, 2011; Aroca *et al.*, 2012).

Similarly, nutrient uptake depends on the mass flow and diffusion of dissolved nutrients in the soil in proximity with the root (Chapman *et al.*, 2012). Such uptake occurs in parallel with water through the apoplastic pathway (Steudle, 2000) and through channels and transporters that control ion uptake (Chapman *et al.*, 2012) (Figure 2b). The latter mechanism is controlled by the expression of transporter-coding genes that vary spatiotemporally in the roots, depending on root developmental stage (Arsova *et al.*, 2020). At the same time, root absorption itself is controlled by shoot growth requirements, nutrient content in the xylem and phloem and plant metabolism (Hermans *et al.*, 2006; Wang *et al.*, 2006). Thus, plants establishing in soils with good edaphic properties (*i.e.*, that provides water, macro- and micro-nutrient content, organic matter, pH, porosity, etc.) (Hulshof & Spasojevic, 2020), would have greater establishment success and therefore increased yield and productivity. Furthermore, a series of phytohormones (*e.g.*, auxins and cytokinin) modulate root growth and branching in response to soil properties (Wang *et al.*, 2006; Wang & Ruan, 2016), following a source-sink dynamic between the shoot and the root (White *et al.*, 2016). Thus, in nutrient deficient soils, lateral root development is induced through increased C allocation to the roots (*i.e.*, C sink), enhancing root growth and its exploration capacity to find nutrient patches (Morgan & Connolly, 2013; Wang & Ruan, 2016; White *et al.*, 2016).

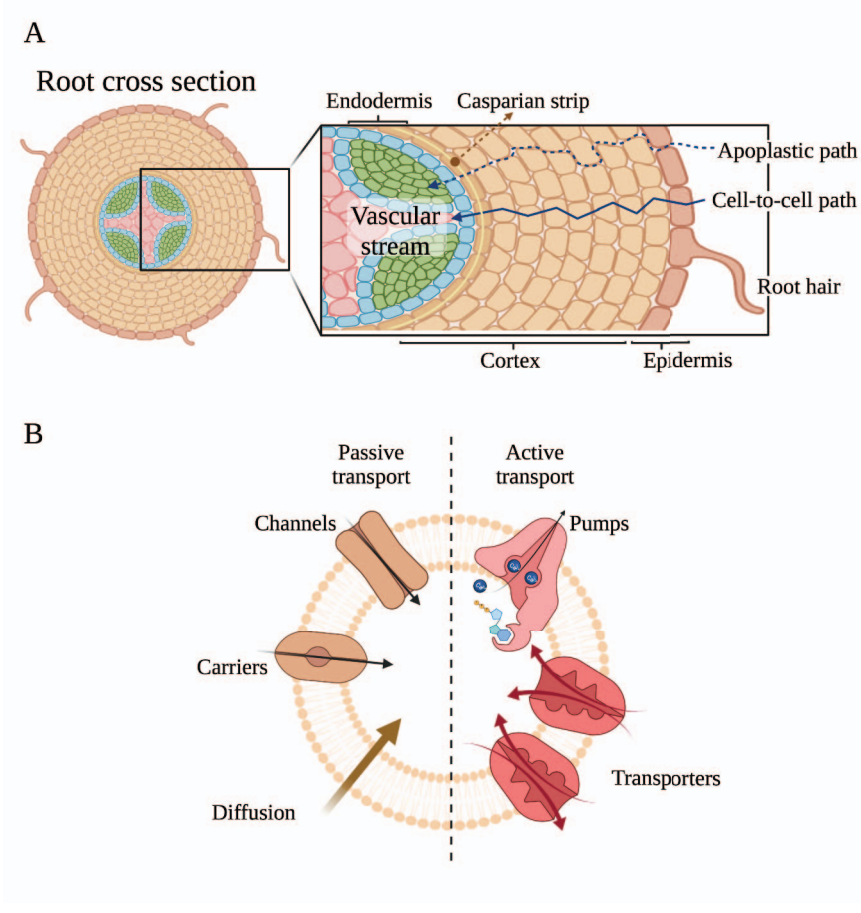

Figure 2. Simplified water movement into the roots to the plant vascular stream. (**A**) cross section depicting the root morphology and the water uptake paths. Apoplastic path, between the cells and cell-to-cell path (*i.e.*, transcellular and symplastic paths). (**B**) Simplified transmembrane transports. Passive transports correspond to small particles movement following a concentration gradient and do not use energy, while active transport moves medium to big particles against gradient, using chemical (pumps) and electrochemical potential (transporters). Transporters can be symporter (two molecules to the same direction) or antiporters (two molecules moves in different direction). Based on Steudle (2000) and Wang *et al.* (2006). Figure created with BioRender.com.

To aid root growth in the soil, the fine roots exude different types of molecules, usually carbon-based, that reduces the friction between the soil and the growing root, aiding root growth (Baetz & Martinoia, 2014). Some other exudates modulate nutrient availability and uptake (Wang *et al.*, 2006). For example, iron (Fe) ion is often found in the soil in Fe^{3+} form that is not

available to plants for assimilation, thus roots exude phytosiderophores, which are iron-chelating compounds that chelate Fe^{3+}, or exude organic acids that locally lower soil pH, increasing the prevalence of Fe^{2+} in the soil and making it available to the plant for assimilation (Wang *et al.*, 2006). Similarly, exudation of malate or citrate can protect plants from aluminium (Al) toxicity, chelating the ion and making it inaccessible to the plants for assimilation (Jung & McCouch, 2013). Some of these exuded compounds, in addition to aiding root penetration of the soil, act as signalling molecules that can attract or repel soil-borne microbes that can have either positive or detrimental impact on nutrient uptake and plant growth. These soil-borne microbes and the potential impact on plant nutrient uptake and growth will be described in sections 1.2 and 1.3, respectively.

1.2 Edaphic properties and the soil microbiome

The soil microbiome corresponds to an array of different microorganisms that include viruses, archaea, bacteria, fungi, nematodes and microarthropods (Buée *et al.*, 2009; Dubey *et al.*, 2019). These microorganisms, and from now on I consider only bacteria and fungi, require a minimal set of conditions to be metabolically active. For instance, soil microbiomes are often less active in bulk soils, as bulk soil has low moisture or SOM, being more active in so-called hotspots (*e.g.*, rhizosphere, mycosphere or nutrient patches) and during hot moments (*e.g.*, after rainfall) (Schloter *et al.*, 2018; Berg *et al.*, 2020) where water and nutrients are easily absorbed. It is in the soil hotspots and hot moments when soil microbes compete for available nutrients (Jansson & Hofmockel, 2018). This active and dynamic modulation between different microbial species, results in community assemblies where different microbes can either be dominant or outcompeted depending in the edaphic properties.

The microbiome harboured by the soil is a key component of the nutrient content of the soil, as they are central to the mineralization-immobilization cycles of nutrients in the soil, especially for C, N and P cycles (Falkowski *et al.*, 2008; Fierer *et al.*, 2012). For instance, as free-living soil microbes break down SOM to sustain their metabolic processes, they improve edaphic properties such as soil aggregation and fertility through, for example, nitrogen-fixation, phosphorus solubilization and siderophore production, increasing N, P, and Fe availability in the soil (Prasad *et al.*, 2015). Thus, the

soil microbiome has a direct impact on soil nutrient dynamics and soil health (Fierer *et al.*, 2012).

1.2.1 Soil microbiome response to edaphic changes

As briefly described at the beginning of this introduction, soils respond to a vertical organization (*i.e.*, horizons) where edaphic properties and microbial functional groups change throughout these horizons (Bünemann *et al.*, 2018; Marupakula *et al.*, 2021). Along this spatial pattern, it has been found that for soil bacterial community composition, SOM quantity and quality and soil pH were the most important factors structuring the soil microbial communities (Fierer, 2017). Similar partitioning has been observed across ecological gradients, where soil relative humidity, soil temperature and soil electric conductance had significant effects on microbial composition (Crits-Christoph *et al.*, 2013; Neilson *et al.*, 2017). Additionally, other studies have shown that C:N ratio, plant community composition, soil nutrient content and plant developmental stage have either direct or indirect effects on soil microbial community composition (Pennanen *et al.*, 1999; Högberg *et al.*, 2006; Chaparro *et al.*, 2012; Mueller *et al.*, 2015). These changes in soil microbial composition in response to changes to edaphic properties are an important component for soil health and fertility, as edaphic selective pressures can enhance soil-borne pathogens or increase mineral immobilization (Chaparro *et al.*, 2012; Schlatter *et al.*, 2017; Schloter *et al.*, 2018).

As previously stated, edaphic properties include a series of characteristics in which SOM is perhaps the most important, as the SOM cycle of immobilization and mineralization are of greater importance for global C storage, as soils globally store more C than aboveground plant biomass and the atmosphere (Scharlemann *et al.*, 2014; Gan *et al.*, 2020; Geyer *et al.*, 2020). The traditional model of SOM formation proposes that plant and animal residues are progressively transformed into humic substances (*i.e.*, three groups of not assimilable C based molecules known as fulvic acid, humic acid, and humin). In recent years, it has been proposed that SOM is a continuum of biopolymers in different decomposition states where microbial metabolism has a central role (see Lehmann & Kleber (2015)). In addition, SOM content is partially controlled by slow, but important, physicochemical processes in the soil, such as soil particle aggregation and mineral adsorption, which ultimately impact SOM content (Lehmann & Kleber, 2015).

SOM accumulation results from the immobilization processes performed by plants and soil microbes (*e.g.*, atmospheric C- and N fixation into biomass), and the inhibition of mineralization processes. Such immobilization and mineralization are partially controlled by the edaphic properties, where nutrient availability can accelerate or delay soil processes (Gan *et al.*, 2020). For example, Pellitier & Zak (2021) found that in low iN soils, the ectomycorrhizal (EcM) fungal community was dominated by fungi with greater decay potential, while in high iN soil, the EcM community was dominated by fungi with less SOM degrading capacities, as nutrient requirements were fulfilled by plant hosts (Pellitier & Zak, 2021). Thus, both soil C and N have an impact on microbial SOM consumption, soil microbial composition, and SOM accumulation.

Changes in SOM have been reported to occur due to excessive atmospheric N deposition, as increased N in the soil enhances plant biomass and plant litter accumulation (Janssens *et al.*, 2010; Entwistle *et al.*, 2018). Globally, N deposition has increased three to five times in the last century, and it has been forecast to further increase 2.5 times by the end of this century in some regions, such as Northern Europe and Asia (Galloway *et al.*, 2004; Janssens *et al.*, 2010). It has been suggested that such increase N content in the soil would have similar impact on plants that N fertilization, with a proportionally less C allocation belowground and changes in SOM quality (Hyvönen *et al.*, 2007; Du & de Vries, 2018). Changes in SOM quality have been shown to have direct impact on soil microbial community (Rousk & Bååth, 2007, 2011; Meidute *et al.*, 2008), having even greater effects than plant community composition (Merilä *et al.*, 2010). Such effects were shown by Averill *et al.* (2018), where they found changes in mycorrhizal community at a continental scale due to N deposition. Such changes in soil microbial community have direct impact on SOM pools, as it has been estimated that soil microbial biomass accounts more than 1000 kg of C per hectare (Fierer, 2017).

Within the global SOM pools, the boreal forest represents one of the largest terrestrial C stores (Bradshaw & Warkentin, 2015). In addition to the high SOM content, the boreal forest is also characterised by nutrient poor soils, being especially deficient in available N (Högberg *et al.*, 2017). To enhance boreal forest productivity, nutrient amendment, often in the form of iN fertilization, is currently used in the final 10 years prior harvest of commercially destined forests (Simonsen *et al.*, 2010; Lindkvist *et al.*, 2011;

Pukkala, 2017). In soils enriched in N, either by N fertilization or N deposition, the proportion of plant C allocated belowground diminishes, modifying the C and N content in the soil and potentially triggering changes in the soil microbial composition (Högberg *et al.*, 2006). In addition, plant diversity further modifies C and N content in N fertilized soil through modification in plant litter, soil pH and soil C trade-off, which alters the microbial community associated to, or in proximity with the roots (Högberg *et al.*, 2006; Zhang *et al.*, 2011; Dawud *et al.*, 2016; Du & de Vries, 2018; Lucas-Borja & Delgado-Baquerizo, 2019; Terrer *et al.*, 2021). The relation between plants and the soil microbiome through the root system, and the potential implications of edaphic properties modifications in such interactions will be described in section 1.3.

1.2.2 Methodologies to study soil microbiome

In the late 19th century, the Russian researcher Sergei Vinogradskii, following the Pasteur and Koch debate about the nature of microbial life, developed a series of experiments that led to the first known evidence of lithotrophy and chemoautotrophy, from his studies with *Beggiatoa* and nitrifying bacteria, respectively (Ackert, 2007). About 1935, using culture methods, he forced soil microbes to compete for nutrients, allowing him to '*capture'* the '*microscopic landscape of the soil*' (Ackert, 2007).

The work of Vinogradskii and others, including Pasteur, Koch, Fleming and Waksman, were the basis to what we know as soil microbiology (Dar, 2009). Their work inspired later works on soil bacterial biomass quantification, including Acridine orange direct count (AODC) for total bacteria, 5-Cyano-2,3-ditolyl tetrazolium chloride (CTC) degrading bacteria, for active bacteria, and (micro-) colony-forming units (CFUs) for viable but not culturable bacteria (microCFUs) and culturable bacteria, respectively (Duineveld & Van Veen, 1999; Insam, 2001; Goldman & Green, 2015). These simple measurements enable estimations of the number of living microbes in a sample, providing advantages compared to cell-counts, which provided the number of both living and dead cells. However, a major limitation of the approach is that it is limited to cultivatable microbes (Goldman & Green, 2015). Due to the unknown extent of species that cannot be cultivated, the soil microbiome has often been referred to as a "*black box*" (Tiedje *et al.*, 1999; Frostegård *et al.*, 2011).

Further studies focused their efforts on key enzymes found in the soil that provided insight into the microbial activity in the soil. For example, peroxidases or chitinases are used as biomarkers to assess microbial activity of soil saprobes (Insam, 2001; Caldwell, 2005; Nannipieri *et al.*, 2018; Bonner *et al.*, 2019). However, soil enzymatic assays often offer misleading results, as abiontic enzyme activity (*i.e.*, exoenzymes) cannot be accurately separated from living cells (see Nannipieri *et al.* (2018)). In addition, in the 1970's, ATP measurements in soil samples was used as molecular biomarker, allowing soil ecologist to estimate soil microbial biomass (Jenkinson & Oades, 1979; Oades & Jenkinson, 1979; Frostegård *et al.*, 2011), and later the application of gas chromatography provided detailed compositional analysis of bacterial and fungal communities using phospholipid-derived fatty acids (PLFAs) (Tunlid *et al.*, 1989; Frostegård *et al.*, 2011). More recent developments in molecular biology added new methods, such as guanine plus cytosine (G+C) content of the soil, terminal restriction fragment length polymorphism (T-RFLP), and amplified ribosomal DNA (rDNA) restriction analysis (Tiedje *et al.*, 1999; Dubey *et al.*, 2019), which enabled researchers to gain insight into microbial community compositional dynamics.

Contemporary to ATP quantification, in 1977 Sanger *et al.* (1977) proposed what now it is known as the Sanger sequencing method or Sanger sequencing, which combines a series of different techniques to obtain a four-coloured plot revealing DNA sequences (Metzker, 2010; Moorthie *et al.*, 2011). This method was the dominant sequencing method for more than two decades (Hutchison, 2007). However, despite improvements over the years, it remains an expensive and laborious method. In addition, the limiting nature of Sanger sequencing to maximum 96 sequences and lower coverage, led to the development of what is known as next-generation sequencing (NGS) (Hall, 2007; Metzker, 2010; Moorthie *et al.*, 2011). Today, two main sequencing technologies are in use, single-molecule and amplification sequencing. Single-molecule sequencing, as its name suggest, corresponds to sequencing of single DNA or RNA molecules, without the need of amplification (Thompson & Milos, 2011). Oxford Nanopore technology is an example of single-molecule sequencing which has recently been used to sequence the complete bacterial metagenome from the human gut (Moss *et al.*, 2020). On the other hand, amplification sequencing requires DNA fragmentation and amplification for sequencing. Illumina/Solexa and

Roche/454 sequencing are examples of this sequencing technology (Hall, 2007; Metzker, 2010). In contrast to single-molecule sequencing, where long-read sequences with few errors are obtained, amplification sequencing produces short-reads with higher sequencing errors produced during amplification. However, those disadvantaged are compensated by high data production and bioinformatic pipelines (Metzker, 2010; Thompson & Milos, 2011).

Further developments included metabarcoding (Bruno *et al.*, 2015; Tedersoo *et al.*, 2015) during amplification of rDNA regions (*e.g.*, 16S and internal transcribed spacer (ITS) for bacterial and fungal amplicon sequencing, respectively. See Beckers *et al.* (2016) for 16S and Santamaria *et al.* (2018) for ITS1) and shotgun metagenomics sequencing (Tedersoo *et al.*, 2015; Quince *et al.*, 2017). Furthermore, the fast advance in sequencing methods led to the development of environmental samples metatranscriptomics analyses (Carvalhais *et al.*, 2012; Moran *et al.*, 2013), which provides empirical evidence of rapid metabolic changes at community level, which has been applied in variety of system including human gut (Jorth *et al.*, 2014; Bashiardes *et al.*, 2016), biotechnology industry (Warnecke & Hess, 2009), marine ecosystem (Moran *et al.*, 2013), soil and rhizosphere (Carvalhais *et al.*, 2012; Turner *et al.*, 2013). These latest development, in addition to comprehensive microbial databases, such as SILVA (Quast *et al.*, 2012) and UNITE (Kõljalg *et al.*, 2005), for small and large subunits of ribosomal RNA (rRNA) and ITS rRNA, respectively, and computational resources such as QIIME (Caporaso *et al.*, 2012) and QIIME2 (Bolyen *et al.*, 2019), have enable us to start '*peeking*' into the black box of environmental soil samples.

Thanks to the listed advances in methods and technologies, is that in the last decade, the integration of metatranscriptomic analyses in complex samples had led to close-to-real-world findings in the plant-microbiome-soil continuum. For example, using Sanger sequencing of cloned 18S rDNA and cDNA libraries from soil extracted RNA from beech and Norway spruce forests, Damon *et al.* (2012) found that about 40 and 60% of soil sequences belonged to fungal RNA, in beech and spruce forest respectively. In addition, they found a significant enrichment in glycoside hydrolases (GH) and total transporters (*i.e.*, sugars, amino acids, P, and N) in the Norway spruce soils, although at the time of the study, the authors suggested more research before attribute the differences in soil function to the plant litter (Damon *et al.*,

2012). Similarly, using RNA extracted from roots and leaves from *Salix purpurea* was used for *de novo* transcriptome assembly to study cross-kingdom responses to petroleum hydrocarbon contaminated soils (Gonzalez *et al.*, 2015, 2018). In the leaves transcriptome, Gonzalez *et al.* (2015) found that *S. purpurea* response to soil contamination enhanced its tolerance to spidermites infection. The authors attribute this to an enhancement in spidermites reads in non-contaminated plants that were found thanks to the *de novo* sequencing (Gonzalez *et al.*, 2015). On the other hand, root transcriptomics analysis showed that abiotic stress in *S. purpurea* enhanced fungal interaction by differential expression (DE) of Sugars Will Eventually Be Exported Transporter (SWEET2) genes (Gonzalez *et al.*, 2018). In addition, they found significant shift in fungal community toward Basidiomycota genera (*i.e.*, *Heboloma*, *Galerina* and *Hypholoma*), with enrichment in monosaccharide transport and cell wall functions (Gonzalez *et al.*, 2018). The authors conclude that *S. purpurea* response to stress included a complex modulation of the belowground interaction rather than abiotic response alone, suggesting that studies of organism, either plant, fungi or bacteria response to stress should be conducted in a wider perspective.

Recently, Geisen (2021) proposed that soil microbiome studies should be performed in a multidimensional manner, including as many aspects of the soil microbiome as possible. As such, he recommends the use of multi-omics approaches in soil microbiome studies, combining different omics, such as metabolomics, proteomics, and metatranscriptomics, to interconnect metabolism, proteins and gene expression, respectively; together with an integrative microbiome view, including the virome, bacteriome, and mycobiome (Geisen, 2021). This multi-omic approach would integrate the functional community assembly with the active metabolism at the moment of sampling, providing functional insights into the different microbes involved in those processes (Geisen, 2021). However, incomplete databases and inconsistency in annotations still limit comprehensive integrative studies (Schneider *et al.*, 2021) (Figure 3).

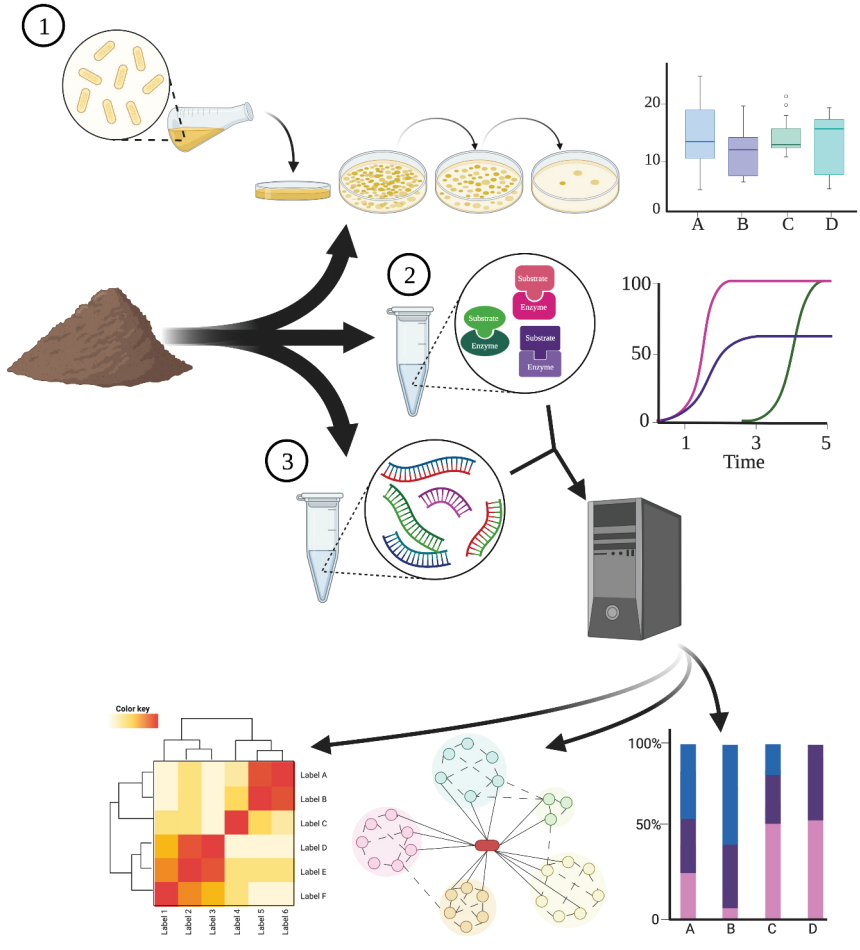

Figure 3. Summary of soil microbiome studies from section 1.2.2. From a soil sample, different methods can be choose depending on the aim of the study. (1) Soil microbes can be grown in nutritive media to then, using serial dilution or selective growing media, establish the colony-forming units. (2) Using total protein extraction, enzymatic assays can be performed to establish the dominant functional group of the soil. (3) Using DNA or RNA extraction method from the soil and sequencing technologies, soil community profiling can be done. Furthermore, combining (2) and (3) methods can lead to stronger community interpretations, correlating composition with interactions and function. Figure created with BioRender.com.

1.3 Soil microbiome and plant interactions

As partially described throughout section one, the root system, through root exudation, locally modifies the properties of the rhizosphere to improve growth and nutrient uptake. For example, white lupins (*Lupinus albus*) exude compounds that decrease rhizosphere pH, both decreasing microbial presence and enhancing P acquisition (Weisskopf *et al.*, 2006). In addition, roots exude a series compounds that act as signalling molecules that are perceived by specific functional groups of microbes with which plants can establish a symbiotic interaction, where plants provide C while the microbial partners provide limiting nutrients (*e.g.*, N, P) and water from sources inaccessible by the plants (Landeweert *et al.*, 2001). Throughout this section, I will describe the interaction between the plants and their root-associated microbiome, some of the potential microbial partners and their implications on plant growth and nutrient dynamics.

1.3.1 Root recruitment and colonization

Daily, between 10 to 40% of photoassimilated C is allocated to the root system (Wang & Ruan, 2016; Hennion *et al.*, 2019). There, C can be retained and utilized by the root for metabolism and growth, transferred to mycorrhizal fungal partners in exchange of nutrients, or be exuded into the rhizosphere (Hennion *et al.*, 2019). While the amount of C exuded is difficult to measure, the nature of these compounds has been estimated in Arabidopsis seedlings to be comprised of about 3% simple sugars, 4% sugar alcohols, 10% amino acids, 19% phenols and more than 50% of unknown compounds (Hennion *et al.*, 2019). In addition to the chemical composition, the exudates have also been characterised on the basis of molecular weight: high-molecular weight exudates (*i.e.,* polysaccharides, enzymes, fatty acids, tannins, flavonoids, growth regulators, nucleotides, steroids, terpenoids, alkaloids, polyacetylenes and vitamins; about 13%); and exudates with low-molecular weight (*i.e.*, mono- and oligosaccharides, amino acids, organic acids, and phenolic compounds; about 10%) (Wang *et al.*, 2006; Herz *et al.*, 2018). Within the exudates some, such as mucilage, function in protection and also stabilize soil aggregation in the rhizosphere, enhancing both root growth and nutrient acquisition (Haichar *et al.*, 2014). At the same time, some other exudates act as signalling molecules. These signals can be attractants and stimulants (*e.g.*, strigolactones, sugars, amino acids, organic acids) for bacteria or mycorrhizal fungi (Baetz & Martinoia, 2014; Haichar

et al., 2014), or inhibitors and repellents (*e.g.*, phenolics and terpenoids compounds, phenylpropanoids) of parasites or pathogens (Baetz & Martinoia, 2014; Haichar *et al.*, 2014).

In addition to the root exudates, both bacteria and fungi secrete lipochitooligosaccharides (LCOs), compounds that enhance root receptiveness. Such compounds are known as nodulation (Nod-) factors in *Rhizobium* and mycorrhization (Myc-) factors in arbuscular mycorrhizal (AM) fungi (Streng *et al.*, 2011; MacLean *et al.*, 2017). Both Nod- and Myc-factors are, chemically speaking, polymers of N-acetyl-D-glucosamine, which vary in the number of units (Limpens *et al.*, 2015). Despite the similarities in these compounds, both Nod- and Myc-factors are perceived by different receptors that induce the nodulation or mycorrhization response. Nod-factor perception is considered a feature unique for legume plants while Myc-factors are broadly spread in plants (Streng *et al.*, 2011). The reception of Nod- and Myc-factors induces the common symbiotic signalling pathway (CSSP) in plants (Parniske, 2008; Genre *et al.*, 2020), which ends with the bifurcate response of nodulation or endomycorrhiza formation. The later evolution of the rhizobial symbiosis suggests that this association has recruited pre-existing components of the AM symbiosis (Oldroyd, 2013; MacLean *et al.*, 2017) (Figure 4).

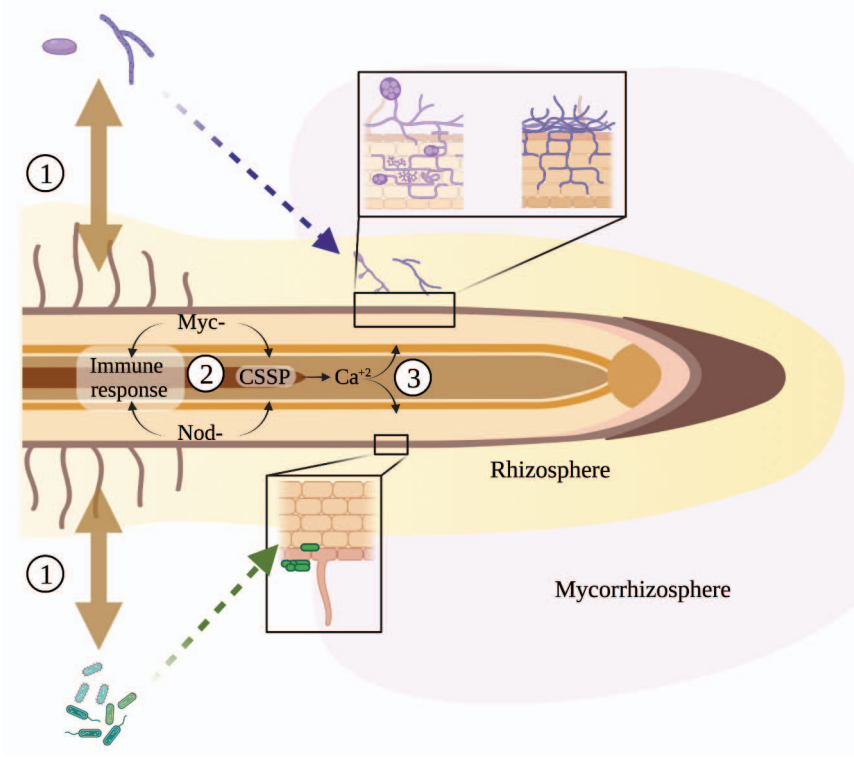

Figure 4. Root colonization by bacteria and mycorrhizal fungi. (1) Microbiome recruitment starts with plant exudation of attractants and stimulants chemical compounds that induces microbial growth toward the roots. (2) Microbes exude lipochitooligosaccharides (LCOs, Myc- and Nod-factors) that prepare the root for the symbiotic interaction. Close, chemically related chitooligosaccharides have been shown to induce immune response rather than CSSP, as fail in induce strong Ca^{+2} signalling. (3) Symbiotic interaction finish with the successful colonization of the rhizoplane or arbuscular or ectomycorrhizal formation. Based on Streng *et al.* (2011), Baetz & Martinoia (2014), Haichar *et al.* (2014), Glick (2015), Limpens *et al.* (2015), MacLean *et al.* (2017) and Hennion *et al.* (2019). Figure created with BioRender.com.

The successful establishment of bacterial and fungal symbiotic interactions with the root systems, and the beneficial impact this has on plant growth, has led to the classification of the bacterial and fungal partners as 'plant growth-promoting microbes' (PGPM), which has recently gained attention given its potential for agronomic production, soil health and climate change mitigation (Naik *et al.*, 2019; Breakfield *et al.*, 2021; Fiodor *et al.*, 2021). Within PGPM, plant growth-promoting bacteria (PGPB), mycorrhizal (or mycorrhiza*tion*) helper bacteria (MHB) and mycorrhizal fungi are among the

most well-known. The interactions between these soil microbial groups have direct impacts on the root recruited community. For example, Nguyen & Bruns (2015) found significant differences in bacterial composition in EcM roots tips compared to non-colonized root tips and the rhizosphere in a monoculture stand of *Pinus muricata* growing on the west coast of the USA. In their results, six out of 10 pairwise comparisons were significantly different in bacterial composition between the five most common EcM fungi present in the EcM root tips, where two *Burkholderia* (*B. phenazinium* and *B. sordidicola*), with potential plant growth-promoting functions (Compant *et al.*, 2008; Lladó *et al.*, 2014), where the most abundant (Nguyen & Bruns, 2015). Similarly, Sakoda *et al.* (2019) found significant differences in Actinomycetes (or Actinomycetales, order of gram-positive, anaerobic bacteria) both outside and inside of *Pinus thunbergia* roots colonized by *Cenococcum geophilum* compared with non-colonized roots, in a coastal forest in central Japan. They found that the root surface bacterial community was dominated by *Streptomyces*, while inside the root the genus *Actinoallomurus* was the dominant (Sakoda *et al.*, 2019). Both bacterial genera have been described to have antibiotic properties (Pozzi *et al.*, 2011; de Lima Procópio *et al.*, 2012). Such results provide evidence of the importance of the microbial interactions in the root vicinity and surface, as synergic functions such as antibiotic protection and enhanced nutrient uptake can occur simultaneously. In the next sections, bacterial and mycorrhizal partners will be further described. MHB will, however, be described together with PGPB, as many bacterial genera present both functions.

Bacterial partners

PGPB, as their name suggests, directly promote plant grow through different mechanism that include modulation of endogenous phytohormones, through bacterial production of, for example, auxins, cytokinin and GA (Kang *et al.*, 2014; Kudoyarova *et al.*, 2017; Iftikhar & Iqbal, 2019; Jochum *et al.*, 2019), enhanced plant stress tolerance through production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase or modulation of endogenous ABA levels (Kang *et al.*, 2014; Cedeño-García *et al.*, 2018), and enhancement of nutrient availability through N fixation, P solubilization and Fe chelation (Kudoyarova *et al.*, 2017; Pahari & Mishra, 2017; Cedeño-García *et al.*, 2018; Matse *et al.*, 2020). Indirect plant growth promotion include production of compounds with antibiotic function, such as antibacterial, antifungal, and phytotoxic compounds, that reduce phytopathogens and

parasites in the rhizosphere (Nejad & Johnson, 2000; Olanrewaju *et al.*, 2017). In addition, some PGPB produce exoenzymes capable of degrading fungal cell walls, which in combination with hydrogen cyanide (HCN) production, has been observed to act as a potent biocontrol agent in the roots (Olanrewaju *et al.*, 2017; Pahari & Mishra, 2017). Similarly, PGPBs occupy the root surface (*i.e.*, niche competition), preventing other bacteria from colonizing the root and limiting the ability of phytopathogens from reaching virulence levels (Bais *et al.*, 2006; Badri *et al.*, 2009; Olanrewaju *et al.*, 2017). In addition to their PGPB properties, some bacterial genera also have MHB properties, enhancing mycorrhizal formation and having synergic positive effects on plant growth (Tarkka & Frey-Klett, 2008; Santoyo *et al.*, 2021).

Mycorrhizal partners

Mycorrhizal fungi correspond to a symbiotic association between a fungus and a plant's root system (Kirk *et al.*, 2008). Broadly, two major types of mycorrhizal fungi exist: endomycorrhizas (EdM) and EcM (Leake *et al.*, 2004). EdMs correspond to a mycorrhizal interaction in which the fungal hyphae penetrate the roots and grow between the plasma membrane and the cell wall of the plant cells, forming specialized exchange structures (*i.e.*, arbuscules, coils & pelotons) (Figure 5a). Within the EdM, three types can be found: AM, ericoid mycorrhiza (ErM) and orchid mycorrhiza (OM) (Perotto *et al.*, 2018; Genre *et al.*, 2020).

AM are the most wide-spread type of mycorrhizal interaction, with an estimated 70 to 90% of all land plants able to host an AM fungi (Parniske, 2008; Genre *et al.*, 2020). Taxonomically speaking, all AM are from the Glomeromycota phylum, and interact with the plant host in a generalist way, having little specificity in their hosts (Parniske, 2008; Oehl *et al.*, 2011; Kehri *et al.*, 2018). This type of mycorrhiza is thought to have a global impact on the C cycle, as about 5 billion tonnes of C per year are delivered from the plant to its AM fungal partners (Parniske, 2008). As endosymbionts, AM cannot complete their life cycle without a plant host (Akiyama & Hayashi, 2006). Their spores can germinate in the soil under favourable conditions and grow a short hyphae that becomes quiescent in the absence of a host (Xie *et al.*, 2010) (Figures 4 and 5).

In addition, ErM and OM are two mycorrhizal associations that are formed with Ericaceae and Orchidaceae, respectively (Perotto *et al.*, 2018). ErM interaction occurs mostly in acidic, poor soils where nutrients are fixed

in hard-to-metabolize organic matter (Kohout, 2017), making ErMs key players in Ericaceae plant nutrition. On the other hand, OM fungi correspond to free-living fungi that do not depend on orchids to complete their life cycle (Jacquemyn *et al.*, 2017). In this mycorrhizal interaction, the orchids need their fungal partners to complete their life cycle, as they are obligatory mycoheterotrophic (*i.e.*, plant-fungi interaction where plants obtain their C from fungal partners) during seed germination, as orchid seeds lack of endosperm (Leake, 2004; Jacquemyn *et al.*, 2017). Interestingly, some OM fungi have been shown to form EcM associations with tree species, as is the case of *Russula* sp. (Genre *et al.*, 2020).

EcM fungi correspond to the most common mycorrhizal formation in temperate and boreal forests, occurring also in large forested zones in tropical and subtropical regions (Kumar & Atri, 2018). Originating from saprotrophic ancestors, EcM can oxidize SOM following Fenton chemistry or releasing oxidative enzymes (Lindahl & Tunlid, 2015; Shah *et al.*, 2016), releasing organic-bound N and delivering it to their plant hosts (Kumar & Atri, 2018). Based on soil properties, the SOM degrading capacity of EcM fungi, coupled to their own metabolic requirements, suggests that EcM fungi can aggravate nutrient limitation (Alberton *et al.*, 2007; Näsholm *et al.*, 2013; Ågren *et al.*, 2019; Pellitier & Zak, 2021). However, the field conditions under which the exacerbation of nutrient limitations can occur requires further study, given the near ubiquitous nature of the EcM symbiosis.

As for other symbionts, EcM have been shown not only to provide nutrients, but also to protect their host from abiotic stressors such as drought (Li *et al.*, 2021), high salt content (Guerrero-Galán *et al.*, 2019), and heavy metal toxicity (Colpaert *et al.*, 2011). Given these beneficial properties, EcM fungi have been proposed as candidates to restore degraded temperate and boreal forest lands (Policelli *et al.*, 2020). However, perhaps the most known beneficial effect of EcM is to provide N and P to their plant host, which enhances C-fixation into biomass (Terrer *et al.*, 2019). Similarly, some EcM such as *Laccaria bicolor* have been shown to simultaneously promote *Pinus massoniana* growth, by providing nutrients (P, calcium and magnesium) while limiting Al content (Gu *et al.*, 2019). Such beneficial effects on stress tolerance and plant nutrient status have been proposed to act as a buffer for plants against extinction (see Bennett & Classen, 2020) under climate change, although more research needed on this ecosystem buffering potential of the plant-EcM association.

As the dominant mycorrhizal group in forests ecosystems, EcM fungi are found in high proportion in the soil. As such, EcM fungi can form symbiotic interaction with different plant species and a single plant can host different EcM (Lofgren *et al.*, 2018) (Figure 5b). The high diversity of EcM fungi, their broad plant host range, and its implications on soil nutrient dynamics, particularly N and C, has global implication on SOM accumulation (Buzzard *et al.*, 2019; Soudzilovskaia *et al.*, 2019; Lindahl *et al.*, 2021; Sokol *et al.*, 2022). The apparent interconnection observed between different plant species through mycorrhizal partners, and the potential effect on forest ecology will be described in the next section.

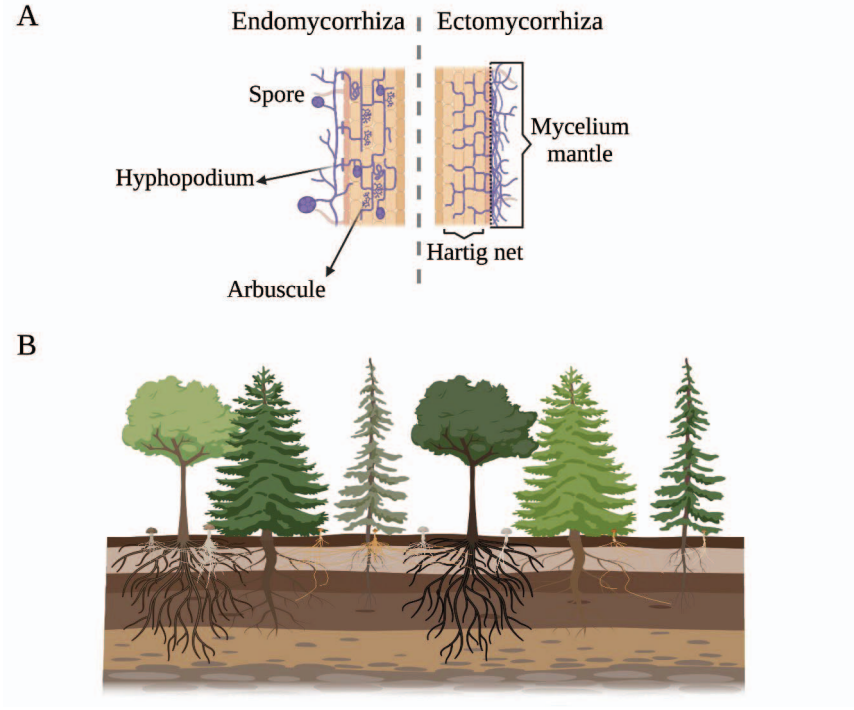

Figure 5. (**A**) Endo- and ectomycorrhizal morphology inside the root. (**B**) Common mycorrhizal network connecting different trees. Figure created with BioRender.com.

1.3.2 Common mycorrhizal network (CMN)

Given the ability of the mycorrhizal fungi to form symbiotic interactions with different plant species, the concept of a common underground network interconnecting different plant species has developed (Whitfield, 2007)

(Figure 5b). This network, known as the common mycorrhizal (or mycelial) network (CMN) suggests that different plant species might be connected at the community level, and be able to share resources according to the needs of the individual within that network (Simard *et al.*, 1997a). Evidence of direct C transfer from a donor plant to a receiver comes from Francis & Read (1984) using autoradiography with labelled ^{14}C. In their work, using *Pinus contorta* and *P. sylvestris*, they tested the principle of C transfer to a shaded (*i.e.*, receiver) plant from an unshaded (*i.e.*, donor) connected by a single mycorrhiza species in controlled chamber conditions (Finlay & Read, 1986). They found that C transfer was significantly increased due to the presence of mycelial connections and suggested that such transfer was induced by a concentration gradient toward the shaded plant (Finlay & Read, 1986). In subsequent studies, the principle was extended to legumes (Bethlenfalvay *et al.*, 1991; Frey & Schüepp, 1992), although in both experimental designs the authors did not find N transfer through mycorrhizal network. Bethlenfalvay *et al.* (1991) tested N transfer from N fertilized soybean plants to maize connected through AM mycelium. In their results, they found increased biomass on the maize plants connected to the fertilized soybean plant, although they argue that such response might be related to plant-to-plant N flux or direct N uptake from the AM fungi in the fertilized compartment more than CMN transfer. Similarly, Ekblad & Huss-Danell (1995), tested N transfer from grey alder inoculated with *Frankia* to Scots pine through *Paxillus involutus* mycelia. In their results, they found no significant differences in the N starved pines. Furthermore, contrasting their results with non-inoculated controls, they found that *P. involutus* mycelial network had negative effects on the inoculated alders, which had lower N content and biomass than non-mycorrhizal controls (Ekblad & Huss-Danell, 1995), suggesting that the CMN was aggravating the nutrient limitation. Later, Simard *et al.* (1997a; b) suggested that the CMN could reciprocally transfer C between *Betula papyrifera* and *Pseudotsuga menziesii*, growing either in pot or in the field. Although they did not find significant differences either in net or bidirectional C transfer compared with the CMN destroyed controls, suggesting potential C soil transfer rather than CMN transfer. After their results, the scope of the CMN was expanded to included water movement (Egerton-Warburton *et al.*, 2007; Prieto *et al.*, 2016) and information transfer (*i.e.*, biotic stress-induced signalling) (Barto *et al.*, 2012; Babikova *et al.*, 2013a; b). The idea of a stable and large belowground network modulating

nutrient and information distribution at an ecosystem level led to some authors to propose the so-called *'Wood-wide web'* (Simard *et al.*, 1997c; Giovannetti *et al.*, 2006; Beiler *et al.*, 2010), where larger trees would facilitate regeneration and ecosystem stabilization.

As positive as the CMN could be, there are some qualifications that must be considered. First, *c.* 15% of all vascular plants are non-mycorrhizal, thus, mycorrhizal fungi are sensed by these species as antagonists (van der Heijden & Horton, 2009). Second, nutrient transfer to the plant host is context dependent, suggesting that in low nutrient environments, mycorrhizal fungi can even aggravate nutrient limitation (Näsholm *et al.*, 2013) following a mutualism-parasitism continuum (Neuhauser & Fargione, 2004), which means that even if connected through the CMN, one plant can be benefited while the second can be negatively affected. Similarly, several studies have found that the CMN increases competitive pressures instead of alleviating them, as adult trees can provide more resources to mycorrhizal fungal partners than seedlings, out competing the seedlings rather than assisting them by providing resources via the CMN (Kytöviita *et al.*, 2003; Merrild *et al.*, 2013; Fellbaum *et al.*, 2014; Weremijewicz *et al.*, 2016). This is evident in experiments where belowground C allocation from adult trees was disrupted, either by canopy gaps or stem girdling, increasing seedling establishment and growth (Axelsson *et al.*, 2014; Pasanen *et al.*, 2016), indicating that a disruption of the CMN is beneficial for seedling establishment.

Using network theory, Southworth *et al.* (2005) tested two alternatives for CMN studies, the phytocentric and the mycocentric. In their study, they sampled roots from 20 trees and fungal morphotypes from each and tested if the network architecture was either random or free-scale for both alternative approaches (Southworth *et al.*, 2005). In their results, they found that from the phytocentric perspective, that the network had a random organization, where the trees had a similar number of links (Southworth *et al.*, 2005). On the other hand, the mycocentric perspective showed a scale-free distribution, suggesting that few fungi had more connections, acting as 'hubs' (Southworth *et al.*, 2005). Both approaches together suggest that all trees have relatively equal importance (*i.e.*, all trees have the same ecological value, having equal chances to be colonized) while few fungi can have a more important role (*i.e.*, few fungi are more successful at colonizing roots) (Southworth *et al.*, 2005; Selosse *et al.*, 2006). A similar analysis suggested

that through the mycocentric perspective, trees would be acting either as resource island (*i.e.*, active nutrient exchange between fungi and host) or a storage unit (*i.e.*, nutrients remains in fungal tissue in the form of storage compounds) of nutrients for the fungi, depending on whether the host is C-rich or C-limited, respectively (Lekberg *et al.*, 2010). Furthermore, following market theory, it has been proposed that mycorrhizal fungi would relocate more to '*good trading partners*' (Kiers *et al.*, 2011; Franklin *et al.*, 2014; Werner *et al.*, 2014). Thus, mycorrhizal networks might not distribute nutrients equally in the system, as nutrients are allocated by the fungi in response to the requirements of the fungus for growth and expansion of its own mycelial network (see Heaton *et al.* (2012)).

The controversy behind nutrient transfer through the CMN and its real biological meaning remains, as inconclusive results keep arising given the difficulties in directly measuring transfer from 'donor-root' to CMN and then to 'receptor-root' (He *et al.*, 2003; van der Heijden & Horton, 2009). In addition, most studies have assumed that the CMN is a long, stable mycelial network that can last indeterminately (Simard *et al.*, 2012), while in reality, the mycelia network is highly dynamic, with a quick turnover (Taylor, 2006). Furthermore, the recent anthropomorphism of the phytocentric perspective of the CMN and the nutrient cycling in the forests (*cf.* Simard (2018)) has led to the notion that the mycorrhizal fungi works as an intermediary between different plant species following a source-sink pattern. As such, little emphasis has been given to the economy associated to nutrient transfer through the mycelia. For example, nutrient transfer within the fungal hyphae follows a mass flow pathway toward the tip of an extending hyphae (Heaton *et al.*, 2012; Werner *et al.*, 2014), which extends in direction to located nutrient patches. Thus, mycorrhizal fungi–plant interactions are sustained by a C economy, where mycelium grows towards nutrients that can further sustain mycelial growth, while decaying where nutrients are not enough to sustain further mycelial grow (Taylor, 2006; Heaton *et al.*, 2012). Furthermore, Akroume *et al.* (2019) found the first evidence that the EcM *Paxillus involutus* can access and mobilize C and N from fungal necromass, adding more complexity to the fungal nutrient transport, as fungal necromass, such as decaying mycelium, can be nutrient patches. This means that a revision of the CMN nutrient transport theory is necessary, as most of the studies interpret their results on nutrient content transfer from donor to receiver plant, assuming that the nutrient mobilization was through CMN.

Throughout this introduction, I have described how changes in plants, microbiome or soil have a direct impact on the other components in this tripartite interaction, where a continuum between the plants, the microbiome and the soil can be established to apply better management strategies in forest production or conservation, exploiting the natural benefits that the local soil microbiome provides to the plants.

2. Objectives

To understand the mechanisms behind the coordinated response of the plant-microbiome-soil continuum, both plant and soil microbiome responses must be quantified with a wide variety of tools. Thus, the aim of my thesis was to establish a comprehensive link between the plant-microbiome-soil continuum, using plant growth measurements, together with next-generation sequencing (NGS) and bioinformatics methods. To achieve this aim, the following specific objectives were addressed through the experiments described by the manuscripts that I have included in this thesis:

1. Establish the effect of edaphic properties on seedling establishment and survival, and microbial recruitment (**Papers I**, **II** and **III**).
2. Determine the effect of different N addition types on Scots pine or Norway spruce seedlings establishment and growth, and its impact on the root-associated fungal recruitment (**Papers II**, **III**, **IV** and **V**).
3. Assess the effect of changing Norway spruce N-status on the root transcriptome and its impact on the coordinated plant-EcM response (**Papers IV** and **V**).

My experiments provide novel insight into the linked response of soil microbiome to newly established seedlings into soils with different properties and nutrients, either by natural variations or by anthropogenic cause.

3. Results and Discussion

The plant-microbiome-soil continuum, and ultimately plant performance, involves an array of components where biotic and abiotic factors vary spatiotemporally. Broadly speaking, edaphic properties, such as water holding capacity, mineral content, particle aggregation, among others, control both soil microbiome diversity and the success of seedling establishment. The soil microbiome, in turn, can either enhance or decrease nutrient and water uptake and seedling survival. In addition, growing plants can locally modify soil properties and recruit soil microbes. The tight interaction between soil properties, the soil microbial community and plant growth has great importance in both ecological and economic contexts. For example, geographical location, site preparation and native biodiversity, all play important roles in seedling establishment, soil health and plant nutrient uptake. Such implications become even more important in northern hemisphere countries, where the boreal forest extends over large parts of Fenno-Scandinavia, Eurasia, and North America, in which the forest industry is often central to these countries' economies. Throughout **Papers I-V** I have evaluated the effects of modifications to edaphic and plant nutritional status, and the role of the associated microbial community on tree seedling establishment and growth.

3.1 Effects of soil properties on plant growth

Edaphic properties group different characteristics that include soil nutrient content, soil particle density, moisture, conductivity and mineral concentration (Hulshof & Spasojevic, 2020). These characteristics, which can vary on very local scales, can be used to predict plant distribution when used along climate gradients at regional scales (Beauregard & de Blois,

2014). Thus, edaphic properties have been recognized as second in importance after climate as a driver for plant distribution (Hulshof & Spasojevic, 2020). Furthermore, Beauregard & de Blois (2014), based on predictive models constructed with climate-, edaphic- and a combination of both variables, found that for some plant species, such as *Sphagnum* sp., edaphic properties may be even more important predictors (*e.g.*, soil drainage and humus content) than climate variables. Thus, the predictive importance of edaphic properties on plant distribution has the potential to provide important information about plant establishment success, as well as for understanding species distribution shrinkage, lack of new recruitment or uncontrolled expansion of invasive plants (Shackleton *et al.*, 2014; Burge, 2020; Hulshof & Spasojevic, 2020). Understanding the interaction between edaphic properties and plant species establishment and success is therefore important for both ecologic and economic purposes. For instance, more than 50% of the biodiversity hotspots of the world are in soils with particular characteristics (Hulshof & Spasojevic, 2020). For example, it has been recently found that magnesium-rich dolomite soils (*i.e.*, sedimentary, double carbonate soils) are widespread around the world (Mota *et al.*, 2021), with many of them located within global hotspots (Hulshof & Spasojevic, 2020). These soils are inhabited by highly specialized flora, having a rich endemism (Mota *et al.*, 2021). On the other hand, other plants species have evolved adaptations to highly specific soils, such as *Jacobaea auricula*, which grows in salt-rich outcrops (Bobo-Pinilla *et al.*, 2021) or gypsophytes, which grow in gypsum (*i.e.*, soft sulphate mineral composed of calcium sulphate dihydrate) soil outcrops (Queiroz *et al.*, 2012). These plants, known as edaphic endemic, require highly specific soil properties to establish and grow (Burge, 2020).

Edaphic endemic plants are a good example of how edaphic properties impact on plant distribution and growth, as well as the degree to which plant species can adapt to extreme soil conditions. However, not all plant species that have a restricted distribution range are edaphic endemics. For example, *Myrcianthes coquimbensis,* an endemic shrub from northern Chile, has a distribution spanning only 60 km along the Chilean coast (García-Guzman *et al.*, 2012). Similarly, *Prosopis reptans*, has only three populations located in the southern limit of the Atacama Desert (Zöllner & Olivares, 2001; Contreras *et al.*, 2020) and *P. tamarugo*, which is limited to the Atacama Desert. Such plant species are at high risk of extinction and thus, identifying

the reasons for their distribution shrinkage, and identifying potential solutions, are needed.

The link between the growth conditions provided by the soil and plant productivity has been tested by soil and forestry scientists, providing a robust theoretical framework on the beneficial effects on seed germination, plant establishment and growth of, for instance, site preparation and nutrient amendment (see section 3.2) (Mäkitalo, 1999; Archibold *et al.*, 2000; Prévost, 2004; Löf *et al.*, 2012; Häggström *et al.*, 2021). Despite this, less is known about the edaphic properties, including the potential changes in soil uses due to human activity, on the soil microbiome and its impact on plant establishment, distribution, and growth. Thus, in this section, I will discuss the main results of the study of the effect of edaphic properties on seedling establishment, growth, and soil microbial recruitment in different soils from different climatic zones in Chile (**Paper I**) and Sweden (boreo-nemoral zone; **Paper II**).

3.1.1 Edaphic properties

Seedling establishment is one of the most critical processes for plant growth. Thus, where a seed falls after dispersion impacts establishment success. Broadly, once a seed is imbibed, a series of transcriptional changes involving ABA and GA and other phytohormones leads to the radicle protrusion. However, this implies that passive water movement through the seed coat can carry ions that are highly abundant in the soil, leaving the embryo vulnerable to osmotic stress or toxic ion concentrations (Li, 2008) (Figure 1). Some plants, known as xerophytes and phreatophytes, have evolved adaptations to tolerate highly toxic levels of ions. For example, *Prosopis* species have documented tolerance to high NaCl concentrations during seed germination (Felker *et al.*, 1981; Westphal *et al.*, 2015). However, despite its high tolerance to stress and documented invasiveness in similar environments (Shackleton *et al.*, 2014), most *Prosopis* species in Chile are threatened or near to extinction (**Paper I, Figure S1**). In **Paper I**, I evaluated the impact of soils with different properties and microbial communities on the establishment of seedlings of two *Prosopis* species from northern Chile. The aim of the study was to determine whether the edaphic properties of the soils, the microbiomes that they harboured or both, were contributing factors to the low natural seedling establishment success of *Prosopis chilensis* and *P. tamarugo*. The results of **Paper I** suggest that soil properties have a major

impact on establishment, with no differences by *Prosopis* species (Figure 6a, Cox proportional hazard *p-value* > 0.05). In the study, Atacama Desert soil, corresponding to the northmost soil type collected (**Paper I, Figure 1**), contained extremely high levels of ions such as chloride, boron, sulphates, sodium, lithium, magnesium, and potassium (**Paper I, Table 1**), which were too high to allow establishment of seedlings of either *P. chilensis* or *P. tamarugo* (Figure 6a).

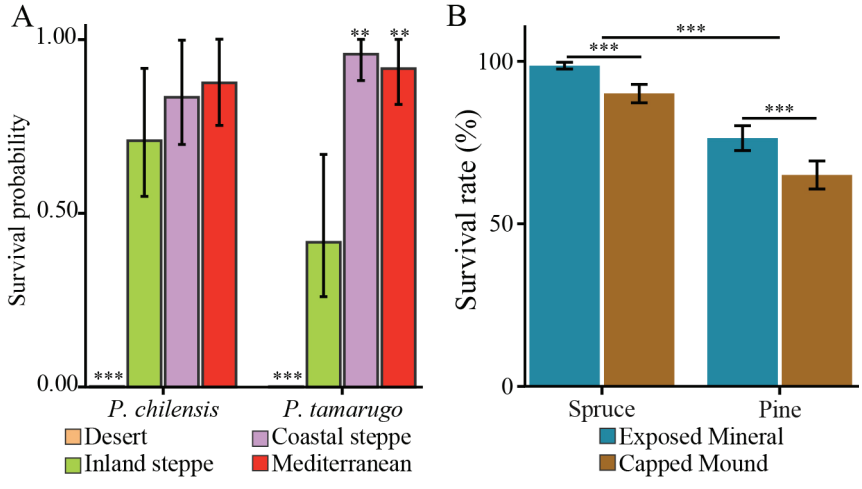

Figure 6. Seedling survival of *Prosopis* species used in **Paper I** (**A**) and conifer species used in **Paper II** (**B**) growing in different soils. Error bars corresponds to 95% confidence interval. Asterisks (*) corresponds to statistical differences after Cox proportional hazard test.

In 1981, Felker *et al.* tested the growth of six *Prosopis* species (*i.e.*, *P. articulata*, *P. chilensis*, *P. glandulosa*, *P. pallida*, *P. tamarugo* and *P. velutina*) in N free media with increasing NaCl concentrations. In their results, they found that all but *P. velutina* were able to grow at 36000 $mg \cdot L^{-1}$ NaCl, (*i.e.*, equivalent to about 600 mM). Similar results were found by Westphal *et al.* (2015) in a germination assay of *P. chilensis* seeds in increasing NaCl concentration, where 600 mM greatly diminished gemination, although still permitting a germination success of around 30%. In our results, the NaCl content of the Atacama Desert soil was equivalent to *c.*1 M of NaCl (**Paper I, Table 1**), which has been observed to induce seed death in *P. chilensis* (Ibáñez, personal communication). These toxic levels of salt are known to trigger the increase of ROS by reducing the activity of

antioxidant enzymes (Dash & Panda, 2001), which induces oxidative damage and apoptosis in the cells (Gomes & Garcia, 2013; Zhang *et al.*, 2015). In addition, *P. tamarugo* had significantly lower survival in coastal steppe soils, which had high content of SO_4^{2-} and K^+, which are known to impair seed germination in *P. strombulifera* (Sosa *et al.*, 2005). However, once established no significant differences were found in the height growth of the *Prosopis* species tested, both species performing equally well in all three soils. In addition, *P. chilensis* had similar shoot water potential values in all three soils, suggesting good adaptation to different edaphic conditions. *Prosopis tamarugo*, on the other hand, performed better in Mediterranean soils, having significantly higher shoot water potential than in coastal and inland steppe soils (**Paper I, Figure 1c**). All together, these findings suggest that *P. tamarugo* may be more sensitive than *P. chilensis* to soil ion content during early establishment stages, showing osmotic stress symptoms (Figure 6a).

As previously stated, edaphic properties can vary on local scales. This is especially true when considering soil horizons, where SOM immobilization and mineralization processes have different depths and rates depending on soil temperature, plant litter and the composition of the soil microbiome (McGuire *et al.*, 2013; Gora *et al.*, 2019). Therefore, forest industries often prepare the soil before planting to improve seedling establishment (Löf *et al.*, 2012). The Swedish forest industry recommends site preparation with disc trenching and mounding (Skogsstyrelsen, 2020; Häggström *et al.*, 2021) because this improves SOM content, soil temperature and reduces exposure to frost damage and flood risk (Häggström *et al.*, 2021). In **Paper II**, I evaluated the effect of planting Scots pine and Norway spruce seedlings into exposed mineral soil and capped mounds on establishment and growth after two growing seasons in central Sweden. One of the aims of the study was to determine the impact of planting position on outplanting success of nursery grown seedlings. After one growing season, seedling survival was significantly higher in exposed mineral soil for both Scots pine and Norway spruce (Figure 6b). Similar results were found by Archibold *et al.* (2000) in the Canadian boreal forest using white spruce (*Picea glauca*), where lower mortality and larger plants were found in drum chopped plus site preparation treated sites. In their experiment, the authors suggest that the combination of reduced competition from drum chopper treatment and better soil properties in the trenches were the main factors aiding survival (Archibold *et al.*, 2000).

Similarly, Hawkins & Moran (2003) found significantly improved establishment and growth of *Abies amabilis* naturally regenerating in a clear-cut sites compared to tree retention and shelterwood treatments located in southwest Canada. Such benefits were further increased when competition with *Vaccinium* sp. was alleviated. The authors suggest that the better establishment and growth of *A. amabilis* was thanks to the reduced competition for nutrients and light (Hawkins & Moran, 2003). In our results, the lower survival observed in both Scots pine and Norway spruce in capped mound soils could be explained by the mechanical turning of the organic layer, which does not always leave the capped mound soil appropriately compacted, leaving 'air pockets' where seedling roots do not have support for growth and are susceptible to faster drying (Löf *et al.*, 2012). During the first month of establishment of the seedlings, the site was unusually dry for the region. This is in agreement with the results of Häggström *et al.* (2021), where survival of Scots pine seedlings in capped mound soils was better in sites with more precipitation. In contrast, as mineral soil remains compact and wet for longer, it provided a better establishment environment for the growing seedling and thus, the higher survival probabilities.

In addition to survival, biomass production (*e.g.*, aboveground volume, *field* root biomass) was measured in both Scots pine and Norway spruce seedlings, with the latter having significantly better outplanting performance on mineral soil, whereas Scots pine seedlings performed equally well in both mineral and capped mound soils (**Paper II, Figure S1**). Häggström *et al.* (2021) reported similar results on Scots pine from 11 different locations in Sweden. As a pioneer species, Scots pine is often one of the first species to colonize disturbed, low-nutrient sites (Houston Durrant *et al.*, 2016). These ecological characteristics support our results of Scots pine growth, where it performed equally well in both soils. On the other hand, Norway spruce is a drought-sensitive species, given its shallow root system (Caudullo *et al.*, 2016), which grows better in wetter, more nutrient rich soils. Furthermore, in our results, no significant differences were found in N and C between mineral and capped mound soils, which suggest that the mineral soil of the site was high in nutrients **(Paper II, Figure 1b-c**). In a study performed along an ecological gradient, testing the effect of mixing Norway spruce stands with European beech (*Fagus sylvatica*), nutrient-rich sites were better for pure Norway spruce stands than mixed, as European beech added competitive pressure to Norway spruce, while in poor-nutrient sites,

European beech alleviated nutrient limitations through litter allocation and enriched decomposition (Pretzsch *et al.*, 2010). This suggests that Norway spruce establishment and growth is highly soil-dependant, where richer- and wetter-sites enhances performance, performing better than pioneer species, such as Scots pine.

Altogether, these results confirm that edaphic properties play a central role in plant establishment and growth and that the intrinsic tolerance and ecology of the different plant species corresponds to responses to such soil conditions. In addition, these results suggest that species with wider distributions (*i.e.*, *Prosopis chilensis* and Scots pine) might have broader physiological tolerances or mechanisms to cope with adverse edaphic properties (*e.g.*, better drought tolerance, water-use-efficiency, lower nutrient requirements) than plants with a smaller distribution range (*i.e.*, *Prosopis tamarugo* and Norway spruce). However, '*soil properties*' does not only include physicochemical characteristics, as soils also harbour thousands of different microbes that have adapted to the soil and may enrich or inhibit plant performance. Thus, in the next section, I will address the impact of edaphic properties on the bulk soil microbiome (*i.e.*, the microbiome presents in the soil without direct root influence) and on the recruited microbiome (*i.e.*, microbiome present in rhizosphere and the root).

3.1.2 Soil microbiome

The diversity and activity of the soil microbiome is closely linked to edaphic properties of the soil, where microbes compete for available organic matter and essential-for-growth nutrients (Jansson & Hofmockel, 2018). In this competitive environment, roots are hotspots where the soil microbiome changes due to the influence of root exudates, which attract potentially beneficial microbes to establish mutualistic interactions. However, although diminished, soil microbes are active in the bulk soil, where they respond to the edaphic properties of the soil. In **Paper I** and **II**, I evaluated the soil microbiome community composition from the different soils where *Prosopis* species (**Paper I**) and Norway spruce and Scots pine (**Paper II**) where establishing. In addition, in **Paper I-III**, I evaluated the impact of the establishing seedlings in the soil microbial community composition and the resulting community recruited in the rhizosphere and the roots. In **Paper III**, using Scots pine seeds germinating in-site using a germination matrix (*i.e.*,

SeedPAD®), I evaluated the fungal recruitment capacity of new germinant establishing in mineral soil from a clear-cut in northern Sweden (**Paper III**).

Bulk soil microbiome

In **Paper I**, in addition to the high ion content and seedling mortality observed, Atacama Desert soil was characterised by a low bacterial and fungal presence, assess by CFUs and microbial richness derived from annotated amplicon data. This resulted in a soil community substantially different from the other three soils in which seedlings did germinate and establish (**Paper I, Figure 3**). Dissimilarity analysis, which provides information about the taxa that make the soil microbiomes different, suggested that overall, the microbial community of each soil is adapted to that soil type or locality. For example, the microbial community of the Mediterranean soil is characterised by the presence of Chitinophagaceae and Thermoleophilia, which are chitin (Rosenberg, 2014) and N-alkane degraders (Gómez-Lama Cabanás *et al.*, 2018; Hu *et al.*, 2019), respectively, and EcM fungi such as *Cortinarius*. This community composition suggests a soil rich in hard-to-metabolize SOM and plant-derived organic matter, which is consistent with the location where the soils where collected. Even the coastal and inland steppe soils, which were geographically close (**Paper I, Figure S1**), had distinctive soil microbial communities, with the coastal steppe having a clear dominance of members of the *Bacillaceae* family, which has several members that are salt- and metal-tolerant (Zhang *et al.*, 2018; Wang *et al.*, 2019). In contrast, the dominant microbes of the nearby inland steppe soil were associated with heat- and salt- resistance, *e.g. Rhodothermaceae* sp. (Genderjahn *et al.*, 2018) and *Bacillales* sp., and chemo-organoheterotrophs such as *Longimicrobiaceae* sp. (Pascual *et al.*, 2016), which reflects microbial adaptations to the high soil conductivity and ion content (*e.g.*, phosphate, sulphate, calcium). Local variations in soil microbial communities have been found to be as close as the centre, margin and outside of Fairy Circles, as found by Ramond *et al.* (2014). In my results, despite being geographically close, inland and steppe soils had substantial differences in mineral content and SOM (**Paper I, Table 1** & **Figure 1**), which clearly exerts selective pressures on the soil communities.

Similar variations in local soil microbial communities were found in the site characterization from **Paper II** and **III**, where the fungal community of the exposed mineral soil and capped mounds (**Paper II**) and scarified mineral soil (**Paper III**) were assessed. In **Paper II,** despite planting position

and site location (Scots pine and Norway spruce blocks in **Paper II, Figure 1**) explaining 28% of the total fungal composition variation, selective pressures toward saprobes and opportunistic fungi were found. For example, principal coordinated analysis of bulk soils suggests a shift in the fungal community from the first growing season toward the second (**Paper II, Figure 5**), where differentially abundant (DA) taxa show an increase in saprobes, especially in capped mound soils of the Norway spruce block (Figure 7). These edaphic selective pressures are common in clear-cut forest sites, as a synergic effect between the reduction of easily metabolizable plant-derived C and the accumulation overtime of hard-to-degrade C from detritus and microbial necromass (Lindahl *et al.*, 2010; Kohout *et al.*, 2018). These results are further confirmed as the relative abundance of saprotrophs and opportunistic fungi increased in either capped mound, mineral soil, or both during the second growing season, while plant-C dependant fungal abundance decreased (Figure 7). Similar results were found in **Paper III**, where the bulk soil was characterized by a rich, diverse community dominated by opportunistic fungi, while symbiotroph fungi occupied less than 5% of the total fungal community, with EcM, such as *Piloderma* and *Russula*, that have been described to have slow decomposition time after root destruction (Gray & Kernaghan, 2020).

Altogether, the microbial composition of the soil samples from **Paper I** to **III** provide information on how edaphic characteristics drive selective pressures that shape the community in bulk soils. As observed in **Paper I**, the different mineral and nutrient contents of the soils created site-specific niches for a portion of the microbial community, exerting selective pressures toward high salt-adapted microbes in the north to diverse C degraders and symbionts in the south, while in **Paper II** and **III**, the *quality* of the nutrient content (*i.e.*, easy- vs hard-to-metabolize SOM) was the driving force of the changes in the soil community composition, as dissolved organic C (DOC) and C stock composition in the soil undergoes significant changes after clear-cutting and site preparation. It is from this resulting microbial community that the establishing seedlings recruit (and are colonized by) different taxa that can either enhance or inhibit plant survival and growth.

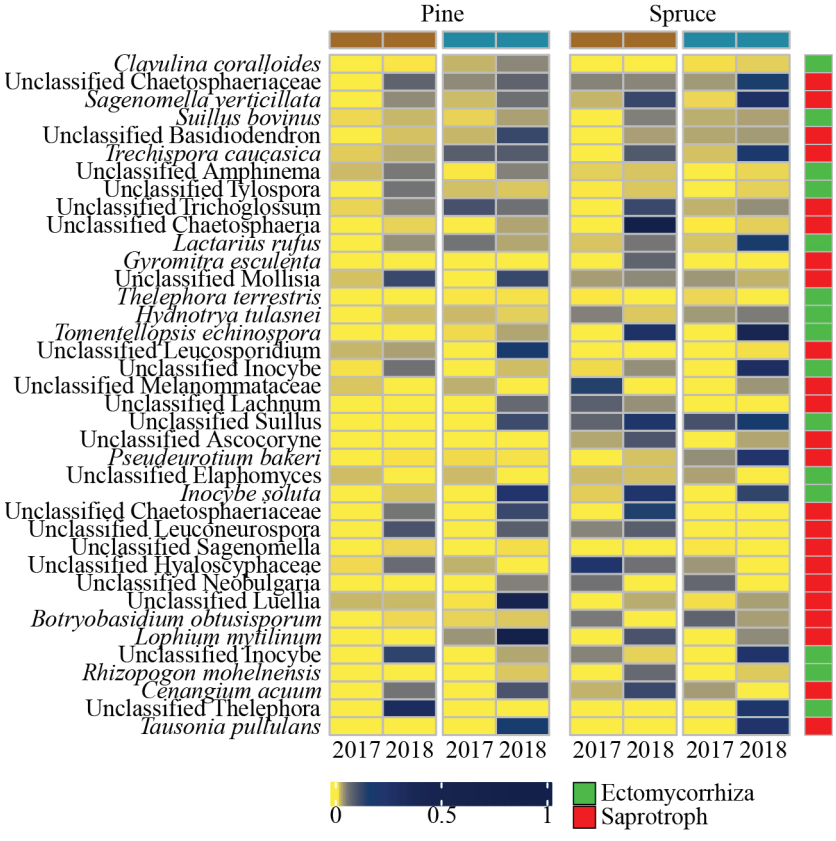

Figure 7. Heatmap with differentially abundant fungal Swarm operational taxonomic units (SOTUs) after one- (2017) and two- (2018) growing seasons in the site's bulk soils for Scots pine and Norway spruce blocks. Upper boxes coloured by planting position (Capped mound in brown and exposed mineral in grey). Right column coloured by trophic mode (Ectomycorrhizal or Saprotroph). Scale was grouped by quintiles for visualization purposes.

Recruited microbial community

In addition to the assessments of the bulk soil microbiome, I evaluated the microbial composition in the rhizosphere and the root-associated microbiomes (*i.e.*, the recruited microbiome) in **Papers I-III**. In the three manuscripts, the root-associated community composition was significantly different from the bulk soil, suggesting that the roots were successfully colonized by specific microbial taxa. In **Paper I**, the initial strong differences between the soil communities of the three locations was significantly decreased by the presence of the growing roots of both *Prosopis* species

(Figure 8a). However, soil-specific, and species-specific patterns in recruitment could be observed (Figure 8a). For instance, *P. tamarugo* recruited more unique bacterial partners than *P. chilensis* when growing in foreign soils (**Paper I, Table 2** & **Figure 5**), whereas greater overlap was observed between the bacterial communities recruited from native soils (**Paper I, Figure 5**). Several studies have reported species-specific microbial recruitment associated to edaphic properties, particularly in saline conditions (Li, 2008). Recently, Ren *et al.* (2020), using maize seedlings growing on one out of three possible soils, followed bacterial recruitment after transplanting the seedlings into a different soil and found that functional compensation (*i.e.*, microbial community recruited to compensate plant functional requirements in unfavourable soil conditions) mechanisms drove the assembly of the bacterial community in the second soil. For example, the authors found that two bacterial taxa, *Klebsiella* and *Pseudomonas* where among the most abundant in the rhizosphere after transplanting into new, poor soils. The authors suggest that these bacteria were recruited to enhance N and P uptake by the maize seedlings (Ren *et al.*, 2020). Later, functional compensation microbial assemblies were identified in phosphorus limited substrates using maize, where it was shown that there was significant recruitment of *Burkholderia gladioli*, which was found to have three acid phosphatase-encoding genes (Shao *et al.*, 2021). In my results from **Paper I**, the composition of the bacterial community recruited by *Prosopis tamarugo* had members of *Streptomyces*, *Bacillus* and *Paenibacillus* among the most dominant bacteria on the roots. In addition, fungal recruitment of *P. tamarugo* suggested an increased abundance of opportunistic fungi, although a few AM and EcM such as *Rhizophagus* and *Cortinarius* were also recruited (**Paper I**). These findings indicate that the microbial partners recruited by *P. tamarugo* seedlings growing in foreign soils might be related to compensatory mechanisms, recruiting high number of beneficial bacteria with antibiotic properties, such as *Streptomyces*, and mycorrhizal partners, to enhance protection against opportunistic fungi colonising the roots.

In **Paper II**, species-specific recruitment patterns were observed, where both plant species root systems having strong influence on the fungal community recruited from both capped mound and mineral soils after one and two growing seasons (Figure 8b). Starting from a nursery-adapted community, where 93 out of 190 fungal SOTUs were common for both Scots pine and Norway spruce, both species showed significant changes in their

fungal community one growing season after planting (**Paper II, Figure 3**). After one growing season, Norway spruce had significantly higher richness and diversity than Scots pine regardless of soil type. In addition, community composition suggests that the Norway spruce root system has significant effects on the rhizosphere, having distinctive fungal communities after one- and two growing seasons (Figure 8b). Scots pine, on the other hand, showed higher similarities between bulk and rhizosphere fungal communities, especially after the second growing seasons, suggesting that Scots pine roots have fewer impacts on the community composition in the rhizosphere than Norway spruce (Figure 8b). These results suggest that Norway spruce invested more resources (*e.g.*, C allocation belowground) in recruiting fungi from the soil than Scots pine (Figure 8b).

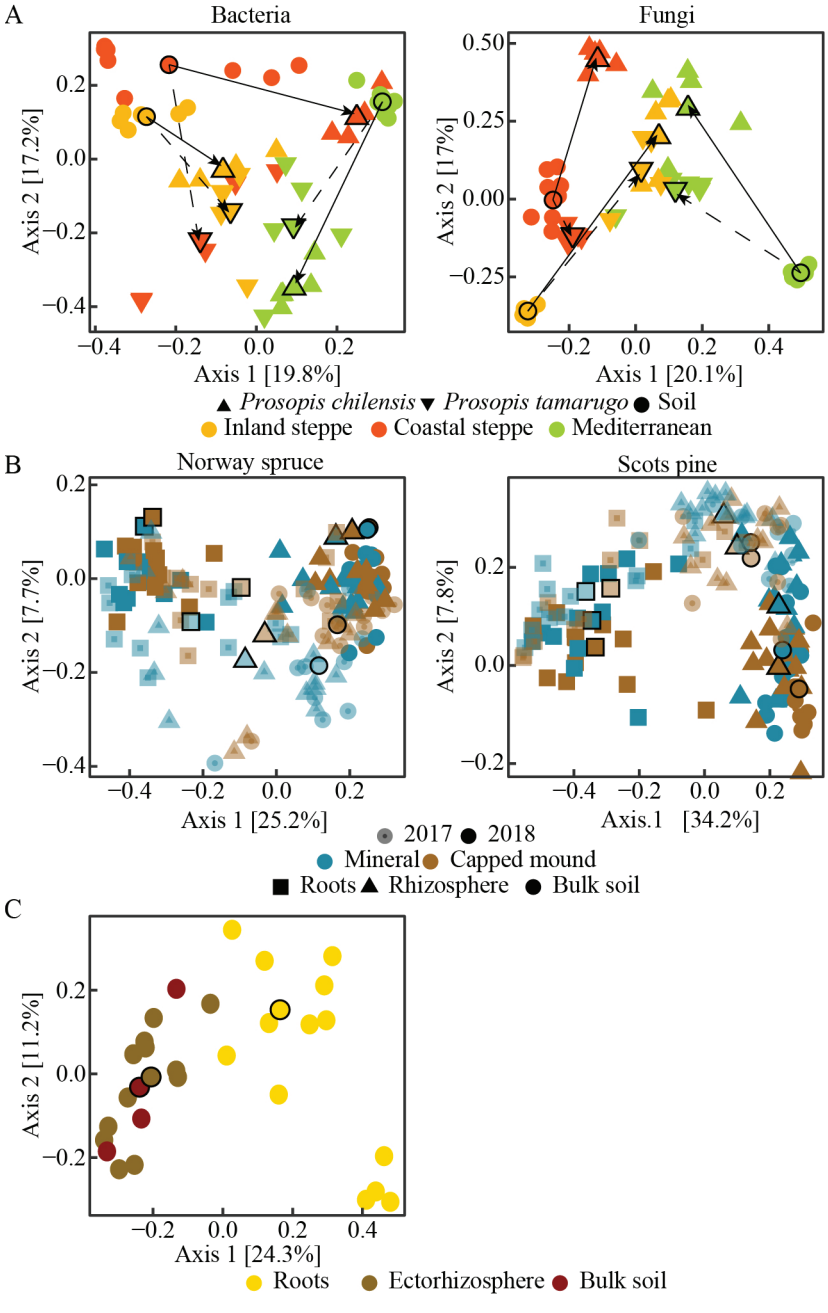

Figure 8. Principal coordinate analysis (PCoA) of *Prosopis* species (**A**) one-year-old Norway spruce and Scots pine (**B**) and in-site grown seedlings (**C**) showing the

compositional differences between roots and soils samples. Black bordered shapes correspond to group centroids (*i.e.*, mean of the variable for the cluster).

In **Paper III**, despite the seedlings being younger than in **Paper II**, similar results were found in the fungal recruitment of Scots pine seedlings after one growing season after germination, with the roots having lower richness and diversity than in the rhizosphere and bulk soil (**Paper III, Figure 3**). However, despite having a poorer and less diverse community, symbiotroph fungi were found in greater abundance on the roots, being two-times higher compared to bulk and rhizosphere soils (**Paper III, Figure S4**). Within the newly recruited fungi, several EcM such as *Russula*, *Piloderma*, *Suillus*, *Lactarius* and *Cortinariaceae*, were enriched in root samples, with some of them being only present in the roots, suggesting rapid colonization. These results are consistent with the hotspot nature of the roots, where a supply of photosynthesised C is constantly allocated belowground to aid root elongation and to sustain symbionts (Baetz & Martinoia, 2014; Hennion *et al.*, 2019) (Figure 8c).

Altogether, **Paper I** to **III** provide evidence of that roots provide hotspots for microbial activity, through root exudates that locally improve soil aggregation, water content, organic matter content and other factors that enhance the presence of soil microbes that have the potential to enhance survival and growth. Whether the resulting microbial community assemblies observed in **Paper I** to **III** are the result of functional compensation, generalist niche competition, active recruitment or all co-occurring at the same time needs further studies including multi-omics approaches, which were not the aim of those experimental designs. However, it is clear that edaphic properties play a key role in the initial microbiome availability.

3.2 Effect of fertilization on recruitment of fungal community

In 1840, Carl Sprengel proposed the law of the minimum, commonly known as Liebig's law, as it was Justus von Liebig who popularized the principle (Grime, 1989). It states that plant growth is controlled by the scarcest resource (*i.e.,* limiting factor). By extension, plant yield can be stimulated by providing the limiting factor (Grime, 1989). In the boreal forest, it is widely known that N is the most limiting factor for plant growth, thus, N fertilization has been seen to improve seedling survival and growth when applied after

planting (Luis *et al.*, 2009; Jonsdottir *et al.*, 2013) and forest yield (Mäkinen *et al.*, 2001; Iivonen *et al.*, 2006). However, quantity and type of iN addition (i.e., NH_4^+ or NO_3^-) has an impact on the soil microbial community and, in addition, can modify soil edaphic properties, which further impacts the microbial community composition, especially in terms of fungal tolerance to C starvation and N availability (Cox *et al.*, 2010; Kranabetter *et al.*, 2015; Hawkins & Kranabetter, 2017). In addition, the changes in microbial community can disrupt the mutualism–parasitism continuum. Thus, understanding the impact of N addition in both plant and soil fungal community simultaneously is fundamental to predict potential harmful effects of fertilization on plant growth and forest productivity.

In **Paper II** and **III**, I studied the impact of the addition of two different N sources on the fungal community, as well as its effects on seedling establishment in two managed boreal forest locations in central (**Paper II**) and northern (**Paper III**) Sweden. Both **Paper II** and **III** aimed to identify the recruitment of fungal community in scarified boreal forest sites using nursery grown one-year old seedlings (**Paper II**) and on-site germinated seeds, using a germination matrix (*i.e.*, SeedPAD®; **Paper III**) when planting material is enriched with a small amount of either organic (*i.e.*, arginine phosphate) or inorganic (*i.e.*, ammonium nitrate) N at the moment of planting in the site. In **Paper II**, Norway spruce had significantly better performance than Scots pine, having higher survival, biomass (*i.e.*, *field* root + shoot biomass and *field* root weight), and *field* root:shoot ratio after planting in mineral soil (**Paper II, Figure 7** & **Figure S10**). In addition, we found significant improvement in Norway spruce growth when seedlings were planted in mineral soil and treated with arginine phosphate (**Paper II, Figure 7**). Scots pine, on the other hand, showed no changes in growth either with iN or oN treatments. As previously stated (section 3.1), exposed mineral soils have better edaphic properties than capped mound soils, having better particle aggregation, thus remaining wetter for longer periods of time (Häggström *et al.*, 2021). Additionally, in a study of photosynthesis in *Pinus taeda* and Norway spruce, the effect of fertilization and wetter soils was additive for both plant species (Ewers *et al.*, 2001). This suggest that the better performance of Norway spruce in mineral soil might be a combination of good edaphic properties and a positive response to fertilization. Similarly, in **Paper III** small amounts of locally applied oN and iN enhanced establishment and survival of recently germinated seedlings (**Paper III,**

Figure 2a). Similar results have been found in calcareous soils, after a single dose of ammonium nitrate (NH_4NO_3) at 15kg·ha^{-1}·a^{-1} (Plassmann *et al.*, 2008), Mediterranean plant species treated with both 25 mM and 50 mM of either potassium nitrate (KNO_3) and NH_4NO_3 (Pérez-Fernández *et al.*, 2006) and in semi-arid grassland species treated with increasing concentrations to up to 40 mM NH_4NO_3 (Zhang *et al.*, 2020), where in all three works the authors found enhanced germination after N addition. However, the positive impact on germination in a managed boreal forest is less known. By using either arginine phosphate or ammonium nitrate in a germination matrix, germinating seeds had about 1.5 times higher survival than control seeds. In addition, surviving seedlings had increased C content in the needles (**Paper III, Figure 2b**), suggesting potentially enhanced photosynthesis (Luo *et al.*, 2015). These results support the positive effects of small-scale N addition on early stages of seedling establishment when seedlings are more sensitive to environmental stressors.

While the small amounts of nitrogen supplied to the seedlings in **Paper II** (40 mg) or the seeds in **Paper III** (10 mg) were enough to enhance plant survival and growth, no significant effects were found to the root-associated and rhizosphere fungal communities. However, taxa specific responses to N supplementation were observed in the relative abundance of the root-associated fungal community. For example, in **Paper II** differentially abundant taxa analysis showed that opportunistic fungi are positively affected by supplementation with a small amount of N. In addition, different responses on abundance are observed depending on soil type. For instance, the mycorrhizal fungi *Rhizopogon verri* increased in abundance in Norway spruce roots growing in mineral soil and treated with arginine phosphate, while in capped mound soils its abundance was unaffected. Similarly, in Scots pine, *R. verii* increased in abundance in response to both N addition treatments in mineral soils, whereas a significant reduction was observed in capped mounds when ammonium nitrate was added (**Paper II, Figure S11**). *Rhizopogon* is an EcM genus which has been described to have highly resistant propagules, as it one of the first genera to dominate in post-fire events (Glassman *et al.*, 2016). At the same time, *Rhizopogon* species have been found to grow in nitrate media, having high rates of mycelial growth (Nygren *et al.*, 2008). Intense fire events have been proposed to have similar effects to site preparation, as fire exposes the underlying mineral soil, increasing iN input in the soil (Pellegrini & Jackson, 2020). In such

conditions, the fungal community is often depleted in EcM species (Sun *et al.*, 2015). Thus, the response of *R. verri* observed in **Paper II** agrees with the findings of Glassman *et al*. (2016) and Nygren *et al.* (2008), where the conditions provided in the mineral soil are adequate for resistant propagules able to grow in iN to reactivate their growth. Similar taxa-specific responses were found in **Paper III**, where *Pyronemataceae* sp. and *Russula* sp. showed significant increase and decrease, respectively, in response to both iN and oN (**Paper III, Figure 5**). *Pyronemataceae* corresponds to the largest family of the Pezizales which include saprotrophic and EcM members (Tedersoo *et al.*, 2006; Perry *et al.*, 2007) while *Russula* corresponds to an EcM genus which has been described as both nitrophilic and nitrophobic (Lilleskov *et al.*, 2011; Davey *et al.*, 2017). *Russula* spp. are thought to have a low-cost lifestyle, based on their short-distance exploration range (Lilleskov *et al.*, 2011), as such, the negative response observed in both N addition types might be related to C-supply interruption to this fungi. However, the mixed response to N addition of the genus suggest that some of the species might have reduced metabolic capacity in response to N (Lilleskov *et al.*, 2011). This is consistent with the reduction of other EcM in the roots, like *Suillus* and the increase of *Piloderma* under oN, given its capacity to uptake oN (Heinonsalo *et al.*, 2015), suggesting that *Piloderma* might had accessed and metabolised part of the oN for its own growth. Altogether, the results from **Paper II** and **III** suggest that the addition of small amounts of either iN or oN at the early establishment stage, has a positive effect on seedling establishment and growth, changing the relative abundance of only a few taxa that are sensitive to the added N.

The taxa specific responses within the root-associated fungal communities observed in **Paper II** and **III**, despite the lack of significant N leakage into the rhizosphere, is indicative of the close relationship that exists between plants and their root-associated fungal community. However, forestry practices often involve the use of high amounts of N during fertilization. To investigate the impact of high rates of fertilization on the composition and activity of the root-associated fungal community, in **Paper IV** and **V** (see section 3.3), we used heavier N fertilization rates of both of young and adult Norway spruce trees. In **Paper IV**, either liquid N added via the stem vascular transpiration stream (500 ml of a 50 mM solution of KNO_3) or ground N fertilization (100 $kg \cdot ha^{-1}$) were used to study the root-associated fungal composition, based on metagenomic and metatranscriptomic

analyses. After one- and four-growing seasons of yearly treatment, no significant effects were found to the composition of the root-associated fungal community, neither by metagenomics nor metatranscriptomic analyses. However, like **Paper II** and **III**, taxa specific responses were found in **Paper IV**. For example, relative abundance changes were found in EcM fungi such as *Tylospora* and *Russula* in the metagenomic analysis of ground fertilized samples (**Paper IV**, **Figure 5a**) and *Lactarius* and *Laccaria* in the metatranscriptomic data after one growing season, in response to both fertilization methods (**Paper IV**, **Figure 5b**). Further changes in relative abundance, without significant changes in community composition, were found after four growing seasons, with previously dominant taxa such as *Oidiodendron* or *Cortinarius* having reduced relative abundance in response to ground fertilization (**Paper IV, Figure 5a**). Similar results were found by Haas *et al.* (2018), where subtle changes in microbial root-associated composition were observed after five years of continuous nutrient optimisation. In **Paper V**, a high-temporal sampling frequency over one growing season was performed on the same experimental forest used by Haas *et al.* (2018), where adult Norway spruce trees had been nutrient optimized for 25 years. In **Paper V**, changes in the root-associated community were attributed, at least in part, to changes in Norway spruce transcriptomic control over C allocation belowground, driving the changes in the metabolism of the fungal partners, favouring those fungi with less dependent lifestyles (see section 3.3; **Paper V**). These results suggest that the root-associated community is highly resilient to short-changes in N content in the soil, where long-term fertilization affect the fungal community indirectly, by altering the tree metabolism. However, different results were found by Marupakula *et al.* (2021) in an adult Scots pine stand in the Lamborn Experimental Forest located in southern Sweden, where strong decrease in richness, evenness and diversity of root-associated fungi after 15 month of a single dose of 150 $kg \cdot ha^{-1}$ of N. Although geographical location, tree species and fertilization type differ to the results found by Marupakula *et al.* (2021) and my results in **Paper V**, these results provide information of how variable the fungal community response can be. Thus, the resilience of the fungal community may be related to a number of environmental factors, to the resilience of the conifer species, edaphic properties, or the additive effect of all three. Altogether, the taxa-specific results observed in **Paper II** and **III**, and partially in **Paper IV** and **V** suggest that while the root-associated fungal

community can be resilient to N addition, community responses cannot be predicted based only on metagenomic results alone, as plant species and its influence on the associated fungal community might drive different metabolic responses in their mycorrhizal symbionts. As such, integrative analyses using multiple 'omics approached should be preferred if possible.

3.3 Coordinated responses of plant and fungal communities

Microbial metagenomics, *per se*, do not provide enough background to the community to fully describe functional changes in response to different stimuli, as the most abundant taxa assessed by metagenomics are not necessarily the most active (Rivera Pérez *et al.*, 2022) and because synergic responses between the different members of the community can be occurring (Gonzalez *et al.*, 2018). As such, recently Lilleskov *et al.* (2019) and Geisen (2021) suggested that phylogenomic studies needed to be integrated with physiological and ecosystem studies, and multiple 'omics data (see review of Lilleskov *et al.* (2019) and commentary of Geisen (2021)). Thus, some studies in microbial composition support their findings with additional enzymatic assays as a proxy of the activities of key functional fungal groups (Nygren *et al.*, 2008; Lindahl *et al.*, 2010; Bödeker *et al.*, 2014; Kohout *et al.*, 2018; Bonner *et al.*, 2019). However, with recent technical developments, new tools such as NGS have opened new possibilities for studying plant-fungi interactions (see Rivera Pérez *et al.* 2022). As I mentioned in section 3.2, boreal forests are widely known to have poor soils, which means that nutrients, especially N, limit plant growth. However, the actual N pool in boreal forest soil is often large, but it is mostly bound into oN compounds that are inaccessible to plants (Fransson *et al.*, 2000; Persson *et al.*, 2000). In boreal forest soils, N mineralization is a slow process mostly performed by soil microorganisms that breakdown organic compounds, releasing iN (*i.e.*, NH_4^+ and NO_3^-) that is either taken up by the plants or given to them by mycorrhizal symbionts in exchange for C (Myrold & Bottomley, 2015). Nevertheless, given the economic importance of boreal forest for northern hemisphere countries, N fertilization has been used to enhance growth and to increase forest profitability by improving stem quality (Pukkala, 2017). Heavy N addition, as discussed in section 3.2, can have a strong impact on the composition and function of the soil microbiome. In

addition to the N input changes, it has been observed that N fertilization alters the soil C pool, inhibiting soil C decomposition and, therefore, increasing SOM accumulation (Vogt *et al.*, 2018; Bonner *et al.*, 2019). Such responses have been attributed to changes in the economy of the fungal metabolism to SOM mineralization, where the relative energy gained through enzymatic degradation of the SOM is reduced under N enriched conditions (Entwistle *et al.*, 2018; Bonner *et al.*, 2019). Non-enzymatic oxidation, also known as Fenton chemistry (Frey, 2019), corresponds to the SOM oxidation through hydroxyl radicals production, releasing hemicellulose which is then enzymatically degraded, in a two-step mechanism (Rineau *et al.*, 2012; Presley & Schilling, 2017; Frey, 2019). Fenton chemistry is known to be slower than enzymatic SOM oxidation (Schilling *et al.*, 2020) that remains energetically favourable under N enriched conditions (Bonner *et al.*, 2019). Thus, in N enriched soils, fungi that can uses Fenton chemistry to access SOM becomes dominant over enzymatic mineralizers.

EcM and trees respond simultaneously to increased N inputs and, thus, the coordinated responses of the plant and fungal partners need to be understood in order to predict ecosystem C and N dynamics. In **Paper IV** and **V**, I evaluated the coordinated response of young and adult Norway spruce trees and their root-associated fungal communities to direct changes in plant N status and to long-term soil nutrient enrichment, respectively, using a combination of amplicon, metatranscriptomic, transcriptomic and physiological approaches. Both projects aimed to identify the mechanisms behind the changes of root-associated fungal community under N addition, and the potential crosstalk between Norway spruce and its root-associated fungal community. It is well-known that traditional N fertilization induces changes in plant biomass and a shift to a nitrophilic soil fungal community. These changes are thought to happen due to increased soil N content and a reduction in C allocation belowground by the trees. However, little is known of the effect of increased plant N status without direct effects on the soil. In **Paper IV**, stem fertilization (*i.e.*, liquid N supplied directly through the stem vascular transpiration stream) was used to evaluate how increased plant N status modifies root transcriptomics and the root-associated fungal community, which has not been impacted by direct N fertilization. Our findings suggest that stem fertilization was an effective method of changing tree N status, based on ^{15}N excess observed in both above- and belowground parts of the treated plants (**Paper IV, Figures 1-2**). Furthermore, stem

fertilization significantly increased %N aboveground in four age-classes of needles, suggesting N assimilation and distribution in aboveground tissues. Parallel ground fertilization, used as positive control for stem fertilization showed similar, although higher, patterns in ^{15}N and %N in aboveground tissues, further confirming stem fertilization as an effective method to change tree N status.

After one- and four growing seasons, neither stem- nor ground fertilization had a significant impact on the whole root gene expression of young Norway spruce trees, with few DE genes found in either stem- and ground fertilized root samples. Similar results were found in a mature Norway spruce forest located in northern Sweden after five years of nutrient optimization (Castro *et al.*, not published). In **Paper V**, where weekly root samples from adult Norway spruce were taken during one growing season, great changes in the seasonal DE genes profiles were found after 25 years of nutrient optimization (**Paper V, Figure 3**), where increased defence responses (*e.g.*, immunity signalling, cell wall thickening) and N assimilation and transport, and reduced transcript abundance of SWEET family transporters were found during nutrient optimization (**Paper V, Figure S6**). Nevertheless, after nutrient optimization period ended, during September, no significant changes were found in expression levels of C transport, N assimilation and N transport (**Paper V, Figure S6**), corresponding to the month when the samples were taken for **Paper IV**. This suggest that Norway spruce responds rapidly to N fertilization, quickly upregulating N assimilation and transport, which is supported by the changes observed in **Paper V**. The simultaneous increase of immune mechanism and reduction of SWEET transporters in the root transcriptome after 25 years of nutrient optimization suggest that Norway spruce undergoes a reshapening of its symbiotic interaction under N fertilization, where more C dependant fungal partners would be negatively affected.

The parallel stem- and ground fertilization used in **Paper IV**, provide a controlled mechanism to test the responses of the root-associated fungal community to N fertilization. Thus, using genomic and metatranscriptomics tools, I evaluated the responses to the fungal community to stem- and ground fertilization. As briefly described in section 3.2, no significant changes in fungal community composition were found using either metagenomics or metatranscriptomic after one or four growing seasons. However, as I showed in **Papers II** and **III**, taxa specific responses were observed in root-

associated communities. For instance, the metatranscriptomic data showed that the EcM fungus *Lactarius* had a clear preference for ground fertilization after one growing season. In addition, *Russula, Cenoccocum* and *Cortinarius*, were among the most active fungi, based on the metatranscriptomic analysis, after one growing season of either N addition treatment, suggesting that these fungal taxa have both resilience to reduced plant-derived C allocation and high soil N content (**Paper IV, Figure 5**). Similar community resilience was found by Rivera Pérez *et al.* (2022) where they found no significant changes in the fungal transcriptome after N addition. Similar to my previous results from **Paper II** and **III**, they found species-specific responses to N addition, where 9 EcM fungi (including *Russula ochroleuca*, *Thelephora terrestris*, *and Cenococcum geophilum*) were involved in two nitrate induced functions annotated in the Eukaryotic Orthologous Groups (KOGs) database. The authors suggest that potential responses of the fungal community might have been masked by the heterogeneous nature of the fungal community of each tree (Rivera Pérez *et al.*, 2022). However, in our results, no differences in fungal composition were found either in metagenomic or metatranscriptomic derived communities under neither N addition treatment after one growing season, suggesting that in the Norway spruce root-associated fungal community is resilient. In addition, after four growing seasons, few subtle changes were observed on the overall abundance and activity of the community. For instance, the relative abundance of the known nitrophobic fungus *Cortinarius* decreased in response to ground fertilization in both metagenomic and metatranscriptomic results, suggesting that *Cortinarius* is sensitive to increases in soil N content. Similar results were found by Kranabetter *et al.* (2015), where a progressive reduction in *Cortinarius* presence and root colonization % was found as mineralizable N in the soil increased, confirming the sensitive nature of *Cortinarius* to N soil content. Previous results have described *Cortinarius* to a sensitive fungus to reduced C allocation belowground, whose presence decreases fast after root destruction (Lindahl *et al.*, 2010; Gray & Kernaghan, 2020). Altogether, the results from **Paper IV** provides evidence of a novel method to modify plant N status in the field without inducing N responses in their associated fungal community, which would allow us to decouple the N and C responses of root-associated fungi. The ability to test independently the EcM response to

N and C in a community context will establish the basis of potential new knowledge in the plant-EcM interaction.

The coordinated nature of N and C metabolism in the plant-fungi interaction is widely known, where mycorrhizal symbionts provide N at exchange of C. However, it has been mostly assumed that such exchange is favourable to the plants. Hasselquist *et al.* (2015) using $^{15}NO_3$ labelling on EcM dominated mor layer, found that shaded Scots pine trees received significantly less ^{15}N from the EcM despite no changes in their C allocation belowground. Their results contrast with previous results showing that EcM fungi can transport nutrients from a donor-tree to a receiver-tree (He *et al.*, 2003; Simard *et al.*, 2012). In **Paper IV**, we showed that after one growing season of stem fertilization, despite significant ^{15}N excess found in the root tips of stem fertilized trees, root-associated mycelia had no higher ^{15}N than the root-associated mycelia from control trees (**Paper IV, Figure 2**). In addition, no increase in ^{15}N was found in any of the plants growing within 2 meters radius of the stem fertilized tree (**Paper IV, Figure 3**). These results suggest that no direct N transfer occurred from N enriched Norway spruce trees to their root-associated fungi nor via the CMN to closely growing trees. The observed distribution of the ^{15}N in the stem- and ground fertilized trees, in addition to the lack of evidence of ^{15}N transfer to neighbouring trees suggest that young Spruce trees with high N status do not act as an N source for low N status neighbouring trees, as has been suggested in greenhouse experiments, where high N plants, either N_2-fixers or N enriched, transferred depending on plant nutrient requirements (He *et al.*, 2003, 2019). Altogether, these results provide direct evidence that Norway spruce trees do not transfer N excess to EcM symbionts, and that the fungal symbionts are not able to take up N directly from the roots, similar to what was recently found by Rivera Pérez *et al.* (2022) in European beech. As such, an alternative mechanism for the potential beneficial effects of CMN observed in previous studies might be related to indirect uptake of labelled ^{15}N by the fungi from, for example, root exudates or direct soil transfer rather than direct N transfer through the CMN (Bethlenfalvay *et al.*, 1991; Ekblad & Huss-Danell, 1995; Simard *et al.*, 1997b).

In **Paper V**, I studied high-resolution coordinated responses of the Norway spruce root transcriptome and the transcriptomes of the root-associated fungal community after 25 years of nutrient optimization. Stronger effects of nutrient optimization in roots and root-associated fungal

metabolism than in **Paper IV** were found in **Paper V**. For example, adult Norway spruce root transcriptome underwent a series of changes that includes the reduction of SWEET transporters and the increase of immunity responses. In addition, metatranscriptomic analysis showed that nutrient enrichment induced significant changes in the number of fungal reads, which is traduced into significant higher diversity in nutrient enriched trees (**Paper V, Figure 1**). Furthermore, metatranscriptomic results suggests that nutrient enrichment induced significant changes in metabolic pathways that were attributable to a few fungal taxa (**Paper V**, **Figure 2**). For instance, in the nutrient limited roots (*i.e.*, non-treated trees), *Piloderma and Cortinarius* were both the most abundant and most active fungi, with functional enrichment of growth and signalling pathways, which were coordinated with plant nutrient transport functions (**Paper V**, **Figure 3-4**). In contrast, under nutrient enriched conditions (*i.e.*, fertilized trees), *Cenococcum* was the most abundant and active taxa, with an enrichment of starvation-responsive genes and alternative energy metabolism pathways. These changes, taken together with the transcriptomic changes of Norway spruce roots suggest that Norway spruce trees redefines its C economy in response to nutrient enriched conditions with its root-associated EcM partners. Notably, *Piloderma* and *Cortinarius* coordinated network modules (*e.g.*, cell cycle and amino acid metabolism) connected with Norway spruce transcriptomics, lost their connections under nutrient optimization, resulting in reduced fungal growth (**Paper V, Supplementary report 1**). On the other hand, 89% of the modules of *Cenococcum* coordinated with Norway spruce modules under control conditions, remained connected. Within these modules, enriched functions associated with metabolism, cell wall biosynthesis and nutrient transport were found. Furthermore, coupled network that were previously associated with *Piloderma* under control conditions were associated with *Cenoccocum* under nutrient optimization (*e.g.*, facilitation of symbiosis), suggesting changes in the symbiotic coordination. These results suggest that under nutrient enrichment, a restructuration of Norway spruce fungal community occurs toward *Cenococcum* partners, which becomes energetically more efficient than *Piloderma* and *Cortinarius*. My results suggested that the success of *Cenococcum* under long-term nutrient enrichment is a combination of metabolic versatility (*e.g.*, lignin degradation), environmental resilience (*e.g.*, melanin biosynthesis) and microbial suppression (*e.g.*, production of antibiotic compounds). In

addition, predicted fungal effectors were highly and positively correlated with metatranscriptomic data, supporting that the molecular dialogue between Norway spruce and its root-associated EcM underwent significant changes under nutrient optimization (**Paper V, Figure 5**).

Altogether, the results of **Paper IV** and **V** provide information on how EcM fungi respond to N addition and C starvation, which in the boreal forest context are key component, given the productive and economic nature of the dominant tree species Norway spruce and Scots pine. In addition, the data of **Paper IV** and **V** show that high resolution sampling and powerful analyses are needed to study the coordinated response of root transcriptome and root-associated fungal metatranscriptomic, as single point might not be sufficient to observe changes.

4. Conclusion and further perspectives

Globally, efforts to develop a more sustainable society are increasing, following the UN Sustainable Development Goals. To fulfil these goals, increasing plant productivity with lower environmental impact is key, as this is either directly or indirectly related with 7 out of the 17 goals. To achieve sustainable, increased plant productivity, a detailed theoretical framework about how to enhance plant survival and growth is therefore needed. Throughout my thesis, I have assessed how the edaphic properties, including amendment of it (*i.e.*, fertilization), impact plant establishment, growth, and the microbial community, to understand the mechanisms behind the coordinated response of the plant-microbiome-soil continuum. I started describing how the inherent characteristic of different soils significantly affect both seedling establishment success and how this modulates the soil microbial community. Then, I examined how the presence of the growing seedling changed the microbial community, and the potential impacts of these newly established rhizosphere and root-associated communities on plant growth. Subsequently, I assessed the impact of nutrient amendment on the plants and its associated microbiome to finally analyse the coordinated plant-microbiome response.

Using a combination of plant growth measurements, soil sampling and molecular tools, in section 3.1, I described the results of how the soil and microbiome partially modulate the establishment and population expansion of highly stress tolerant *Prosopis* species from northern Chile (**Paper I**) and the establishment success of economically important conifers in Sweden (**Paper II** and **III**). These results highlighted the importance of the edaphic properties on the microbiome available for the plants to recruit and how it can influence plant distribution and growth. In addition, the results provided information of how closely related plant species respond differently to soil

characteristics (*i.e.*, both edaphic and soil microbiome). Such data provides useful information that can be used in conservation as well as productivity programs. In section 3.2, the results presented suggests that small-scale N amendment, while enhancing seedling survival and biomass, does not have a substantial effect on soil N content or on the whole fungal community, but there were taxa-specific effects (**Paper II** and **III**). These results further provide information for developing potential new, more sustainable, forest management methods to increase seedling establishment success during the early stages of the rotation, where plants are more sensitive to biotic and abiotic stressors. Finally, in section 3.3, where the coordinated response of the plant-microbiome was assessed (**Paper IV** and **V**), the results suggest that long-term nutrient optimization conditions are required to induce changes in the plant-EcM interactions, where plants can redefine their C economy toward more beneficial microbial partners, with a consequent restructuration of metabolic pathways.

The results obtained on my thesis provide new insights into plant-microbiome interactions into a plant-microbiome-soil continuum frame that, hopefully, will help in the decision-making process for future, more sustainable ways of managing agro-forestry sites without impairing soil health. This, in the frame of the expected changes due to global warming, where substantial soil temperature increment would accelerate soil mineralization rates and increase the frequency of drought events, which would directly affect the belowground microbial community, might help reducing the potential risks of raised temperatures, based on close-to-reality predictions. As such, the plant-microbiome-soil continuum, and the combination of multiple omics data to assess it have greater potential for further studies for a wide range of applications.

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Popular science summary

Plants have co-evolved beneficial interactions with members of the rhizospheric microbiome, which increase their access to nutrients and provides them with protection against antagonistic microbes, at the cost of photosynthetically derived carbon. During my PhD, I have studied the interconnected relationship between soil properties, soil microbes and plants to understand how altering one of these three factors can impact the other two. I have applied novel methods such as directly altering tree nutrient status independent of the soil, and combined technologies such as soil DNA metagenomics, root transcriptomic and root-associated fungal meta-transcriptomic to study the tightly coordinated interactions between plants and soil microbiome. My results provide new insight into the coordinated response of plants and their associated microbiome to changes in soil nutrient availability and the potential implications on the soil nutrient cycles.

Populärvetenskaplig sammanfattning

Växter har utvecklat fördelaktiga symbioser med mikroorganismer i jorden, som höjer växternas tillgång till näringsämnen och förbättrar skyddet mot antagonistiska mikrober, i utbyte mot kol från fotosyntes. Under min doktorandtid har jag undersökt samspelet mellan markegenskaper, jordmikrober och växter för att förstå hur en förändring av en av dessa faktorer påverkar de andra två. Jag har använt nya metoder som att direkt modifiera ett träds näringstillstånd oberoende av marken, och har kombinerat teknologier som DNA metagenomik på jordprover eller RNA sekvensering av trädrötter och associerade svampar för att studera det finkoordinerade samarbetet mellan växter och deras mikrobiom i marken. Mina resultat tillhandahåller nya insikter om den koordinerade reaktionen som växter och deras mikrobiom visar på förändringar i näringstillgänglighet i marken, och potentiella implikationer detta har för näringscykler.

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III

forests

MDPI

Article

Effects of Early, Small-Scale Nitrogen Addition on Germination and Early Growth of Scots Pine (*Pinus sylvestris*) Seedlings and on the Recruitment of the Root-Associated Fungal Community

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Abstract: Scots pine (*Pinus sylvestris* L.) is one of the most economically important species to the Swedish forest industry, and cost-efficient planting methods are needed to ensure successful reestablishment after harvesting forest stands. While the majority of clear-cuts are replanted with pre-grown seedlings, direct seeding can be a viable option on poorer sites. Organic fertilizer has been shown to improve planted seedling establishment, but the effect on direct seeding is less well known. Therefore, at a scarified (disc trencher harrowed) clear-cut site in northern Sweden, we evaluated the effect of early, small-scale nitrogen addition on establishment and early recruitment of fungi from the disturbed soil community by site-planted Scots pine seeds. Individual seeds were planted using a moisture retaining germination matrix containing 10 mg nitrogen in the form of either arginine phosphate or ammonium nitrate. After one growing season, we collected seedlings and assessed the fungal community of seedling roots and the surrounding soil. Our results demonstrate that early, small-scale N addition increases seedling survival and needle carbon content, that there is rapid recruitment of ectomycorrhizal fungi to the roots and rhizosphere of the young seedlings and that this rapid recruitment was modified but not prevented by N addition.

Keywords: boreal forest; clear-cut; fungal community composition; ectomycorrhiza; Scots pine; nitrogen addition; mycobiome

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1. Introduction

Swedish forested land makes up part of the wider boreal forest area, which is the largest terrestrial biome covering more than 10% of the land surface, extending across Eurasia and North America between 45° to 70° northern latitude [1–3]. It is characterized by nitrogen (N) limitation, slow regeneration, low plant community species richness and one of the largest carbon (C) pools in the world [1,3–5]. Plants growing in the boreal forest form symbioses with ectomycorrhizal fungi (ECM), one of the most diverse groups of soil organisms that establish mutualistic interactions with plants [6,7]. ECM are generally considered to enhance nutrient and water uptake by plants, seedling establishment and survival, and increased plant resilience against environmental stressors [8]. However, the ecological role of ECM can vary from mutualistic to parasitic [9–11], and in situations such as strong N limitation, mycorrhizal fungi can immobilize available N and aggravate plant N limitation [12–14].

In Sweden, forested land accounts for approximately 60% of the country [15], and forestry is one of the most important export industries [2], with the conifers Norway spruce

(*Picea abies* (L.) H. Karst.) and Scots pine (*Pinus sylvestris* L.) being the ecologically dominant and economically most important species [16,17]. The current Swedish silvicultural strategy, which has been used since the mid 20th century, focuses on clear-cutting mature Norway spruce and Scots pine forests that are re-planted within a few years of harvesting [18,19]. Using this method, approximately 50,000 to 70,000 individual clear-cuts, covering between 150,000–300,000 ha, are created each year [18]. The majority of clear-cuts are replanted with pre-grown seedlings, but direct seeding can be a viable alternative for low-nutrient sites with adequate moisture, limited competing vegetation, and where *Hylobius abietis* attacks are minimal [20,21]. Prior to any type of re-planting, site soils are scarified to improve soil properties (e.g., soil temperature and porosity) [22]. In addition, soil scarification increases natural regeneration [23] and seedling survival [24] by reducing above- and belowground competition with older trees [25,26]. Despite the benefits of soil scarification, it also increases nutrient leaching and site water loss [24,27,28]. Moreover, clear-cutting these N-limited boreal forests leaves the root-associated ECM fungi without photosynthetically fixed C, leading to a rapid decrease in biomass within the soil community [29,30]. In addition, scarification disturbs the upper, organic layer of the soil to expose the deeper mineral horizon [31,32], further disrupting the mycorrhizal network and increasing fungal necromass [30,33]. Scarification also exposes dead fine roots and complex C, increasing available carbon sources for opportunistic saprotrophs, which have been shown to increase in abundance in soil communities after both clear-cutting and forest fires [34–37]. Climate change will continue to drive increases in air and soil temperatures and the frequency and severity of drought in boreal forests [38]. These ongoing changes to the boreal environment also have the potential to alter plant–ECM interactions, with higher soil temperatures known to reduce ECM formation [39,40] at a time when the establishment of ECM symbiosis has the potential to enhance seedling drought tolerance [41,42].

In order to alleviate N limitation in the Swedish boreal forest soils, fertilization with inorganic N has been one of the most used strategies to improve tree growth and forest yield when used in the final 10 years prior to harvest [43–45]. Inorganic N addition has also been used extensively to produce high-quality seedlings in nurseries, but this often results in a reduction of the seedling root to shoot ratio [46,47]. Recently, it has been found that the production of seedlings using an organic N fertilizer produces high-quality seedlings with a well-developed root system, which improves out-planting performance [46,48]. However, it is less well known whether direct, small-scale N addition to site-planted seeds can improve early establishment and survival, and how this impacts early ECM fungal recruitment. Here, we used orchard-produced seeds supplied with controlled addition of inorganic and organic N sources supplied in a moisture-retaining and biodegradable germination matrix (seedPAD^{TM}; Figure S1), to investigate the role of early, small-scale N addition on seed germination and seedling establishment and fungal recruitment in situ on a scarified clear-felling site.

2. Materials and Methods

2.1. Site Description

The field site is located at 63°28′ N, 17°29′ E in the Svanatjärn area in northern Sweden (Figure S2A) and was part of a production forest belonging to Holmen Skog AB that was harvested in 2014, followed by scarification during the autumn of 2016. Prior to harvest, the forest was a mix of Scots pine and Norway spruce, and the trees were between 90 to 110 years old, growing on a podzol soil. The topography of the site is relatively flat with a slight slope toward the Svanatjärn lake shore, where water saturates the soil. Monthly mean temperatures at the site during the growing season range from 14 °C in July to 4 °C in October. In 2017, the mean temperature in summer was approximately 0.5 °C higher than normal, while in autumn it was approximately 0.7 °C lower. Precipitation at the site ranges from 56 mm per month in June, increasing to 78 mm in July and decreasing to 45 mm in October (Figure S3). The observed precipitation in 2017 was 30% lower in June,

approximately 20% higher during July and August, and around 50% lower in September (Figure S3).

2.2. Planting, Treatment and Sampling

In September 2014, an area of approximately 2.4 ha was harvested and subsequently scarified using a disc trencher to prepare for manual planting. From the site, a smaller area of about 0.9 ha was selected and on 15 June 2017, 300 seedPADs (seedPAD^{TM}, Arevo AB, Umeå, Sweden), each containing one Scots pine (*Pinus sylvestris*) seed, were placed on top of the exposed mineral soil. These seedPADs represented three different treatments, with 100 seedPADs containing 10 mg nitrogen (N) in the form of arginine phosphate, 100 seedPADs containing 10 mg N in the form of ammonium nitrate, and 100 controls containing no added N (Figure S1). They were distributed across three different sub-plots within the selected planting area (Figure S2B). At the end of the first growing season, on 20 October 2017, all surviving seedlings were collected without damaging the root system. The roots were washed with distilled water and separated from the shoot, and both root and shoot were weighed. These were then stored separately at −80 °C until further processing. The soil surrounding the root system (from now on referred to as the 'ectorhizosphere') was also collected and stored separately at −80 °C. In parallel, soil samples from the scarified site were collected to assess the effect of clear-cutting and scarification on the fungal community (hereafter 'scarified soil'). Soil was sampled from the upper 10 cm of the soil profile, and three cores in a triangle of around 15 cm side length were pooled for one sample.

2.3. Carbon and Nitrogen Content

Needle samples were ground to a fine powder in liquid nitrogen and air-dried at 70 °C until constant weight. Soil samples were air-dried at 70 °C until constant weight and sieved (<2 mm). The C and N content of 5 mg of the dried needle and soil samples were determined by conversion to CO_2 and N_2 by combustion and measured with an isotope ratio mass spectrometer (DeltaV, Thermo Fisher Scientific, Bremen, Germany), following the method of Werner et al. [49].

2.4. Genomic DNA Extraction

Genomic DNA from the rinsed roots was extracted using a cetyl trimethylammonium bromide (CTAB) based method [50]. Briefly, mortar-ground samples were homogenized and washed for cleaning with chloroform:isoamyl alcohol (24:1). After cleaning, the genomic DNA was precipitated with cold isopropanol and cleaned further with 80% ethanol before being resuspended in 10 mM TRIS buffer (pH 8.5). Co-extracted RNA was eliminated with RNase A (10 mg/mL). The ectorhizosphere and scarified soil samples were freeze-dried prior to DNA extraction. Soil DNA was extracted using the DNeasy PowerLyzer PowerSoil Kit (QIAGEN, Hulsterweg, The Netherlands) following the manufacturer's instructions. Purity was assessed using a NanoDrop 2000 Spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) and the concentration was quantified using a QuBit 2.0 fluorometer (ThermoFisher Scientific). Before PCR amplification, DNA was diluted to a concentration of 5 ng/µL.

2.5. PCR Amplicon and Sequencing

The amplification protocol was based on the procedure proposed by Beckers et al. [51]. Briefly, PCR amplification was performed in a two-step PCR; in the first step, the primers gITS7 and ITS4 (Table S1) targeting for the internal transcribed spacer (ITS) region were used to amplify the fungal ITS2 region [52,53]. The PCR reactions were performed using HotStar HiFidelity Kit (QIAGEN, The Netherlands) according to the manufacturer's instructions, and 0.5 ng/µL of bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO, USA) in a final volume of 25 µL. The PCR amplification was run in triplicate for each sample. Five min at 95 °C were used for initial activation of the polymerase, then 15 s at 94 °C for denaturation, 1 min at 55 °C for annealing, and 45 s at 72 °C for extension, repeated 35 times;

for final extension, 10 min at 72 °C were used. Triplicate PCR products were then pooled, and PCR amplification success was confirmed using a 1.2% agarose gel. Twenty microliters of each sample was used for clean-up using AMPure XP magnetic beads (Beckman Coulter Genomics, Danvers, MA, USA) following the manufacturer's instructions, and 2.5 µL of the clean product was used for the second PCR step, where each sample was assigned a unique pair of barcodes (Table S2). The PCR amplification program used was the same as before, except 20 cycles were used instead of 35. Five microliters of each sample was pooled into one library, and the resulting pool was cleaned with AMPure XP magnetic beads. Library concentration was assessed with Qubit 2.0 and further purified to the desired fragment length (350–800 bp, accounting for variability of the ITS region) using BluePippin (Sage Science, Beverly, MA, USA) following the manufacturer's instructions. Final concentration was measured with QuBit 2.0, and the library was diluted to 10 nM. A fungal mock community, described in Haas et al. [54], was used as a positive control, and water samples as negative controls; both mock and water were treated as samples through the amplification and sequencing process.

The library pools were sequenced at the Science for Life laboratory in Stockholm, using an Illumina MiSeq and 600 cycles, yielding paired end reads of 300 bp length. Raw data were demultiplexed and quality filtered at the sequencing facility prior to delivery.

2.6. Sequence Analysis

The Illumina data were processed using QIIME2 [55] version 2019.1. Raw sequence data were imported using the q2-import plugin using the setting for Illumina fastq files, followed by denoising with Dada2 [56] via q2-dada2 denoise-paired, setting truncation at 301 bp for forward read and 300 for reverse. Taxonomy was assigned with q2-feature classifier [57] plugin using the UNITE database [58] (version 8.0) at a 97% similarity and dynamic level. Amplicon sequence variants (ASVs) were aligned with MAFFT [59] and used to infer a phylogenetic tree using FastTree [60], both processes via q2-phylogeny pipeline align-to-tree-mafft-fasttree using default settings.

To assign fungal functional guilds, we used the FUNGuild [61] Python script Guilds_v1.1.py (available at https://github.com/UMNFuN/FUNGuild, accessed on 17 November 2021) using the author's default settings. Taxa not classified by FUNGuild were completed manually through literature searches, wherever possible.

2.7. Statistics

The "kruskal" function of the agricolae package [62] was used to assess the effect of arginine phosphate or ammonium nitrate fertilization on C and N content of the soil, ectorhizosphere and needles (C or N content ~ Fertilization type) and seedling growth (root/shoot mass ~Fertilization type). The function provides Fisher's least significant difference (LSD) as post hoc analysis and statistical grouping based on Bonferroni correction when significant differences are detected in the Kruskal–Wallis test ($\alpha = 0.05$). Statistical differences in the survival rate were tested using a Cox proportional hazards regression model using the "coxph" function from the survival package [63,64] to assess the hazard ratio of the arginine phosphate- or ammonium nitrate-treated seedlings compared to the control.

Sequence analysis outputs were performed in R (Version 3.5.3) [65] and analyzed with the phyloseq package [66]. Prior to any analysis, ASVs with lower than 10 sequences were removed from the dataset using the "filterfun" function from the genefilter package [67]. Additionally, ASVs with an abundance lower than 0.005% per sample type were also removed. By doing this, 1214 low abundant ASVs were removed from the dataset. Finally, one sample with a library size lower than 10,000 filtered reads was removed from the dataset.

After filtering, species richness was estimated using the "pd" function of the picante package [68] after rarefaction of the samples to 10,894 reads, which was the minimum number of reads of a sample in the dataset. The Shannon diversity index was estimated

from raw, non-rarefied samples using the "diversity" function from the vegan package [69]. The "kruskal" function [62] was used to perform the Kruskal–Wallis rank sum test to assess the effect of arginine phosphate or ammonium nitrate fertilization on the fungal diversity (Shannon Index ~ Fertilization type). At the same time, the effect of the different sample types on the fungal diversity was tested (Shannon Index ~ Sample type).

Beta diversity was tested using the "ordinate" function from the phyloseq package, using Bray–Curtis to build the distance matrix with the rarefied samples and principal coordinate analysis (PCoA) for visualization. Changes in the community composition were tested using PERMANOVA with the vegan package [69], testing the effect of sample type and fertilization. Kruskal–Wallis was used to test any further differences between the different levels. Statistical groups were assigned based on Fisher's least significant difference (LSD) test.

To test for significant differences in abundance of the ASVs, we used the DESeq function of DESeq2 package [70] to estimate a sequencing library size factor and variance dispersion prior to fitting a generalized linear model (GLM) using the non-normalized data. Results of pairwise comparison of sample types with Log2fold change > 0.5 and adjusted *p-value* < 0.01 were used for visualization.

3. Results

3.1. Site Description

To characterize the mineral soil exposed by scarification, soil carbon (C) and nitrogen (N) content were measured at three locations within the experimental site covering the areas where the different seedPAD treatments were placed. The C and N content were highly variable in the scarified soil samples, indicative of mixing of the organic and mineral soils during scarification (Figure 1D,E). To assess whether N supplementation from the seedPADs altered soil N or C, the soil surrounding the seedling root systems (i.e., ectorhizosphere) was measured. The ectorhizosphere soil was less variable than the more mixed scarified soil but was not statistically different from it (Figure 1A–C; Kruskal–Wallis *p-value* > 0.05). Furthermore, there were no significant differences between the ectorhizosphere soil from the different treatment subsites (Figure 1D–F; Kruskal–Wallis *p-value* > 0.05). Thus, the soil across the different subsites was generally similar in N and C content and there was no evidence of significant N enrichment from the SeedPADs to the ectorhizosphere 4 months after planting.

3.2. Nitrogen Addition Increases Seedling Survival but Not Early Biomass

Seed germination and seedling survival increased 1.5-fold relative to the control when seeds were supplemented with either arginine phosphate or ammonium nitrate (Cox proportional hazard *p-value* < 0.05; Figure 2A). Furthermore, supplementation with either arginine phosphate or ammonium nitrate increased total needle C content (Kruskal–Wallis *p-value* > 0.05; Figure 2B). Although the higher survival and increase in needle C of N-enriched seedlings was not reflected in statistically greater overall shoot or root biomass, supplementation with arginine phosphate and ammonium nitrate did tend to increase shoot biomass compared to the control (Figure 2E,F).

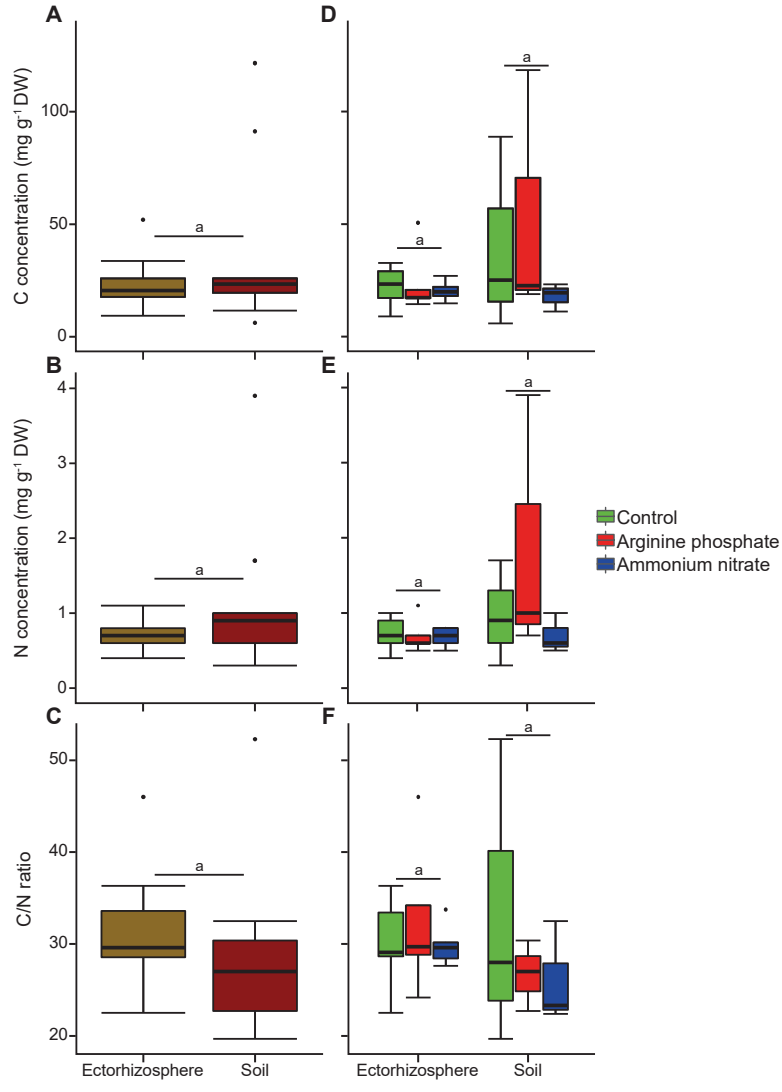

Figure 1. Boxplots of the mass fraction of carbon (**A**,**D**), nitrogen (**B**,**E**) and C to N ratio (**C**,**F**) in ectorhizosphere and scarified soil samples. Panels (**A**–**C**) depict the nutrient content per sample, and panels (**D**–**F**) depict the same data split by fertilization treatment. The lowercase letters in the upper part of the boxplots represent Fisher's least significant difference (LSD) statistical grouping testing sample type effect. Whiskers represent 1.5 × inter-quartile range (IQR).

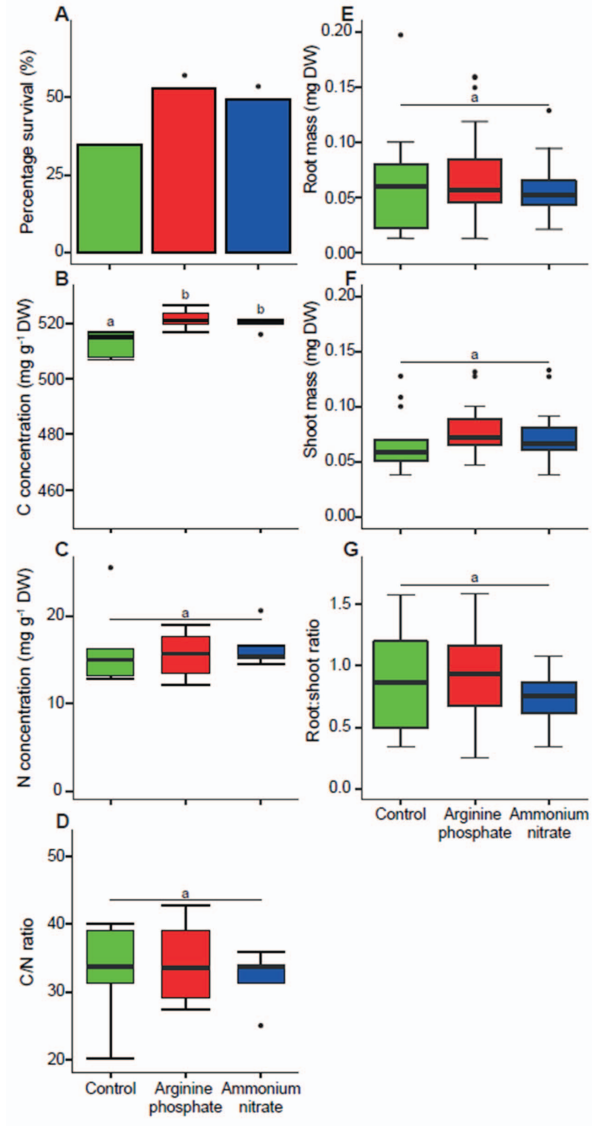

Figure 2. Barplot of the survival rate (**A**) and boxplots of the mass fraction of needle carbon (**B**), nitrogen (**C**), C-to-N ratio (**D**), root weight (**E**), shoot weight (**F**) and root-to-shoot ratio (**G**) of SeedPAD-planted Scots pine seedlings growing in scarified soil or supplied with either arginine phosphate or ammonium nitrate prior to germination. Asterisks represent statistical differences of Cox proportional hazard regression for $n = 100$. Lowercase letters in the upper part of the boxplot represent Fisher's least significant difference (LSD) statistical grouping testing treatment effect. Whiskers represents 1.5 × inter-quartile range (IQR). $n = 22$ (arginine phosphate), 18 (ammonium nitrate) and 13 (control).

3.3. Early Established Seedlings Are Colonized by Fungi Regardless of Nitrogen Supplementation

ITS2 amplicon sequence analysis was used to evaluate the fungal communities of the scarified soil, ectorhizosphere and washed roots. In control samples, the scarified and ectorhizosphere soils had, on average, two- and three-times higher richness compared to the roots (Kruskal–Wallis *p-value* < 0.001; Figure 3A). Diversity indicators showed that both scarified and ectorhizosphere soils had similarly structured fungal communities both in terms of evenness (Kruskal–Wallis *p-value* > 0.05; Figure 3B) and diversity (Kruskal–Wallis *p-value* > 0.05; Figure 3C). Both soil types had more even (Kruskal–Wallis *p-value* < 0.05) and more diverse (Kruskal–Wallis *p-value* < 0.05) communities than the roots, which had a community dominated by fewer ASVs.

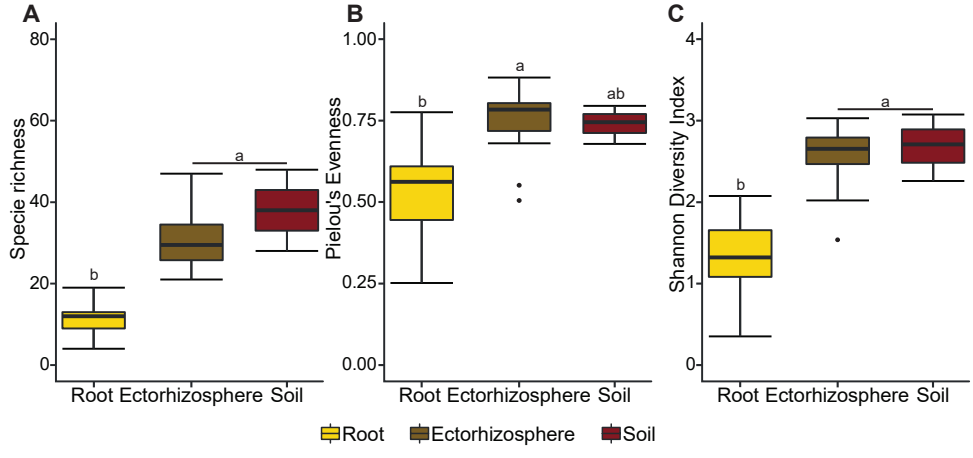

Figure 3. Boxplots of (**A**) species richness, (**B**) Pielou's evenness, and (**C**) Shannon diversity of control samples of roots, ectorhizosphere and scarified soils. Lowercase letters in the upper part of the boxplot represent Fisher's least significant difference (LSD) statistical grouping testing scheme 1.5 × inter-quartile range (IQR).

While species richness, Pielou's evenness and Shannon diversity all provide broad information about community structure, they do not provide information about community composition. An analysis of the community composition indicated that while the scarified soil and ectorhizosphere fungal communities were similar (PERMANOVA; adjusted *p-value* > 0.05), they harbored different dominant ASVs (Figure 4). For example, within the scarified soil community, the most dominant ASV was identified as belonging to the opportunistic taxon *Helotiales* sp., while in the ectorhizosphere *Leotiomycetes* sp., together with ASVs assigned to known saprotrophic taxa such as *Penicillium*, *Umbelopsis*, *Mortierella* and *Mycena* along with the ericoid mycorrhiza *Oidiodendron*, were dominant (Figure 4). In contrast, the community composition of the roots was significantly different from both the scarified soil (PERMANOVA adjusted *p-value* < 0.005) and the ectorhizosphere (PERMANOVA adjusted *p-value* < 0.005), with the root-associated fungal community strongly enriched for ectomycorrhizal symbiotrophs such as *Russula, Piloderma* and *Suillus*, but depleted in saprotrophic fungi (Figure 4).

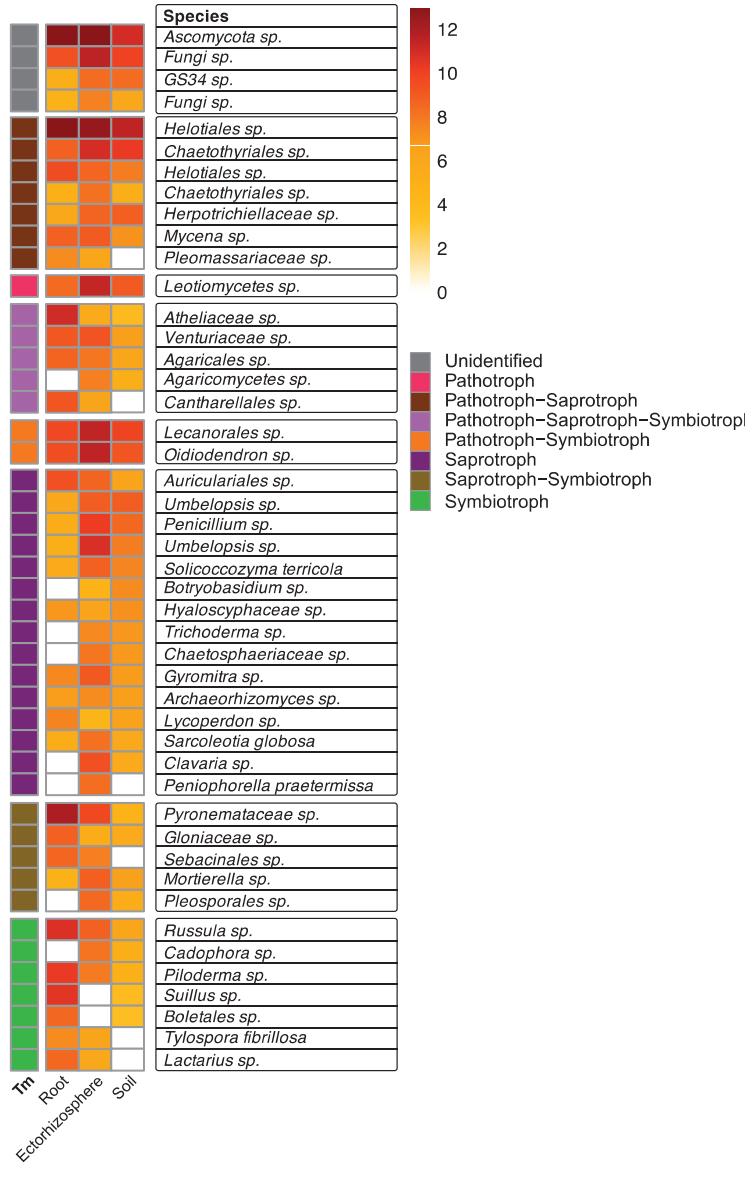

Figure 4. Heatmap depicting the most abundant amplicon sequence variants (ASVs) adding up to a total abundance of 50% of the total community of roots, ectorhizosphere or scarified soils. Left column colored by trophic modes annotated on FUNGuild V1.1. Scale is $\log_{10}$ of the sequencing reads. The complete list of ASVs can be found in Table S3.

When looking closer at the abundances of ECM fungal ASVs in roots, ectorhizosphere and bulk soil, we observed three differing patterns of occurrence (Figures 4 and S4).

(i) Several ectomycorrhizal ASVs in both types of soil samples, belonging to *Russula*, *Piloderma*, *Suillus* and *Cortinariaceae*, were enriched in root samples (Symbiotrophs in Figure S4), indicating their presence across the site as well as a rapid colonization of seedling roots. (ii) Two ASVs identified as *Cortinarius* sp. and *Russula densifolia* were present in bulk soil samples but never close to or on the seedling roots, which might indicate a preference for other host species or older trees. (iii) Several ectomycorrhizal ASVs (*Inocybe soluta*, *Tomentellopsis echinospora*, *Tylospora fibrillosa*, *Amphinema byssoides*, *Lactarius* sp.) were found only on roots and the rhizosphere, potentially indicating that these fungi very specifically grew to colonize the seedlings, with dormant spores in bulk soil staying below the detection threshold (Figures 4 and S4).

PCoA analysis of the N-supplemented samples indicated the presence of a core community that was persistent regardless of N-supplementation treatments. This community included ASVs identified as symbiotrophs, such as *Tylospora* and *Amphinema*, as well as ASVs identified as 'pathotroph-symbiotrophs' (e.g., *Lecanorales* and *Oidiodendron*) and a large number of unidentified Ascomycota and opportunistic *Helotiales* and saprotrophs (e.g., *Umbelopsis* and *Penicillium*) (Figure S4). Supplementation with arginine phosphate increased the number of unique ASVs in the root-associated community, accounting for 28.5% of the root-associated community (Figure S5). Despite this increase, no significant differences were found in diversity indexes (Figure S6; Kruskal–Wallis $p\text{-value} > 0.05$) nor at compositional level (PERMANOVA adjusted $p\text{-value} > 0.05$).

To further test the effect of N supplementation on the root-associated fungal community recruited by the roots of newly germinated pine seedlings, ectomycorrhiza fungi abundance was estimated based on the ASV counts and statistical significance (Figure 5). From the results, ASVs of the family *Pyronemataceae* were positively increased by supplementation with either arginine phosphate or ammonium nitrate (Figure 5, Table S4). Another ASV assigned to the genus *Russula*, which contains both nitrophilic and nitrophobic species [71], showed a significant negative response to N supplementation (Figure 5; Table S4). An ASV assigned to the mostly N-tolerant genus *Lactarius* was present only in the arginine phosphate-treated samples (Figure 5). ASVs assigned to the nitrophobic taxon *Piloderma* showed an increase in abundance in the presence of arginine phosphate, while *Suillus* showed reduced abundance in response to both N-supplementation treatments (Figure 5). Interestingly, a member of the *Cortinariaceae* family, which is known to be nitrophobic [71], was only present on roots of fertilized seedlings (Figure S7).

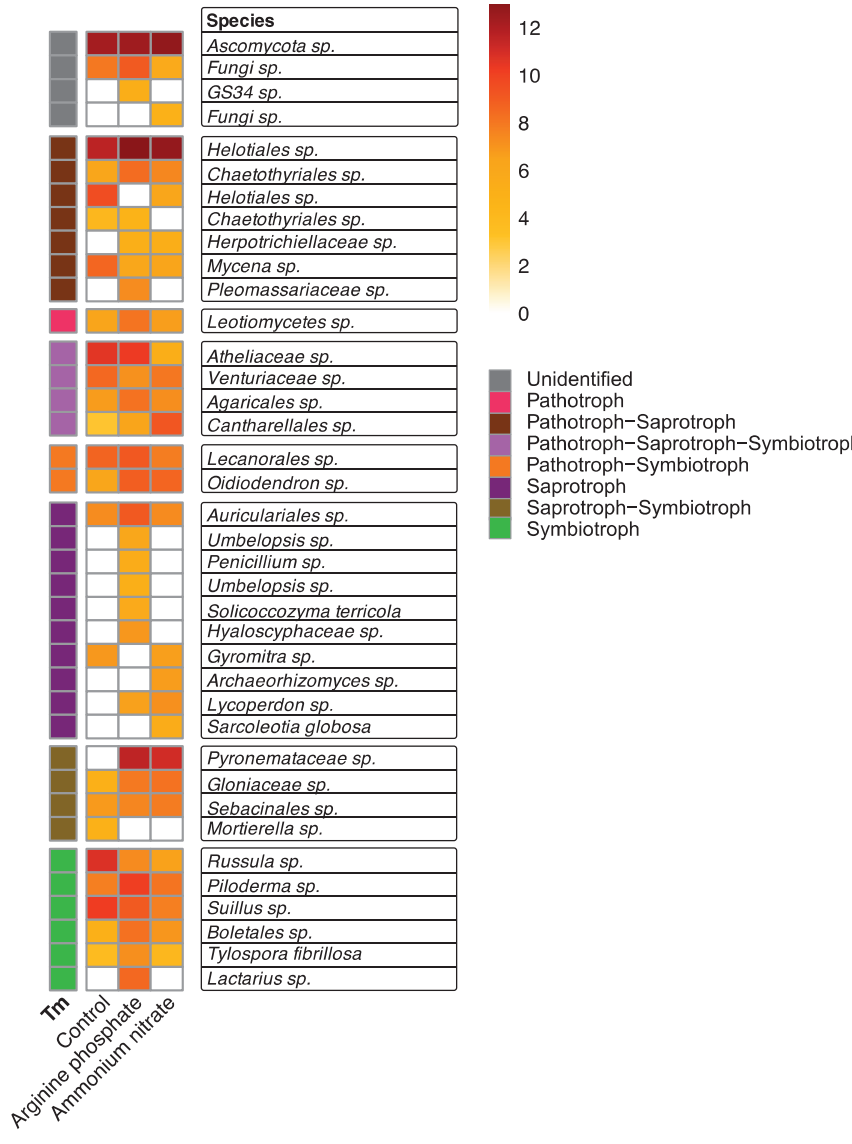

Figure 5. Heatmap depicting the most abundant amplicon sequencing variants (ASVs) with a cumulative presence of 50% of the total community on roots. Left column colored by trophic modes annotated on FUNGuild V1.1. Scale is $\log_{10}$ of the sequencing reads.

4. Discussion

In the Swedish boreal forest, soil scarification is the current practice for site preparation after clear-cut timber harvesting. Clear-cutting stops the transport of current photosynthate to roots and the associated rhizospheric community, which leads to a drastic shift in soil

fungal communities, reducing ECM abundance [30]. The subsequent soil scarification further disrupts the ECM, leaving only fragments of the previous ECM networks. These scarified clear-cuts are typically replanted with nursery-produced seedlings 2 years after harvesting or, on specific sites, directly sown with orchard-produced seeds. The boreal forest is generally characterized by nutrient-poor soils, with N being the most limiting nutrient [15], and nursery-produced seedlings are deliberately loaded with nutrients during production to improve initial seedling survival and growth [72–74]. However, it is not known whether the same positive effect of N supplementation can be achieved upon direct seeding. Our results show that a small addition of either organic or inorganic N did increase seedling survival (p-*value* < 0.05) and carbon accumulation aboveground (p-*value* < 0.05) (Figure 2). Furthermore, we provide evidence for a strong early effect on fungal recruitment by the root, with an enrichment of ECM fungi in the saprobe-dominated, disturbed soil after only a few months (Figure 4).

Reduction in recent plant-sourced C in the soil caused by site preparation (i.e., tree removal and soil scarification) reduces ECM dominance, favoring saprobes [30,75]. Kohout et al. [29] found a progressive shift from an ECM- to saprotrophic-dominated soil community in a spruce forest over a timeframe of around 20 months after harvesting, which corresponds to the time that usually passes between harvest and replanting. Our data are consistent with this finding, showing that saprotrophs were the most dominant trophic mode in the scarified soil and ectorhizosphere (Figure 4; Table S3), accounting for about 15% of the whole community. However, while greatly diminished, ECM were not completely removed from the scarified soil community of our study site, accounting for about 5% of the community (Figure S4). The persistence of these ECMs might be explained by hyphae, sclerotia or spores remaining in the soil [76]. These different types of propagules are believed to remain dormant and can be induced to restart growth or germinate, respectively, by the presence of closely growing roots [76,77]. Our results clearly show this stimulating effect of the exploring root (Figure 4; Figure S4), with ECMs associated with roots being twice as abundant relative to either the ectorhizosphere or scarified clear-cut soils. Similar results were found by Kyaschenko et al. [78] in a study performed in an age-gradient Scots pine stand, where 1 year after site preparation the soils were saprobe-dominated, while the abundance of ECM was generally low, with a dominance of species such as *Piloderma* and *Tylospora.* They proposed that these ECM fungi from the *Atheliaceae* family are favored by the high levels of soil inorganic N and host-derived C in the soils of the younger stand [78]. We also found that *Atheliaceae* such as *Piloderma, Tylospora* and *Amphinema* were among the dominant ECM families in the root samples (about 25%; Figure S7), supporting the view that they are among the first ECM colonizers of Scots pine seedlings germinating in disturbed soils, and that recruitment by these seedlings occurs very rapidly despite the saprobe-dominated community of the disturbed scarified soil.

Growing in association with already-established ECM is often positive for seedlings, and established ECM fungi can enhance survival at the plant community level [79]. However, it has been observed that under some environments, such as nutrient-poor soils, belowground interactions limit seedling growth due to their low competitive strength against adult trees [14,25,80,81]. Thus, although seedlings that germinate in undisturbed boreal forest soils have access to established mycorrhiza, from which they may gain valuable nutrients [82], seedling establishment in such undisturbed forests is often poor, and these seedlings are generally smaller compared to those that establish in scarified soils [25]. This was illustrated by Axelsson et al., [25] who performed an experiment in a Scots pine stand where they observed that the seedlings successfully established and grew larger, both when C flux belowground was eliminated by girdling the adult trees, or in a clear-cut ca. 10 m away from the forest edge [25]. Similar results were found by Pasanen et al., [26] in artificial canopy gaps with soil preparation in a Scots pine-dominated forest, where most of the establishing seedlings were found in the disturbed soil plots [26]. Thus, in highly disturbed soils, such as a scarified clear-cut site, where mycorrhizal networks and plant-derived C flux have been heavily disrupted, initial seedling establishment is enhanced and

resistant propagules (i.e., sclerotia and spores) become the dominant mycorrhizal inoculum source [76,83,84]. This might explain why we found some ECM fungi such as *Inocybe soluta*, *Tomentellopsis echinospora* or *Amphinema byssoides* at relatively high abundances in root and rhizosphere samples, while being absent from bulk soil samples (Figure S4). Their apparent absence in the bulk soil might be due to these propagules falling below the detection threshold of our profiling method, while other ectomycorrhizal ASVs belonging to *Russula* and *Piloderma* were still present in the disturbed soil with relatively higher biomass abundance, possibly due to higher resilience to disturbance or higher abundances before clear-cutting.

The addition of a small amount of N to the germinating seedling did not significantly alter the already-strong selective pressure of the roots on the fungal community (Table S4). However, we did find evidence of initial changes in the abundance of specific fungal ASVs, particularly in *Pyronemataceae* sp. and *Russula* (adjusted *p-value* < 0.01; log2Fold > 2). Although the specific nature of most *Pyronemataceae* is unknown, different members of the family have been characterized as saprobes or ectomycorrhizal [85,86], while *Russula* is a large ECM genus with both nitrophobic and nitrophilic species, and is typically dominant in older stands [71,78,87]. Within the other ASVs recruited by the roots, the nitrophobic *Suillus* was negatively affected by both arginine phosphate and ammonium nitrate (Figure 5) while *Piloderma*, which has the ability to take up, use and deliver amino acids to Scots pine [88], responded positively to the addition of arginine phosphate but not ammonium nitrate (Figure 5). While previous large-scale studies have shown that short-term N addition to undisturbed boreal forest soils had no significant influence on root-associated fungal communities [54,89], our study showed that small, localized applications of either organic or inorganic N in disturbed soils both increases seedling survival and facilitates the rapid recruitment of some taxa of ECM fungi, such as members of *Pyronemataceae*, *Cortinariaceae* and *Piloderma*.

5. Conclusions

Traditional forest management includes clear-cutting of mature trees and subsequent soil scarification for new seedling plantations. While this increases seedling establishment success and growth, its collateral effect is the local disruption of the soil, altering nutrient equilibrium and microbial composition. Further complicating future management decisions, the impacts of climate change-related increases in air and soil temperatures and increasing drought on the plant–soil community interaction are uncertain. While increases in soil temperature are expected to enhance seed germination and survival in the northern hemisphere [90–92], higher soil temperatures are also known to reduce ECM formation [39,40], potentially leaving the roots more vulnerable to pathogen attacks [93] or drought. Our study provides evidence that small-scale N addition to onsite-planted seeds can be used to enhance seedling establishment without N leakage into the soil, and that such small-scale N addition does not significantly alter the strong selective pressure of the growing root in rapidly reshaping the root-associated fungal community from the opportunistic dominated community of the scarified soil into an ECM-dominated community.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12111589/s1, Figure S1: SeedPAD^{TM} germination matrix, Figure S2: Site description, Figure S3: Site temperature and precipitation data, Figure S4: Whole fungal community for roots, ectorhizosphere and soil samples, Figure S5: Venn diagram of root ASVs by treatment, Figure S6: Violin plot of richness, Pielou's evenness and Shannon diversity of root samples by treatment, Figure S7: Whole root fungal community by treatment, Table S1: ITS2 Primer sequence, Table S2: Barcode combination used by sample, Table S3: Whole community taxonomy, Table S4: Differentially abundant taxa results.

Author Contributions: Conceptualization, V.H., N.R.S. and T.N.; methodology, V.H., N.R.S., T.N. and M.H.; software, D.C.; validation, D.C. and A.N.S.; formal analysis, D.C.; investigation, D.C. and A.N.S.; resources, D.C. and A.N.S.; data curation, D.C.; writing—original draft preparation, D.C. and A.N.S.; writing—review and editing, D.C., A.N.S., V.H., N.R.S. and T.N.; visualization, D.C.;

supervision, V.H.; project administration, V.H., N.R.S., T.N. and M.H.; funding acquisition, V.H., N.R.S. and T.N. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The dataset generated and analyzed in this study is available in the European Nucleotide Archive (ENA; https://www.ebi.ac.uk/ena/browser/home, accessed on 17 November 2021) and is available under the accession number PRJEB48062.

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Conflicts of Interest: The authors declare no conflict of interest.

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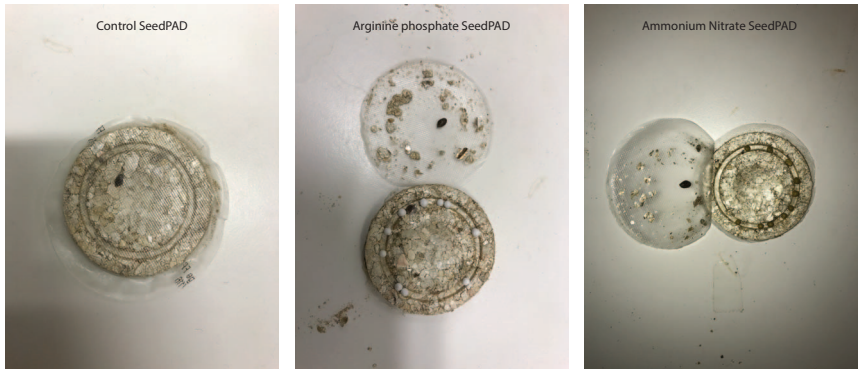

Figure S1. SeedPAD® with Scots pine seed without (left panel) and with arginine phosphate or ammonium nitrate.

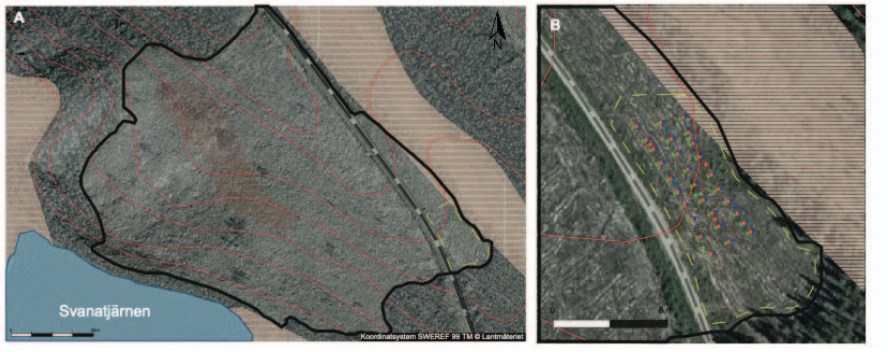

Figure S2. (**A**) Clear-cut field site where the experiment was done, in the Svanatjärn area. Topographical characteristic of the site, like plateaus,altitude, road and water are presented as lines or colored areas. The complete clear-cut is delimited with a black continuous line and the experimental site is delimited with a yellow dashed line. (**B**) Experimental site detailed view, sub-sites where the SeedPAD's were planted are delimited with a dotted line. Schematic planting are marqued with colored dots and scarified soil samples are m; control (green), arginine phosphate (red) and ammonium nitrate (blue). Original maps taken from Lantmäteriet.se on May 2020.

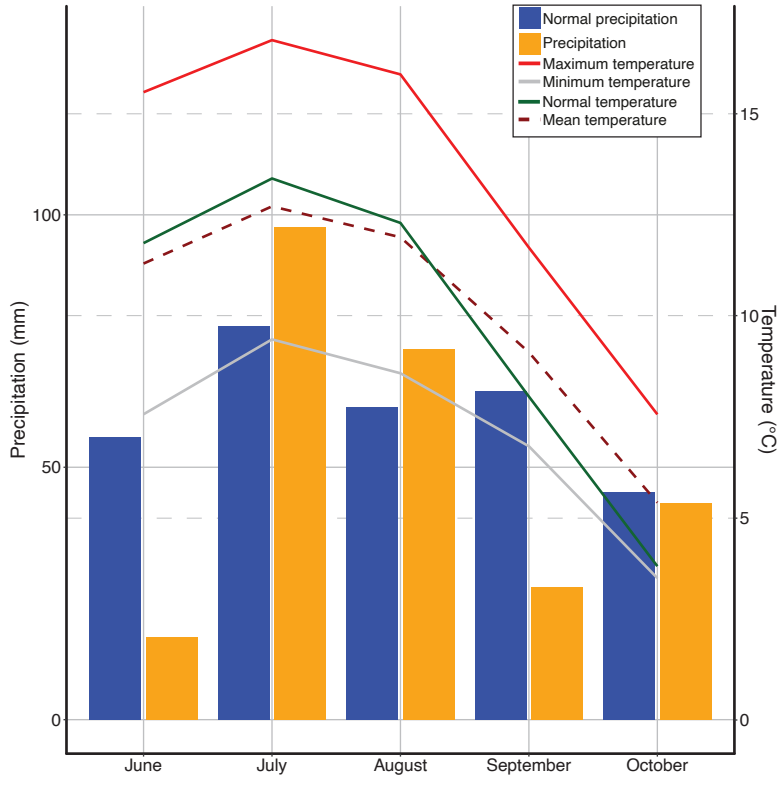

Figure S3. Temperature and precipitation. Normal temperature in continuous green line, mean temperature in dashed red line. Maximum in continuous red line and minimum in gray continuous line. Normal precipitation in bluebars, observed precipitation in orange bars. Data was imported from SMHI database from Frösön station, located approximately 150 km west-southwest from the clear-cut. 'Normal' data is calculated with data collected from 1961 to 1990.

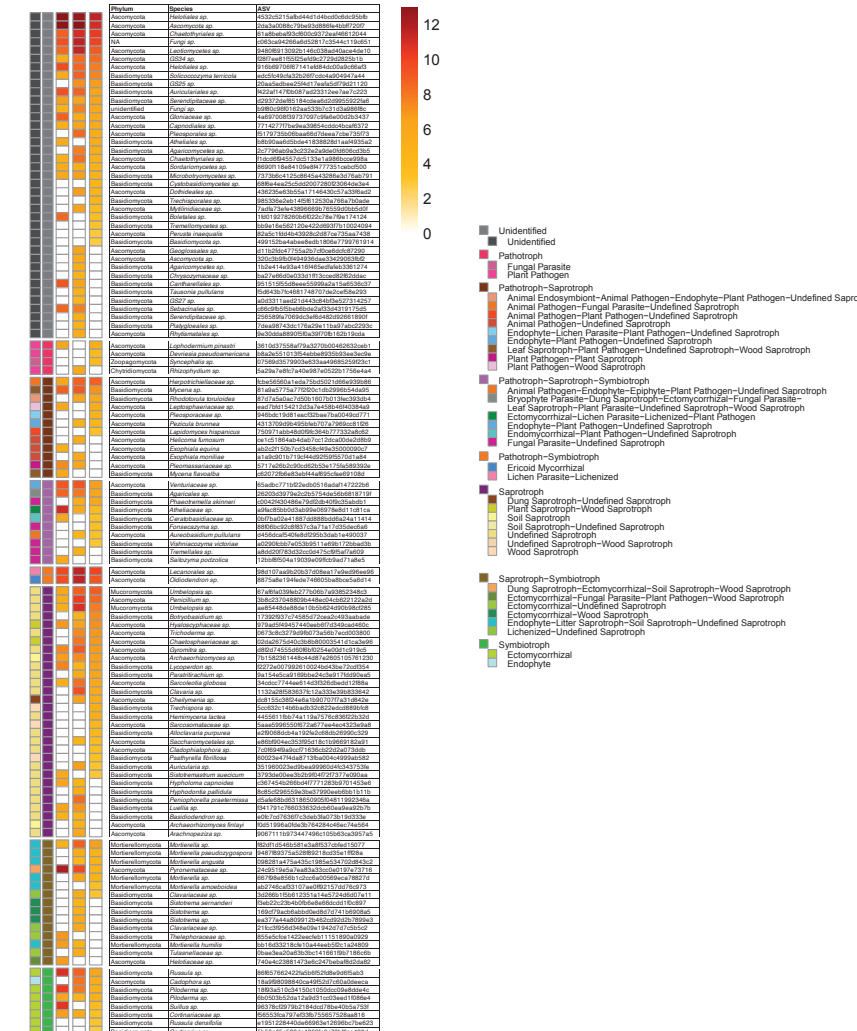

Figure S4. Heatmap of whole fungal community of root, ectorhizosphere and soil samples. Guild and Trophic mode columns correspond to functional annotation from FunGuild. Scale is $\log_{10}$ of the sequencing reads.

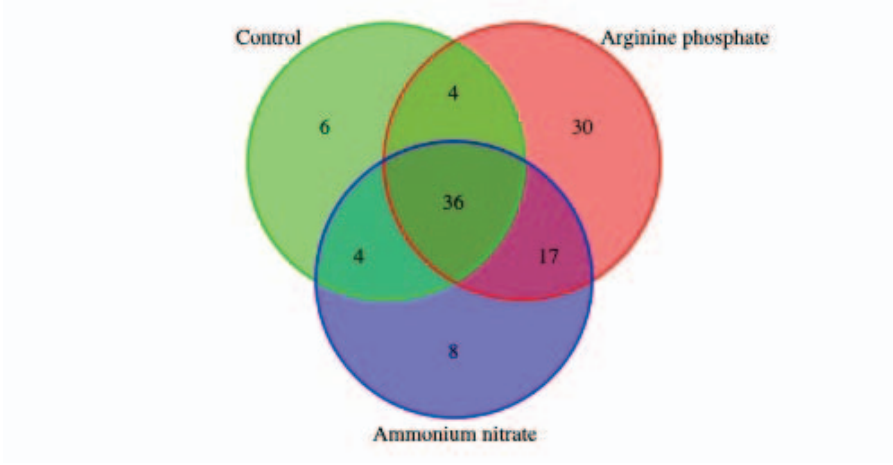

Figure S5. Venn diagram showing numbers of common and unique fungal ASVs of Scots pine roots. Circles coloured by treatment.

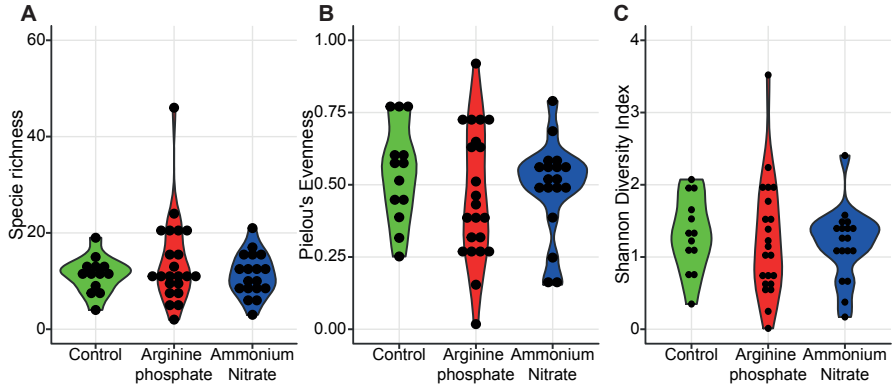

Figure S6. Violin plot showing fungal richness (**A**), Pielou's evenness (**B**) and Shannon diversity index (**C**) of Scots pine root samples. Samples are shown as black dots. Violin shape coloured by treatment

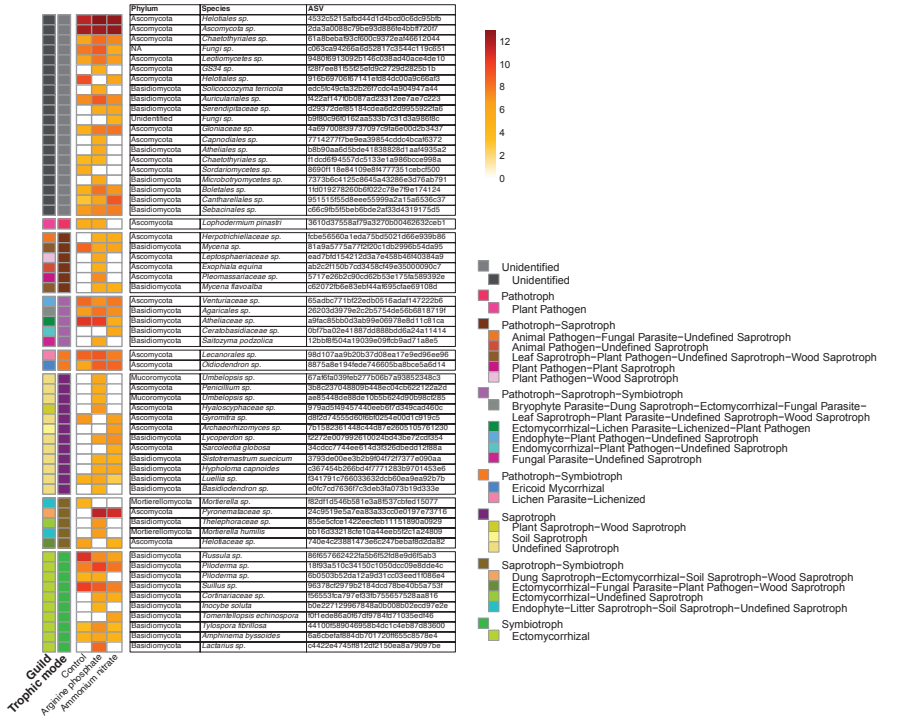

Figure S7. Heatmap of whole root fungal community by treatment. Guild and Trophic mode columns correspond to functional annotation from FunGuild. Scale is $\log_{10}$ of the sequencing reads.

Table S1. Primers used for ITS2 amplification PCR of DNA extracted from root, ectorhizosphere and soil samples.

Amplicon PCR				
Primer Name	Overhang	N	Locus specific sequence	Concatenated primer
ITS2				
gITS7	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG		GTGARTCATCGARTCTTTG	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGARTCATCGARTCTTTG
gITS7N	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	N	GTGARTCATCGARTCTTTG	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNGTGARTCATCGARTCTTTG
gITS7NN	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	NN	GTGARTCATCGARTCTTTG	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNGTGARTCATCGARTCTTTG
gITS7NNN	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	NNN	GTGARTCATCGARTCTTTG	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNGTGARTCATCGARTCTTTG
ITS4	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG		TCCTCCGCTTATTGATATGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTCCGCTTATTGATATGC
ITS4N	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	N	TCCTCCGCTTATTGATATGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNTCCTCCGCTTATTGATATGC
ITS4NN	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	NN	TCCTCCGCTTATTGATATGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNTCCTCCGCTTATTGATATGC
ITS4NNN	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	NNN	TCCTCCGCTTATTGATATGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNTCCTCCGCTTATTGATATGC

Table S1. Primers used for indexing PCR amplified ITS. Continues from amplicon PCR

Barcoding PCR					
Primer Name	P5/P7 element	Index	Overhang-Complement	Concatenated primer	Index Sample Sheet
Index1-1	CAAGCAGAAGACGGCATACGAGAT	TCGCCTTA	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGG	TAAGGCGA
Index1-2	CAAGCAGAAGACGGCATACGAGAT	CTAGTACG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGG	CGTACTAG
Index1-3	CAAGCAGAAGACGGCATACGAGAT	TTCTGCCT	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGG	AGGCAGAA
Index1-4	CAAGCAGAAGACGGCATACGAGAT	GCTCAGGA	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGG	TCCTGAGC
Index1-5	CAAGCAGAAGACGGCATACGAGAT	AGGAGTCC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGG	GGACTCCT
Index1-6	CAAGCAGAAGACGGCATACGAGAT	CATGCCTA	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGG	TAGGCATG
Index1-7	CAAGCAGAAGACGGCATACGAGAT	GTAGAGAG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGG	CTCTCTAC
Index1-8	CAAGCAGAAGACGGCATACGAGAT	CAGCCTCG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGG	CGAGGCTG
Index1-9	CAAGCAGAAGACGGCATACGAGAT	TGCCTCTT	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGG	AAGAGGCA
Index1-10	CAAGCAGAAGACGGCATACGAGAT	TCCTCTAC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGG	GTAGAGGA
Index1-11	CAAGCAGAAGACGGCATACGAGAT	TCATGAGC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTCATGAGCGTCTCGTGGGCTCGG	GCTCATGA
Index1-12	CAAGCAGAAGACGGCATACGAGAT	CCTGAGAT	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATCCTGAGATGTCTCGTGGGCTCGG	ATCTCAGG

Barcoding PCR					
Index1-13	CAAGCAGAAGACGG-CATACGAGAT	TAGC GAGT	GTCTCGTGGGC TCGG	CAAGCAGAAGACGGCATACGA-GATTAGCGAGTGTCTCGTGGGCTCGG	ACTCGCT A
Index1-14	CAAGCAGAAGACGG-CATACGAGAT	GTAG CTCC	GTCTCGTGGGC TCGG	CAAGCAGAAGACGGCATACGA-GATGTAGCTCCGTCTCGTGGGCTCGG	GGAGC-TAC
Index1-15	CAAGCAGAAGACGG-CATACGAGAT	TACT ACGC	GTCTCGTGGGC TCGG	CAAGCAGAAGACGGCATACGAGAT-TACTACGCGTCTCGTGGGCTCGG	GCG-TAGTA
Index1-16	CAAGCAGAAGACGG-CATACGAGAT	AG-GCTC CG	GTCTCGTGGGC TCGG	CAAGCAGAAGACGGCATACGAGA-TAGGCTCCGGTCTCGTGGGCTCGG	CGGAGCC T
Index1-17	CAAGCAGAAGACGG-CATACGAGAT	GCAG CGTA	GTCTCGTGGGC TCGG	CAAGCAGAAGACGGCATACGA-GATGCAGCGTAGTCTCGTGGGCTCGG	TAC-GCTGC
Index1-18	CAAGCAGAAGACGG-CATACGAGAT	CTGC GCAT	GTCTCGTGGGC TCGG	CAAGCAGAAGACGGCATACGAGATCTGCG-CATGTCTCGTGGGCTCGG	ATGCG-CAG
Index1-19	CAAGCAGAAGACGG-CATACGAGAT	GAGC GCTA	GTCTCGTGGGC TCGG	CAAGCAGAAGACGGCATACGA-GATGAGCGCTAGTCTCGTGGGCTCGG	TAGCGCT C
Index1-20	CAAGCAGAAGACGG-CATACGAGAT	CGCT CAGT	GTCTCGTGGGC TCGG	CAAGCAGAAGACGGCATACGA-GATCGCTCAGTGTCTCGTGGGCTCGG	ACTGAGC G

Table S1. Primers used for indexing PCR amplified ITS. *Continuation*

Barcoding PCR					
Index2-1	AATGATACGGCGACCAC-CGAGATCTACAC	CTCT CTAT	TCGTCGG-CAGCGTC	AATGATACGGCGACCACCGAGATCTACAC-CTCTCTATTCGTCGGCAGCGTC	ATAGA-GAG
Index2-2	AATGATACGGCGACCAC-CGAGATCTACAC	TATC CTCT	TCGTCGG-CAGCGTC	AATGATACGGCGACCACCGAGATCTACAC-TATCCTCTTCGTCGGCAGCGTC	AGAG-GATA
Index2-3	AATGATACGGCGACCAC-CGAGATCTACAC	GTAA GGAG	TCGTCGG-CAGCGTC	AATGATACGGCGACCACCGAGATCTACAC-GTAAGGAGTCGTCGGCAGCGTC	CTCCTTA C
Index2-6	AATGATACGGCGACCAC-CGAGATCTACAC	CTAA-GCCT	TCGTCGG-CAGCGTC	AATGATACGGCGACCACCGAGATCTACAC-CTAAGCCTTCGTCGGCAGCGTC	AG-GCTTAG
Index2-7	AATGATACGGCGACCAC-CGAGATCTACAC	CGTC TAAT	TCGTCGG-CAGCGTC	AATGATACGGCGACCACCGAGATCTACAC-CGTCTAATTCGTCGGCAGCGTC	ATTAGAC G

Table S3. Barcode combination for each sample.

Sample	Index1	Index2
Ctrl.1	Index1_1	Index2_1
Ctrl.2	Index1_2	Index2_1
Ctrl.3	Index1_3	Index2_1
Ctrl.4	Index1_4	Index2_1
Ctrl.5	Index1_5	Index2_1
Ctrl.6	Index1_6	Index2_1
Ctrl.7	Index1_7	Index2_1
Ctrl.8	Index1_8	Index2_1
Ctrl.9	Index1_9	Index2_1
Ctrl.10	Index1_10	Index2_1
Ctrl.11	Index1_11	Index2_1
Ctrl.12	Index1_12	Index2_1
Ctrl.13	Index1_13	Index2_1
Osm.1	Index1_14	Index2_1
Osm.2	Index1_15	Index2_1
Osm.3	Index1_16	Index2_1
Osm.4	Index1_17	Index2_1
Osm.5	Index1_18	Index2_1
Osm.6	Index1_19	Index2_1
Osm.7	Index1_20	Index2_1
Osm.8	Index1_1	Index2_2
Osm.9	Index1_2	Index2_2
Osm.10	Index1_3	Index2_2
Osm.11	Index1_4	Index2_2
Osm.12	Index1_5	Index2_2
Osm.13	Index1_6	Index2_2
Osm.14	Index1_7	Index2_2
Osm.15	Index1_8	Index2_2
Osm.16	Index1_9	Index2_2
Osm.17	Index1_10	Index2_2
Osm.18	Index1_11	Index2_2
Arg.1	Index1_12	Index2_2
Arg.2	Index1_13	Index2_2
Arg.3	Index1_14	Index2_2
Arg.4	Index1_15	Index2_2
Arg.5	Index1_16	Index2_2
Arg.6	Index1_17	Index2_2
Arg.7	Index1_18	Index2_2
Arg.8	Index1_19	Index2_2
Arg.9	Index1_20	Index2_2
Arg.10	Index1_1	Index2_3
Arg.11	Index1_2	Index2_3
Arg.12	Index1_3	Index2_3
Arg.13	Index1_4	Index2_3
Arg.14	Index1_5	Index2_3
Arg.15	Index1_6	Index2_3
Arg.16	Index1_7	Index2_3
Arg.17	Index1_8	Index2_3
Arg.18	Index1_9	Index2_3
Arg.19	Index1_10	Index2_3
Arg.20	Index1_11	Index2_3
Arg.21	Index1_12	Index2_3
Arg.22	Index1_13	Index2_3
S.arg.1	Index1_15	Index2_6
S.arg.2	Index1_16	Index2_6
S.arg.3	Index1_17	Index2_6
S.ctrl.1	Index1_2	Index2_7
S.ctrl.2	Index1_3	Index2_7
S.ctrl.3	Index1_4	Index2_7
S.osm.1.1	Index1_9	Index2_7
S.osm.1.2	Index1_10	Index2_7
S.osm.2	Index1_11	Index2_7
S.osm.3	Index1_12	Index2_7

Table S3. Taxonomy table.

AVS	King-dom	Phylum	Class	Order	Family	Genus	Specie
02da2675d40c3b8b80003541d1ca3e96	Fungi	Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	NA	NA
0673c8c3279d9fb073a56b7ecd003800	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	NA
07569d3579903e633aa49685259f23c1	Fungi	Zoopagomycota	Zoopagomycetes	Zoopagales	Piptocephalidaceae	Syncephalis	unidentified
0912ada1f2253cefc9ee566d2a99613e	Fungi	Ascomycota	Sordariomycetes	NA	NA	NA	NA
098281a475a435c1985e534702d843c2	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	Mortierella angusta
0a3e2dfb5c79d603a8ee85c8e20d0ec7	Fungi	Basidiomycota	Tremellomycetes	NA	NA	NA	NA
0bae3ea20a63b3bc141661f9b7186c6b	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Tulasnellaceae	unidentified	unidentified
0bf7ba02e41887dd888bdd6a24a11414	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae	NA	NA
1132a28f583637fc12a333e39b833642	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavaria	NA
12bbf8f504a19039e09ffcb9ad71a8e5	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Trimorphomycetaceae	Saitozyma	Saitozyma podzolica
169cf79acb6abbd0ed8d7d741b6908a5	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Cantharellales_fam_Incertae_sedis	Sistotrema	unidentified
17392f937c74585d72cea2c493aabade	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Botryobasidiaceae	Botryobasidium	NA
18a9f98098840ca49f52d7c60a0deeca	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiales_fam_Incertae_sedis	Cadophora	unidentified
18f93a510c34150c1050dcc09e8dde4c	Fungi	Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	Piloderma	NA
18ffbcc8c367bfe6945249fbf7a6eac9	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Omphalotaceae	Gymnopus	Gymnopus perforans
1b2e414e93a416f465edfafeb3361274	Fungi	Basidiomycota	Agaricomycetes	unidentified	unidentified	unidentified	unidentified
1fd019278260b6f022c78e7f9e174124	Fungi	Basidiomycota	Agaricomycetes	Boletales	NA	NA	NA
20aa5adbee25f4d17eafa5df79d21120	Fungi	Basidiomycota	GS25	GS25	unidentified	unidentified	unidentified
21fcc3f956d348e09e1942d7d7c5b5c2	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	NA	NA
24409e996e4f6ba03540356871675735	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	Cortinarius	Cortinarius subtortus
24c9519e5a7ea83a33cc0e0197e73716	Fungi	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	NA	NA
256589fa7069dc3ef6d482d92661890f	Fungi	Basidiomycota	Agaricomycetes	Sebacinales	Serendipitaceae	unidentified	unidentified
26203d3979e2c2b5754de56b6818719f	Fungi	Basidiomycota	Agaricomycetes	Agaricales	NA	NA	NA
2c7796ab9a3c232e2a9de0fd606cd3b5	Fungi	Basidiomycota	Agaricomycetes	NA	NA	NA	NA

AVS	King-dom	Phylum	Class	Order	Family	Genus	Specie
2da3a0088c79be93d886fe4bbff720f7	Fungi	Ascomycota	NA	NA	NA	NA	NA
320c3b9fb0f494936dae33429063fbf2	Fungi	Ascomycota	unidentified	unidentified	unidentified	unidentified	unidentified
34cdcc7744ee614d3f326dbedd12f88a	Fungi	Ascomycota	Geoglossomycetes	Geoglossales	Geoglossaceae	Sarcoleotia	Sarcoleotia globosa
351960023ed9bea99960d4fc343753fe	Fungi	Basidiomycota	Agaricomycetes	Auriculariales	Auriculariaceae	Auricularia	unidentified
3610d37558af79a3270b00462632ceb1	Fungi	Ascomycota	Leotiomycetes	Rhytismatales	Rhytismataceae	Lophodermium	Lophodermium pinastri
3793de00ee3b2b9f04f72f7377e090aa	Fungi	Basidiomycota	Agaricomycetes	Trechisporales	Trechisporales_fam_Incertae_sedis	Sistotremastrum	Sistotremastrum suecicum
3b8c237048809b448ec04cb622122a2d	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Penicillium	NA
3d266b1f5b612351a14e5724d6d07e11	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	unidentified	unidentified
40ec0449ec7e642dbdd74aa8d5509a0b	Fungi	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	Pseudotomentella	NA
4313709d9b495bfeb707a7969cc81f26	Fungi	Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	Pezicula	Pezicula brunnea
436235e63b55a17146430c57a33f6ad2	Fungi	Ascomycota	Dothideomycetes	Dothideales	NA	NA	NA
44100f589046958b4dc1c4eb87d83600	Fungi	Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	Tylospora	Tylospora fibrillosa
4455611fbb74a119a7576c836f22b32d	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	Hemimycena	Hemimycena lactea
4532c5215afbd44d1d4bcd0c6dc95bfb	Fungi	Ascomycota	Leotiomycetes	Helotiales	NA	NA	NA
47468fb13141bd2a2294a1feb6c91c89	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Capronia	unidentified
48eb29e4f552e888c1b4b2e7ccae3070	Fungi	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	Otidea	Otidea tuomikoskii
499152ba4abee8edb1806e7799761914	Fungi	Basidiomycota	unidentified	unidentified	unidentified	unidentified	unidentified
4a697008f39737097c9fa6e00d2b3437	Fungi	Ascomycota	Dothideomycetes	Mytilinidales	Gloniaceae	NA	NA
4a95ef50d7eeb406454385db68a1f0a2	Fungi	Basidiomycota	Agaricomycetes	Russulales	Albatrellaceae	Byssoporia	Byssoporia terrestris
51f84c0b1b7b9622b6600e90013c53c3	Fungi	Ascomycota	Leotiomycetes	Helotiales	Sclerotiniaceae	NA	NA
5666c7fcd4c19ffda98e9a25de6750e6	Fungi	Basidiomycota	Agaricomycetes	Polyporales	Xenasmataceae	Xenasmatella	unidentified
5717e26b2c90cd62b53e175fa589392e	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleomassariaceae	NA	NA
5a29a7e8fc7a40e987e0522b1756e4a4	Fungi	Chytridiomycota	Rhizophydiomycetes	Rhizophydiales	Rhizophydiaceae	Rhizophydium	unidentified
5aae5996550f672a677ee4ec4323e9a8	Fungi	Ascomycota	Pezizomycetes	Pezizales	Sarcosomataceae	unidentified	unidentified
5b56a65c528de4266fa9c73bf6ca490d	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	Cortinarius	NA
5cc632c14b6badb32c822edcd889bfc8	Fungi	Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	Trechispora	NA

AVS	King-dom	Phylum	Class	Order	Family	Genus	Specie
60023e47f4da8713fba004c4999ab582	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	Psathyrella	Psathyrella fibrillosa
601997d30e638e77884bb4bf331cabda	Fungi	Basidiomycota	Agaricomycetes	Hymenochaetales	unidentified	unidentified	unidentified
6157e6a8b700121d5a0e570f9473379b	Fungi	Basidiomycota	Agaricomycetes	Boletales	Boletaceae	Leccinum	Leccinum scabrum
61a8bebaf93cf600c9372eaf46612044	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	NA	NA	NA
65adbc771bf22edb0516adaf147222b6	Fungi	Ascomycota	Dothideomycetes	Venturiales	Venturiaceae	NA	NA
667f98e856b1c2cc6a00569eca78827d	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	unidentified
67af6fa039feb277b06b7a93852348c3	Fungi	Mucoromycota	Umbelopsidomycetes	Umbelopsidales	Umbelopsidaceae	Umbelopsis	NA
68f6e4ea25c5dd2007280f23064de3e4	Fungi	Basidiomycota	Cystobasidiomycetes	NA	NA	NA	NA
6a6cbefaf884db701720ff655c8578e4	Fungi	Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	Amphinema	Amphinema byssoides
6b0503b52da12a9d31cc03eed1f086e4	Fungi	Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	Piloderma	unidentified
6b42810619842c34febfd5b430d0edb0	Fungi	Basidiomycota	Pucciniomycetes	Pucciniales	unidentified	unidentified	unidentified
72ed3c9da81296b1988029e817d200ee	Fungi	Basidiomycota	Agaricomycetes	Hymenochaetales	Hymenochaetales_fam_Incertae_sedis	Peniophorella	Peniophorella pallida
7373b6c4125c8645a43286e3d76ab791	Fungi	Basidiomycota	Microbotryomycetes	NA	NA	NA	NA
740e4c23881473e6c247bebaf8d2da82	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	NA	NA
750971abb48d0f9fc364b777332a8c62	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Lapidomyces	Lapidomyces hispanicus
7596add17d508705117ac14b220e14f9	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	NA	NA
7714277f7be9ea39854cddc4bcaf6372	Fungi	Ascomycota	Dothideomycetes	Capnodiales	NA	NA	NA
79c9358813d49b653ab62aec07e8a2ea	Fungi	Ascomycota	Dothideomycetes	Botryosphaeriales	unidentified	unidentified	unidentified
7adfa73efe43896669b76559d0bb5d0f	Fungi	Ascomycota	Dothideomycetes	Mytilinidiales	Mytilinidiaceae	NA	NA
7b1582361448c44d87e2605105761230	Fungi	Ascomycota	Archaeorhizomycetes	Archaeorhizomycetales	Archaeorhizomycetaceae	Archaeorhizomyces	unidentified
7c0f694f9a9ccf71636cb22d2a073ddb	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Cladophialophora	unidentified
7dea98743dc176a29e11ba97abc2293c	Fungi	Basidiomycota	Pucciniomycetes	Platygloeales	unidentified	unidentified	unidentified
81a9a5775a77f2f20c1db2996b54da95	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	Mycena	NA
82a5c1fdd4b43928c2d87ce735aa7438	Fungi	Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	Perusta	Perusta inaequalis
855e5cfce1422eecfeb11151890a0929	Fungi	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	NA	NA
866a83706c1a192f2a67e63be525ec5c	Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Trichomonascaceae	Sugiyamaella	Sugiyamaella paludigena

AVS	King-dom	Phylum	Class	Order	Family	Genus	Specie
8690f118e84109e8f4777351cebcf500	Fungi	Ascomycota	Sordariomycetes	unidentified	unidentified	unidentified	unidentified
86f657662422fa5b6f52fd8e9d6f5ab3	Fungi	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	Russula	NA
87d7a5a0ac7d50b1607b013fec393db4	Fungi	Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	Rhodotorula	Rhodotorula toruloides
8875a8e194fede746605ba8bce5a6d14	Fungi	Ascomycota	Leotiomycetes	Helotiales	Myxotrichaceae	Oidiodendron	NA
88f06bc92c8f837c3a71a17d35dec6a6	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleraceae	Fonsecazyma	unidentified
8997d142fbe128fa26a72231b8ddb3c0	Fungi	Basidiomycota	Tremellomycetes	Trichosporonales	Trichosporonaceae	Apiotrichum	Apiotrichum gamsii
8c85cf296559e3be37990eeb6bb1b11b	Fungi	Basidiomycota	Agaricomycetes	Hymenochaetales	Schizoporaceae	Hyphodontia	Hyphodontia pallidula
9067111b973447496c105b63ca3957a5	Fungi	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	Arachnopeziza	unidentified
916b69706f67141efd84dc00a9c66af3	Fungi	Ascomycota	Leotiomycetes	Helotiales	unidentified	unidentified	unidentified
92d050c5c3031a37d3bb4ce75d4b021e	Fungi	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	Otidea	Otidea leporina
946bdc19d81eacf32bae7ba0049cd771	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	unidentified	unidentified
9480f6913092b146c038ad40ace4de10	Fungi	Ascomycota	Leotiomycetes	NA	NA	NA	NA
9487f89375a528f89218cd35e1fff28a	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	Mortierella pseudozygospora
951515f55d8eee55999a2a15a6536c37	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	unidentified	unidentified	unidentified
956cd66f87ea96b953095d7ce932b4ee	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Rhinocladiella	unidentified
96378cf2979b2184dcd78be40b5a753f	Fungi	Basidiomycota	Agaricomycetes	Boletales	Suillaceae	Suillus	NA
979ad5f49457440eeb6f7d349cad460c	Fungi	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	NA	NA
985336e2eb14f5f612530a766a7b0ade	Fungi	Basidiomycota	Agaricomycetes	Trechisporales	unidentified	unidentified	unidentified
98d107aa9b20b37d08ea17e9ed96ee96	Fungi	Ascomycota	Lecanoromycetes	Lecanorales	unidentified	unidentified	unidentified
9a154e5ca9169bbe24c3e917fdd90ea5	Fungi	Basidiomycota	Tritirachiomycetes	Tritirachiales	Tritirachiaceae	Paratritirachium	unidentified
9d1ef68fdeb72d030e1f75f27773fe16	Fungi	Chytridiomycota	unidentified	unidentified	unidentified	unidentified	unidentified
9e30dda8890f5f0a39f70fb162b19cda	Fungi	Ascomycota	Leotiomycetes	Rhytismatales	NA	NA	NA
9ea3265ee0d67745bd8b43a361e7a65a	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiales_fam_Incertae_sedis	Leptodontidium	unidentified
a0290fcbb7e053b9511e69b172bbad3b	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	Vishniacozyma victoriae
a0d3311aed21d443c84bf3e527314257	Fungi	Basidiomycota	GS27	GS27	unidentified	unidentified	unidentified
a1a9c901b719cf44d92f59f5570d1a84	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Exophiala	Exophiala moniliae

AVS	King-dom	Phylum	Class	Order	Family	Genus	Specie
a8dd20f783d32cc0d475cf9f5af7a609	Fungi	Basidiomycota	Tremellomycetes	Tremellales	NA	NA	NA
a9fac85bb0d3ab99e06978e8d11c81ca	Fungi	Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	NA	NA
aa424ee0f0e4bc1e97f4fdc1cceab223	Fungi	Basidiobolomycota	Basidiobolomycetes	Basidiobolales	Basidiobolaceae	Basidiobolus	Basidiobolus magnus
ab2746caf33107ae0ff92157dd76c973	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	Mortierella amoeboidea
ab2c2f150b7cd3458cf49e35000090c7	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Exophiala	Exophiala equina
ae85448de88de10b5b624d90b98cf285	Fungi	Mucoromycota	Umbelopsidomycetes	Umbelopsidales	Umbelopsidaceae	Umbelopsis	unidentified
b0e227129967848a0b008b02ecd97e2e	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	Inocybe	Inocybe soluta
b8a2e551013f54ebbe8935b93ee3ec9e	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	Devriesia	Devriesia pseudoamericana
b8b90aa6d5bde41838828d1aaf4935a2	Fungi	Basidiomycota	Agaricomycetes	Atheliales	NA	NA	NA
b9f80c96f0162aa533b7c31d3a986f8c	Fungi	unidentified	unidentified	unidentified	unidentified	unidentified	unidentified
ba27e66d0e033d1ff13cced82f62ddac	Fungi	Basidiomycota	Microbotryomycetes	Microbotryomycetes_ord_Incertae_sedis	Chrysozymaceae	NA	NA
bb16d33218cfe10a44eeb5f2c1a24809	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	Mortierella humilis
bb9e16e562120e422d693f7b10024094	Fungi	Basidiomycota	Tremellomycetes	unidentified	unidentified	unidentified	unidentified
c0042f430486e79df2db40f9c35abdb1	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Phaeotremellaceae	Phaeotremella	Phaeotremella skinneri
c063ca94266a6d52817c3544c119c651	Fungi	NA	NA	NA	NA	NA	NA
c367454b266bd4f7771283b9701453e6	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	Hypholoma	Hypholoma capnoides
c4422e4745ff812df2150ea8a79097be	Fungi	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	Lactarius	NA
c62072fb6e83ebf44af695cfae69108d	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	Mycena	Mycena flavoalba
c66c9fb5f5beb6bde2af33d4319175d5	Fungi	Basidiomycota	Agaricomycetes	Sebacinales	NA	NA	NA
c951303cae0c0c2ee0aac91ac6f4a690	Fungi	Ascomycota	Sordariomycetes	Microascales	Microascaceae	Cephalotrichum	Cephalotrichum stemonitis
ce1c51864ab4dab7cc12dca00de2d8b9	Fungi	Ascomycota	Dothideomycetes	Tubeufiales	Tubeufiaceae	Helicoma	Helicoma fumosum
d11b2fdc47755a2b7cf0ce6dcfc87290	Fungi	Ascomycota	Geoglossomycetes	Geoglossales	NA	NA	NA
d29372def85184cdea6d2d9955922fa6	Fungi	Basidiomycota	Agaricomycetes	Sebacinales	Serendipitaceae	NA	NA
d456dcaf540fe8df295b3dab1e490037	Fungi	Ascomycota	Dothideomycetes	Dothideales	Aureobasidiaceae	Aureobasidium	Aureobasidium pullulans
d5afe68bd6318650905f04811992346a	Fungi	Basidiomycota	Agaricomycetes	Hymenochaetales	Hymenochaetales_fam_Incertae_sedis	Peniophorella	Peniophorella praetermissa
d8f2d74555d60f6bf0254e00d1c919c5	Fungi	Ascomycota	Pezizomycetes	Pezizales	Discinaceae	Gyromitra	NA

AVS	King-dom	Phylum	Class	Order	Family	Genus	Specie
dc5b0f84a2d87d78b59b57dc6f712154	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unidentified	unidentified
dc8155c38f24e6a1b90707f7a31d842e	Fungi	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	Cheilymenia	NA
e09079c42123656448bc883b5230112d	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	unidentified	unidentified	unidentified
e0fc7cd7636f7c3deb3fa073b19d333e	Fungi	Basidiomycota	Agaricomycetes	Auriculariales	Exidiaceae	Basidiodendron	NA
e1951228440de66963e12696bc7be623	Fungi	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	Russula	Russula densifolia
e2f9068dcb4a192fe2c68db26990c329	Fungi	Basidiomycota	Agaricomycetes	Hymenochaetales	Rickenellaceae	Alloclavaria	Alloclavaria purpurea
e5d52acc034b8a5409b93c3775f9c952	Fungi	Basidiomycota	Agaricomycetes	Boletales	Paxillaceae	Paxillus	Paxillus involutus
e86bf904ec353f95d18c1b9669182a91	Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	unidentified	unidentified	unidentified
e9138b2538a7941393948213598bf541	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	NA	NA
ea377a44a809912b462cd92d2b7899e3	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Cantharellales_fam_Incertae_sedis	Sistotrema	NA
ead7bfd154212d3a7e458b46f40384a9	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Leptosphaeriaceae	NA	NA
edc5fc49cfa32b26f7cdc4a904947a44	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	Solicoccozyma	Solicoccozyma terricola
f0d51996a0fde3b764284c46ec74e564	Fungi	Ascomycota	Archaeorhizomycetes	Archaeorhizomycetales	Archaeorhizomycetaceae	Archaeorhizomyces	Archaeorhizomyces finlayi
f0f1ede86a0f67df9784fd71035edf46	Fungi	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	Tomentellopsis	Tomentellopsis echinospora
f1dcd6f94557dc5133e1a986bcce998a	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	unidentified	unidentified	unidentified
f2272e007992610024bd43be72cdf354	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Lycoperdaceae	Lycoperdon	NA
f28f7ee81f55f25efd9c2729d2825b1b	Fungi	Ascomycota	Xylonomycetes	GS34	unidentified	unidentified	unidentified
f341791c766033632dcb60ea9ea92b7b	Fungi	Basidiomycota	Agaricomycetes	Trechisporales	Hydnodontaceae	Luellia	NA
f3eb22c23b4b0fb6e8e66dcdd1f0c897	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Cantharellales_fam_Incertae_sedis	Sistotrema	Sistotrema sernanderi
f422af147f0b087ad23312ee7ae7c223	Fungi	Basidiomycota	Agaricomycetes	Auriculariales	unidentified	unidentified	unidentified
f5179735b06baa66d7deea7cbe735f73	Fungi	Ascomycota	Dothideomycetes	Pleosporales	NA	NA	NA
f56553fca797ef33fb755657528aa816	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	NA	NA
f5d643b7fc4681748707de2cef58e293	Fungi	Basidiomycota	Tremellomycetes	Cystofilobasidiales	Mrakiaceae	Tausonia	Tausonia pullulans
f604d9754447470d71b2ef50a4910235	Fungi	Mucoromycota	Umbelopsidomycetes	Umbelopsidales	Umbelopsidaceae	Umbelopsis	Umbelopsis gibberispora
f75b065037ce51403f6cdf61caa886e7	Fungi	Ascomycota	Orbiliomycetes	Orbiliales	Orbiliaceae	NA	NA
f7606a7ac44ef74f4a08c0122e8e77b3	Fungi	Basidiomycota	Tremellomycetes	Cystofilobasidiales	Mrakiaceae	Mrakia	Mrakia frigida

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f82df1d546b581e3a8f537cbfed15077	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	NA
fcbe56560a1eda75bd5021d66e939b86	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	unidentified	unidentified

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Plants are sessile organisms that rely on their co-evolved interactions with certain groups of bacteria and fungi that provide nutrients and water in exchange for Carbon. This symbiotic interaction is central to plant establishment and survival, where edaphic properties exert selective pressures. This thesis work investigates the impact of the soil properties on plant establishment and how this affects the ability of the plant to recruit a root-associated microbial community, with a focus on the ectomycorrhizal fungi.

David Castro received his graduate education at the Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Sweden. He completed his Master of Biology with Specialization in Arid Zones at Universidad de La Serena, Chile.

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