Ectomycorrhizal fungal endemism and rainforest nutrition in Pacific Northeast

by

Claire McPolin
BScH, Queen's University, 2017

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Claire McPolin
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Supervisory Committee

Dr. Barbara J. Hawkins (Department of Biology)
Supervisor

Dr. J. Marty Kranabetter (Department of Biology)
Departmental Member

Dr. Greg Owens (Department of Biology)
Departmental Member

Abstract

Considerable research exists on how niche processes and spatial trade-offs structure the species richness observed in ectomycorrhizal fungal (EMF) communities, but little attention has been paid to how this might relate to the high levels of endemism reported in these communities. As endemism is typically associated with habitat specialization, I anticipated that EMF species endemic to the distinct high available nitrogen (N), low available phosphorous (P) soils of the Pacific Northeastern (PNE) temperate rainforests would display greater macronutrient concentration, indicative of superior nutrient exploitation. I measured both the sporocarp nutrition and the root tip abundance of EMF species on a mature forest (CWHVm biogeoclimatic zone) of Sitka spruce and western hemlock and determined fungal endemism using UNITE database. Endemic species, representing close to 50% of species found on root tips, had significantly higher sporocarp N, K and Mg concentrations than cosmopolitan species, but comparable P levels. Sporocarp N and P were strongly correlated, and species with higher N levels showed an increasing N:P ratio, supporting existing evidence for the N cost of organic P-acquiring enzymes. Endemics were more likely to occur on western hemlock (a coastally restricted genus) plots than Sitka spruce (a circumpolar genus) plots and became more frequent on root tips as inorganic P levels in the soil decreased. Endemics represented a diverse group, with moderate but non-random dispersion across the phylogeny; The Inocybaceae family were predominantly endemic, while Cortinariaceae was largely cosmopolitan, highlighting some role of phylogenetic niche conservatism in certain lineages, but not as an overall pattern. I conclude that endemic EMF account for a significant portion of fungi most well adapted to PNE coastal soils, have preference for coastally restricted *Tsuga*, and are expected to provide superior N nutrition to their tree hosts.

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1. Introduction

1.1 The relevance of microbial ecological strategy to ecosystem conservation

Across environmental and successional gradients, it is commonly observed that many species remain constant, persisting across a wide diversity of habitats, while others are replaced along the gradient. Understanding these patterns, visible across a range of scales, is key to the conservation of species and ecosystems (Escoriza 2020; Toledo et al. 2012; Isermann 2011; Szabo et al. 2009). Species distributions are driven in part by (i) differences in niche breadth between species, where distributions are governed deterministically by differences in competitive ability, and (ii) differences in dispersal, also known as neutral or random processes, where distributions are determined by the arrival/ arrival order of propagules (Hubbell 2001; MacArthur and Levins 1967). While the former is commonly associated with evolved traits and the latter with stochastic processes, an evolutionary lens considers how the two can be related. This perspective considers two divergent strategies for a species to maintain fitness in any given region; one of which is to be more competitive for a narrower breadth of resources, while the other is to capitalize on a wider breadth of resources but be less competitive for any given one. The fitness of specialists thus depends on competitive ability, while generalist species rely more on order of arrival (priority effects) or frequent disturbance (Pandit et al. 2009). As this model predicts, a trade-off between competitive ability and colonization investment has been observed empirically in a wide variety of taxa (Zinger et al. 2019; Guejiman et al. 2013; Armsworth et al. 2008; Hadany et al. 2004; Durrett and Levin 1998; Tilman et al. 1994). Examining patterns in species distribution across different scales may be a useful means to elucidate groups of species with distinct evolutionary strategies (Kunin and Gaston 1993), through linking the often-separate fields of community ecology and biogeography (Kissling et al. 2011).

Identifying generalist and specialist species holds tangible applications to ecosystem conservation. There is considerable evidence that these specialist and generalist groupings respond differently to climate change (Silva et al. 2017). Generalists have an advantage under increasing climatic variability and are often better equipped to handle stressors such as drought. Specialist species, on the other hand, depend on a narrower habitat range and are more vulnerable to extinction under habitat loss or alteration (Olden and Rooney 2006). Specialists are reported to represent a dominant portion of microbial communities, which are increasingly gaining attention in their relevance to ecosystem function (Graham et al. 2016; Graham et al. 2014; Griffiths et al. 2001). Analyses of large data sets have revealed that microbial specialists are often locally abundant (Mariadassou et al. 2015) and can represent keystone species in soil ecosystems (Xu et al. 2022). In communities of mutualistic microbes, specialists have been observed to offer hosts the greatest benefit, accredited to greater investment in acquiring local resources (Meeds et al. 2021; Angell and Rudy 2020). As ecosystem conservation requires (i) identifying key species relevant to conservation and (ii) elucidating the processes governing their distributions, it is a worthwhile pursuit to identify microbial ecological strategy types and characterize their relationship to ecosystem function.

1.2 The utility of identifying ecological strategy in EMF

As the forestry sector faces increasing land use intensity and climate threats, interest in the role of species within a mutualist community of ectomycorrhizal fungi (EMF) is increasing. These fungi exist in an obligate symbiosis with most tree species, making up to 99% of the nutrient absorbing area in a tree, and exchanging most of their nutrient uptake for host carbon (Hawkins et al. 2015; Rousseau et al. 1994). Now recognized as critical to the nutrition and health of temperate forest ecosystems, EMF fungal conservation efforts are overwhelmed by the number of species coexisting in a community. For example, Kranabetter et al. (2018) found approx. 360 EMF species likely coexist on mesotrophic sites in a single watershed.

Communities of EMF, despite associating with only 2% of plant species, are over 100 times more speciose than arbuscular mycorrhizal fungi (AMF), which exist in an analogous symbiosis with 80-90 % of terrestrial plant species (Smith and Read 2010). While the latter represent a monophyletic group (Corradi and Bonfante 2012), EMF span over 80 clades, all of which evolved independently from a saprotrophic lifestyle (Tedersoo 2009). A proposed reason for this asymmetry in diversity between the two mycorrhizal types is that EMF fungi obtain nutrients from organic matter, requiring a diverse pallet of enzymes, while AMF provide conduits for nutrients already mobilized by saprotrophs (Phillips et al. 2013). While some functional redundancy is reported to exist in EMF species communities, wide variation in traits related to the breakdown of organic matter is well recognized (Crowther et al. 2014). As forests mature, and competition for nutrients increases, a diverse EMF community is beneficial for tree growth, as many species specialize on different nutrient forms (Kranabetter et al. 2004; Baxter and Dighton 2001).

The knowledge that a diverse EMF community is beneficial to tree health and nutrition has prompted the use of diversity indices as tools to infer community responses to different forestry practices (Dejene et al. 2021; Tomao et al. 2020). While diversity indices are the most common descriptor of biological communities, their use in isolation may not hold sufficient predictive power to inform land management decisions (Bestelmeyer et al. 2003; Hulbert 1971). Identifying EMF species groups with different ecological strategies can bolster this method, as habitat specialists may offer greater benefit for the host tree than generalists (Meeds et al. 2021; Kranabetter 2019a), and the two could respond differently to forestry practises, even if the diversity index remains constant (Matthews et al. 2014; Tjorve 2010). Identifying and assigning “generalist” and “specialist” terms to species is complicated by spatiotemporal scales, particularly in a microbial community where specialization can exist at the level of the microsite, host, successional stage, or wider bioclimatic zone. Nonetheless, scale-invariant processes are reported, demonstrating correlations between local and global species assembly patterns. The relationship between niche breadth and colonization investment visible on a community scale can determine species distributions globally, in so that range restricted species represent specialists, with a narrow niche and low dispersal investment, and globally widespread species represent generalists, investing more highly in dispersal (Hargreaves et al. 2014; Devictor et al. 2010; Gaston 1996). While the determinants of fungal range sizes are under-studied, the role of niche and neutral processes in structuring EMF communities at the scale of the host and community is well documented (Web of Science search “ectomycorrhizal fungi community ecology” (1189 results) versus “ectomycorrhizal fungi biogeography” (245 results), 2022). Illuminating a connection between generalist versus specialist strategies and species ranges could produce a useful deductive tool in EMF community conservation.

1.3 EMF community assembly

Many EMF species are considered host generalists, with the exception of select clades such as *Rhizopogon* and *Suillus*, that exhibit associations with a narrower range of hosts (Kretzer et al. 1996; Molina and Trappe 1994). While host generalism and specialization are typically defined as binary in the literature, EMF species can display distinct asymmetry in fitness on different hosts. For example, Ning et al. (2020) found *Rhizopogon* released substantially more nitrogen (N) and phosphorous (P) acquiring enzymes when paired with a native pine species than an exotic species, highlighting the role of plant-fungal coadaptation in structuring these communities. In contrast, many other EMF groups, such as the Thelephoraceae family, are successful across a wide range of distantly related plant hosts (Bruns et al. 2002; Horton et al. 1999; Taylor and Bruns 1999). The concepts of specialism and generalism are often discussed in context of host specificity for EMF, despite most species being host generalists. Species distribution patterns however, might suggest that these terms are more applicable to differences in abiotic niche space.

The impressive diversity of EMF species observed at community scales is maintained by the abiotic heterogeneity of soil, as well as turnover in root tips creating new habitat (McCormack et al. 2012). This fine scale variation in abiotic soil properties permits different outcomes in competitive interactions (Kennedy 2010), while the turnover of host root tips allows competitively inferior or equivalent species to be maintained through dispersal and priority effects (Jumpponen et al. 2012; Kennedy et al. 2009; Jumpponen 2003). Across wider environmental or temporal gradients however, the role of niche filtering is more pronounced. The roles of N and P availability and source play a critical role in determining local species pools. On Vancouver Island, a study examining the effects of a P deficiency gradient on EMF community structure noted high species turnover linked to exoenzyme functions, with species expressing the most P mobilization enzymes (e.g. *Lactarius subviscidus*) limited to low P sites, only (Meeds et al. 2021).

Asymmetrical niche breadth can also be observed along a chronosequence. In young forests, it is typical to find a homogenous EMF community on root tips, consisting of species such as *Amphinema, Cenococcum,* and *Thelephora.* This group remains constant across environmental gradients, as well as different hosts (Dickie et al. 2013). In contrast, the fungi found only in association with mature forests show high degrees of turnover along environmental gradients as well as greater host preference, indicative of a narrower niche (Walker and Jones 2013; Dickie et al. 2013). While the early seral species do not disappear as forests mature, their abundance on root-tips declines throughout forest succession as they are outcompeted by late-stage fungi (Dickie et al. 2013). The appearance of these late seral fungi in mature forests is thought to be due to changing abiotic conditions throughout succession (Walker and Jones 2013; Ding et al. 2011; Kranabetter et al. 2009), as well as the later arrival of propagules (Peay et al. 2010; Dickie and Reich 2005). Moreover, a late successional forests favours EMF species using vegetative, as opposed to spore-based dispersal, due to higher root density. Root tips of planted seedlings surrounding mature trees have shown distinct EMF composition from distant seedlings (Kranabetter 1999), while seedlings within the forest canopy have shown similar EMF composition to the surrounding forest (Kennedy 2001). This structuring suggests the preference of root-to-root vegetative spread by late-colonizing EMF species, and wider wind-based dispersal by early colonizing species.

1.4 Linking ecological strategy to geographic range in EMF

The transition from wider to narrower species niche breadth throughout EMF succession can also be understood in relation to life history strategy. While microbial dispersal limitation is often considered neutral (Becking 1931), traits influencing colonization investment have shown heritability and affiliation with life history strategy in fungi, where ruderal species invest more in dispersal and long lived, stress-tolerant spores (Halbwachs et al. 2015; Tullos 2005). In EMF, the species investing most in dispersal have demonstrated competitive disadvantages. Tree seedlings in closer proximity to a mature forest edge host an EMF community that more closely resembled the mature forest, while the abundance of early seral species increased with seedling distance from the forest edge. While all EMF species had the highest spore density closest to the mature forest, the high dispersing species were successful in occupying seedling root tips only where the spore density of other species was low. Species with a close range of spore dispersal showed the opposite trend, highlighting a trade-off between colonization investment and competitive ability in this guild (Smith et al. 2018; Kranabetter et al. 1999; Kranabetter and Wylie 1998). Through this lens, changes in EMF fungal community over succession can be understood as generalist species, the first to arrive, being replaced by specialist species, superior competitors. This perspective provides an elegant explanation as to why early seral EMF species tend to persist and colonize a diversity of habitats, while late seral fungi tend to favour spore based dispersal less, and outcompete early seral species (Moeller and Peay 2016; Peay et al. 2007; Dickie et al. 2002; Kranabetter 1998).

As species' geographic ranges are largely influenced by dispersal processes and niche compatibility (Brown et al. 1996), it is logical to consider that the ecological strategies described in EMF on the community scale may be relevant to the distribution of these species across the globe. EMF communities typically have high levels of endemism, as well as species spanning many bioclimatic zones across the world (Tedersoo et al. 2022; Miyamoto et al. 2018; Talbot et al. 2014; Timling et al. 2012; Branco 2011). A 2010 survey conducted on southern tip of Vancouver Island revealed approximately 50% of the EMF species to be endemic to dry, maritime *Pseudotsuga menziesii* stands. It was surmised that these endemic fungi were late-successional, while species with cosmopolitan distributions dominated in younger forests, suggesting that these ranges reflect ecological strategies (Kranabetter et al. 2018; Dickie et al. 2008). An analysis of how the endemic status of species influenced spatial assembly on the community scale demonstrated more clustering in regionally endemic species, supporting the link between localized dispersal and geographic range restriction in this guild (McPolin and Kranabetter 2021). To expand on this finding and characterize ecological strategy in relationship to range, the present study asks if endemic EMF display superior competitive ability than cosmopolitan species.

1.5 Defining competitive ability in EMF

The term "competitive" has a variety of definitions in the mycorrhizae literature. A simple and common definition is the degree of species dominance on host root tips. In the field, this is complicated by priority effects, where it is impossible to disentangle whether a species has colonized more root tips because it is a superior competitor or because it was the first to occupy the site (Kennedy et al. 2009). The inference of competitive ability from number of colonized root tips is further complicated by observed differences in carbon (C) exchange rate between fungal species, when occupying a single host (Bidartondo et al. 2001). While some believe this phenomenon to be host independent (Valtanen et al. 2014), recent research has revealed that C acquisition of the EMF species is directly coupled to nutrient provisions. A 2017 study conducted by Hortal et al. observed that the presence of a competitor EMF species increased the amount of N provided to a host by a *Pisolithus* isolate. In return, the isotopic C acquired from the host increased in the mycelium of the isolate. This finding was corroborated by Bogar et al. (2012 and 2019), who found that EMF N provision predicted C acquisition from the host. Similarly, Arguello et al. (2016) found plants received more P from an AM fungi in the presence of an AMF competitor. Hortal et al. (2017) also found that host plants targeted defense compounds directly to the root tips colonized by EMF species providing less N, both when fungal isolates shared and were separated by compartments.

These results support a model built by Franklin et al. (2014), proposing that fungal C competition is directly linked to their N uptake, promoted indirectly through the host. Interestingly, investment in nutrient acquisition comes at a C cost, as a large-scale meta-analysis on 600 strains of fungi showed resource acquisition had a negative relationship with both rapid growth and stress tolerance (Lovero and Treseder 2021). This implies that EMF can be competitive for root tips by investing more in either nutrient acquisition or biomass accumulation, the latter of which would require early arrival to be effective. Considering that species providing superior nutrition to hosts may be more competitive long term (Bogar et al. 2019; Bogar et al. 2022; Horton et al. 2017), it is important that the measurement of competitive ability in EMF expand beyond root tip colonization to include the acquisition of nutritional resources.

While other studies have not found support for preferential C allocation (Valtalen et al. 2014; Albaracin et al. 2013), the role of EMF nutrient acquisition in predicting root tip colonization is evident in field studies. Zheng and Song (2022) found N and P status of the soil predicted a 90% turnover in the EMF community, likely determined by adaptation to nutrient acquisition in the particular habitat (Meeds et al. 2021; Kranabetter et al. 2015). In the lab, competitive outcomes between species show high sensitivity to conditions in the medium. An eight-degree difference in medium temperature, and a two-unit change in pH has been shown to dramatically change the outcome between EMF species competing for pine roots (Erland and Finlay 1992; Mahmood 2003). Additionally, Erland et al. (1990) found that adding N to a medium changed which EMF species of *Piloderma* colonized more root tips. This observed sensitivity of competitive outcomes to local conditions both in the field and lab underscores how competitive ability must be understood in the context of adaptation to the specific environmental conditions in which it is measured.

1.6 Research objectives

In this thesis, I define competitive ability based on superior nutrient acquisition as well as presence on host root tips. In the first chapter, I explore how endemism to the Pacific Northeast (PNE) coastal temperate rainforest predicts EMF acquisition of major nutrients, hypothesizing that they represent a group of species with greater investment in the nutrient acquiring traits relevant to this particular ecosystem. This definition is useful ecologically, as it implies greater nutrition for host trees and overall ecosystem functioning (Hawkins et al. 2015; Rousseau et al. 1994). In the second chapter, I measure EMF abundance on root tips to observe how PNE endemism relates to community assembly in this region; i.e., if endemic species have distinct assembly patterns from cosmopolitan species in response to variation soil properties and host species. I predict that if endemic species are more competitive, they will have greater nutrient concentrations, and show increasing dominance on root tips where soil profiles are most characteristic of the PNE coast. I will also explore the phylogeny of endemic species, to see if any taxonomic groups are disproportionately represented. This work can inform ecological theory, regarding the relationship between species ranges and competitive ability in EMF. Its implications are relevant to the understanding of the processes governing range restriction in this guild, as well as the utility of endemic/cosmopolitan status in predicting community level-assembly processes and ecological function.

2. Sporocarp nutrition

2.1 Introduction

An important aspect of functional diversity of EMF is differences in nutrient acquisition pathways, reflecting distinct extracellular enzyme activities, and nutrient assimilation pathways between species (Kranabetter et al. 2015; Jones et al. 2012; Jones et al. 2010). These differences reflect the evolutionary history of EMF, with enzyme loss occurring in the transition from a saprotrophic lifestyle (Martin et al. 2016). As EMF occupy two niches, soil and plant host, their evolution reflects the selection pressures associated with both the soils they inhabit, as well as the host plant. Functional turnover in EMF communities in response to soil nutrient forms and quantities emphasizes the role of habitat filtering in determining species success (Tedersoo et al. 2014). Moreover, species able to accrue more nutrients provide host trees with superior nutrition (Kranabetter et al. 2019), which may offer an indirect, host-mediated selection advantage. Recent studies demonstrate the role of the tree host in determining partnership quality through preferential inhibition. Hosts have been observed to select against root tip colonization by nutrient-poor fungi through the upregulation of defense compounds (Hortal et al. 2017) or to preferentially allocate carbon to EMF species providing greater quantities of N (Bogar et al. 2019).

As EMF assembly is largely influenced by adaptation to host and soil profile (Tedersoo et al. 2014), one might expect ecozones with particularly distinct edaphic profiles and plant species to host a uniquely adapted fungal community. A recent review by Tedersoo et al. 2022 also revealed endemicity of EMF to peak in tropical and temperate rainforests, a pattern observed across other plant and animal biota (Brown 2014). The temperate rainforests of the Pacific northeastern coast (PNE), spanning from Alaska to northern California, represent an unparalleled ecosystem. With a cool wet climate and rich soils, these conifer forests are some of the most productive in the world, with entire stands existing for up to 2000 years. The biota of the region is extremely unique, with the predominant tree species, such as Sitka spruce (*Picea sitchensis* [Bong,] Carr.), western hemlock (*Tsuga heterophylla [Raf.] Sarg.)* and western redcedar (*Thuja plicata Donn ex D. Don*), being bound to this zone. It is also home to many endemic and threatened epiphytes as well as animal species (Dunbar et al. 2014; Dellasalla 2011). Though climate and soil vary within this ecozone, two general classifications are delineated based on degree of rainfall and temperature; a drier, seasonal region and a wetter, perpetually humid region (perhumid). The perhumid temperate rainforest describes the most distinct climate and edaphic properties in comparison to surrounding inland forests. It receives 1500-3000 mm of rainfall annually, with relatively uniform temperatures throughout the year (small diurnal fluctuation and rarely below freezing) (Dunbar et al. 2014; Delasalla 2011). While soil available N and acidity are relatively high throughout the PNE rainforest, the perhumid soils undergo substantial podzolization resulting in a distinct edaphic profile, with low available P, Mg^{2+}, K^{+} and Ca^{2+} relative to other forested ecosystems (Carpenter et al. 2014; Mainwaring et al. 2014; Singleton and Lavkulich 1987).

The relatively high inorganic N available in PNE rainforest soils is likely to favour different fungal N-acquisition strategies than in surrounding N-limited forest habitats, where N is mostly bound in organic matter (Reich and Oleksyn 2004; Vitousek et al. 2002; Chadwick et al. 1999). While many EMF species increase in abundance with declining inorganic N (Read et al. 2004), recent work has revealed a distinct group of species that increase in abundance with increasing soil inorganic N (Kranabetter et al. 2015), suggesting divergent selection pressures on traits involved in the uptake of inorganic and organic N sources. Moreover, weathering under the particularly high rainfall in the perhumid region causes P bound to primary minerals to solubilise and become immobilized, increasing the formation of secondary mineral P, and resulting in distinctly low Pi availability in these soils (Carpenter et al. 2014). Previous work has demonstrated EMF species turnover without a change in richness in response to Pi levels in the organic layer, suggesting that some species have traits conferring an advantage when phosphorous is less available (Meeds and Kranabetter 2021; Zavisic et al. 2016). Leaching under high rainfall and low pH also decreases the availability of cations in the soils along the Pacific coast (Carpenter et al. 2014). To mobilize cations in such conditions, EMF are known to weather minerals through chemical force, releasing protons and organic acids to hydrolyze bonds in the crystal lattice (Smits and Wallander 2017). The production levels of these organic acids have been shown to vary significantly between species (Peng et al. 2020), indicating variation in the traits involved in the acquisition of these cations.

Considering the existing variation in nutrient-acquiring traits between EMF species, together with the distinctive Pacific temperate rainforest niche and the role of pedological filtering in EMF community assembly; this study is designed to determine if fungi endemic to this region can accrue and provide host trees with superior nutrition to cosmopolitan species. A plethora of methods are available for testing and comparing the nutrient acquisition and transfer abilities of ectomycorrhizal fungi species. One means is to measure the expression levels and K_m of enzymes involved in nutrient uptake and utilisation (Walker et al. 2016). Another common and useful method is to use microelectrodes, which can be placed at the hyphal-root tip interface, quantifying the rate of nutrient flux to the tree host (Hawkins and Kranabetter 2017). While these methods could directly measure our traits of interest, they limit the scale of the study to a narrow range of enzymes and nutrients, as well as EMF species.

The chemical concentration of fungal tissue is recognized as a meaningful tool with which to characterize nutrient dynamics in forest ecosystems (Meeds et al. 2021; Kranabetter et al. 2019; Zhang and Elser 2017; Cleveland and Liptzin 2007). Widespread analyses of soil microbial nutrition have found stoichiometric averages converging to the Redfield indicator, commonly used as an indicator for marine systems, highlighting conserved stoichiometric ratios for biological functioning. The homeostasis displayed in microbial nutrient ratios with respect to the environment permits the interpretation of microbial nutrition and identification of the most limiting nutrient. Microbial N:P is considered the best indicator of biological nutrient limitation in terrestrial ecosystems. On average, N:P values have demonstrated the most consistency with the Redfield indicator of balanced stoichiometry, where N:P ratios >16 signify P-limitation, and N:P ratios < 14 signify N limitation (Cleveland and Liptzin 2007). In EMF, N:P measurements of host foliage directly predicted phosphorous acquiring enzyme activity (Meeds et al. 2021).

Fungal stoichiometric ratios have shown conservation at the level of the guild, as EMF on average display distinctly lower C:N, and higher N:P ratios than saprotrophic and pathogenic fungi (Zhang and Elser 2017). This has also been noted at the level of the EMF species, and even strain (see Kranabetter et al. 2019; Mouginot et al. 2014). EMF stoichiometry and nutrient concentration levels of EMF tissue (such as sporocarps) are proportional indicators of what the host tree receives (Meeds et al. 2021; Kranabetter et al. 2019; Leberecht 2016; Valtanen et al.

2014). This consistency in relative nutrient content within species and direct prediction of host nutrition together support the use of EMF sporocarps as an indicator of fungal species' nutrient acquisition traits.

This study used sporocarp nutrition as a proxy for the efficacy of EMF species' nutrient acquisition and transfer. Employing this method permitted characterization of the acquisition of all relevant nutrients at a community scale, to effectively compare ecological strategies between endemic and cosmopolitan fungi. The study took place within the CWHvm (Green and Klinka 1994), where unique soil properties (high Ni, low Pi) are pronounced. Sporocarp nutrient data was collected on two host species that are dominant across PNE temperate rainforests, Sitka spruce and western hemlock, in an effort to characterize the wider regional community. This study will be a novel test of whether species endemic to the PNE coast have higher nutritional concentrations (N, P and cations) than the co-existing cosmopolitan species of this community, thus indicating a greater fitness in the local edaphic environment. Results of this study will indicate if geographic range can predict the nutritional quality of EMF symbionts.

2.2 Methods

2.2.1 Site descriptions

The study was conducted on five sites (Table 1) that are part of a long-term silvicultural trial (BC Ministry of Forests E.P. 571) located on the south-west side of Vancouver Island. These sites are in the Coastal Western Hemlock very wet maritime subzone (CWHvm; Green and Klinka 1994) (Table 1), and are approximately 60-year-old secondary forest stands, planted in 1962 following the harvest and slashburn of old growth stands in 1961 (Omule 1988).

On each site, two plots were selected, one of 100% Sitka spruce and the other 100% western hemlock. Spacing is consistent between plots chosen within each site but varies between sites. Plot pairs on three sites have 4.6 x 4.6 m spacing (Klanawa, Br 247, Br 167), while plots on the other two sites have 3.7 x 3.7 m and 2.7 x 2.7 m spacing (WC1000 and Br 136, respectively). All plots consist of 81 trees in 9 rows and columns, and plots vary in size according to tree spacing.

Soil profiles are characteristic of the podzols in the CWHvm biogeoclimatic (BEC) zone, with textures ranging from sandy loam to loam, and clear Humo-Ferric or Ferro-Humic podzol horizons (Soil Classification Working Group 1998). Soils at all sites have intermediate stone content, and the parent material originates from glacial deposition (morainal, fluvial or colluvial).

Previous work demonstrated that these plots have nutrient regimes that are characteristic of this BEC zone; Forest floor and mineral soils are acidic, with high N and C content but low levels of inorganic P (Table 2) (Kranabetter et al. 2020).

Table 1. Mean annual temperature (MAT) and precipitation (MAP) between 1961–1990, with latitude, longitude and elevation of the five study sites from E.P. 571, obtained through ClimateWNA version 4.72 (Wang et al. 2012).

Site	Latitude (N)	Longitude (W)	Elev. (m)	MAT (°C)	MAP (mm)
WC1000	48°33′12″	124°21′02″	250	8.1	3,443
Br136	48°53′52″	124°54′41″	140	8.8	3,189
Br 247	48°51’00″	124°53’02″	265	8.2	3,557
Klanawa	48°49′11″	124°46′29″	95	8.9	3,258
Br 167	48°54′51″	124°49′21″	220	8.4	3,448

1.1 Soil nutrient sampling

The availability of cation and anion nutrients were measured in each plot using Plant Root Simulator (PRS ®) probes (Western Ag Innovations, Saskatoon, Canada). In early May 2020 12 pairs of probes (one anion and one cation) were installed at each plot, with the membrane placed 6-12 cm deep in the ground, placing it in the mineral soil or forest floor, depending on the depth of the organic layer. They were placed at 12 random locations on each plot and retrieved after 12 weeks.

Once scrubbed of all adhering soil debris and rinsed with distilled water, probes were returned to the manufacturer to be analysed. Both cation and anion probes were combined in groups of four for analysis, resulting in three subsamples of probe pairs per plot.

2.2.2 Sporocarp nutrition

EMF sporocarps were collected in the fall of 2020, for the purpose of nutritional and molecular analysis. Each plot was visited three times at three-week intervals between early October to mid-November, and thoroughly searched for intact EMF mushrooms. A minimum of three representative sporocarps per EMF species were collected per plot, with more collected for species with smaller fruiting bodies. Following collection, mushrooms were cleaned of debris and dehydrated in a 60 °C convection oven for 24 h. A small piece of each dried sporocarp was set aside for molecular analysis, and the rest was used for nutritional analysis. All sporocarp tissue was ground to <2 mm for both analyses.

Sporocarp P, Mg, Ca, and K concentrations were analysed using microwave digestion, followed by ICP-mass spectrometry analysis (Agilent Technologies, Santa Clara, CA, USA; Nakagawa et al. 1995). Sporocarp C and N concentrations were determined by elemental combustion, using a Fisons/Carlo-Erba NA-1500 NCS analyzer (Thermo Fisher Scientific, Waltham, MA, USA; Carter and Gregorich 2008). Following field grouping by phenotype, fungal species were determined by molecular analysis, following the method of Kranabetter et al. (2015).

2.2.3 Range designation

Using the ITS region, the sequence from each sporocarp morphotype was matched to a species hypothesis on the UNITE database. To assign endemism status, each species hypothesis (SH) was observed at a 99% species hypothesis threshold (Garnica et al. 2016). If all accessions under this SH had only been found west of the Coastal Mountain range between Northern California and Alaska (within temperate rainforest), it was considered endemic. Accessions that had occurred elsewhere, such as eastern North America, Europe or Asia were designated cosmopolitan.

2.2.4 Statistical analysis of soil properties

Previously collected nutritional data on mineral soil and forest floor (Kranabetter et al. 2020) was analysed along with PRS probe data to test if soil properties varied between spruce and hemlock plots. All statistical analyses were conducted in R (v 4.0.3, R Core Team 2020). A mixed linear model was applied to test all soil properties (function *lmer* in the *lme4* package) (Bates et al. 2015); Tree species was a fixed effect, site a random intercept, and tree species was a random slope varying by sites. Data was log transformed prior to analyses if normality assumptions were not met by the Shapiro-Wilk test (Shapiro and Wilk 1965).

2.2.5 Statistical analysis of sporocarp chemistry across host and range

A linear mixed model was used to test the effect of fungal species range (endemic vs cosmopolitan) and tree host (Sitka spruce or western hemlock) on sporocarp nutrition. Site, the effect of site on host, and effect of host on range were treated as random effects, while holding range and host as fixed effects. Significance and coefficients were determined using type II ANOVA, and the LRT method in the *afex* package in R (Singmann et al. 2016). Nutrient concentrations were log transformed if they did not pass the Shapiro-Wilk test for normality prior to modelling.

As not all EMF species were found on every plot, average nutrient concentration was compared between endemic and cosmopolitan groups using a paired two-way t-test. This was conducted to account for the potential effect of species co-occurrence patterns at different sites. The t-test was run for two separate datasets, one including all recorded species, and another including only species with a minimum of two sporocarp collections.

2.2.6 Statistical analysis of sporocarp nutrient correlations

To observe correlations between nutrient concentrations in the sporocarps, a Pearson product-moment correlation was conducted across all samples. Preliminary tests were conducted to test the reliability of the correlation value after site and host species groupings were included. This was done using separate ANOVA models for each nutrient, with a site*species interaction term, and examining the significance of the correlation of residual models for each nutrient. If the residuals from the two nutrient models were highly correlated and significant, then the significance of the Pearson correlation coefficients across the whole data set were observed, as suggested by Bland and Altman (1994). To control for the effect of species identity, nutrient concentration was also averaged for each species with a minimum of two occurrences. A Pearson product- moment correlation was conducted across the average nutrient concentrations for each EMF species.

2.3 Results

2.3.1 Soil properties

No significant differences between spruce and hemlock plots were found for any of the recorded soil properties sampled in 2020 (Kranabetter et al. 2020) (Table 2). The PRS probe data revealed significantly greater amounts of available NH_4^+ and K^+ in hemlock plots (p= 0.003, 0.008, respectively), while spruce plots demonstrated significantly higher levels of Mg^{2+} (p= 0.005). The difference in NH_4^+ was the most distinct between the two hosts, with an effect size of 0.3, and a marginal R^2 value of 0.27 (Table 3).

Table 2. Mean bulk nutrient concentrations of paired western hemlock and Sitka spruce plots, with standard error in brackets. P values reflect the significance of the comparison of nutrient means between the two host species. Data collected by Kranabetter et al. (2020)

	Mineral soil									**Forest floor**	
	Carbon (%)	Nitrogen (%)	C:N ratio (molar)	Pi (mg kg^{-1})	Po (mg kg^{-1})	Mg (mg kg^{-1})	Ca (mg kg^{-1})	K (mg kg^{-1})	pH	Depth (cm)	C:N ratio (molar)
Western hemlock											
WC1000	8.2 (0.3)	0.36 (0.02)	22.9 (0.63)	121 (14)	613 (14)	0.19 (0.05)	0.23 (0.05)	0.04 (0.005)	4.27 (0.1)	1.0 (0.2)	30.1 (1.4)
Branch 136	10.3 (0.9)	0.39 (0.03)	26.0 (0.5)	133 (16)	419 (15.6)	0.34 (0.09)	0.84 (0.3)	0.097 (0.039)	4.51 (0.16)	4.7 (1.1)	35.3 (3.7)
Branch 247	10.8 (0.4)	0.50 (0.03)	21.9 (0.5)	91 (1.5)	816 (77)	0.22 (0.02)	0.33 (0.05)	0.085 (0.01)	4.25 (0.05)	9.1 (1.3)	24.2 (0.5)
Klanawa	9.6 (1)	0.43 (0.04)	22.6 (0.5)	87 (8)	801 (90)	0.22 (0.05)	0.42 (0.1)	0.079 (0.009)	4.22 (0.07)	6.9 (0.5)	27.1 (3)
Branch 167	11.7 (10.8)	0.44 (0.03)	26.8 (0.3)	126 (17)	368 (22)	0.93 (0.2)	4.87 (1.6)	0.098 (0.01)	4.64 (0.04)	3.5 (0.5)	36.4 (1.1)
Mean	**10.1**	**0.42**	**24**	**112**	**603**	**0.38**	**1.34**	**0.021**	**4.4**	**5.1**	**30.6**
Sitka spruce											
WC1000	7.8 (0.2)	0.33 (0.01)	23.6 (0.1)	77 (5)	274 (35)	0.16 (0.05)	0.54 (0.15)	0.052 (0.007)	4.74 (0.03)	1.6 (0.4)	25.8 (0.6)
Branch 136	10.8 (0.4)	0.50 (0.02)	21.6 (0.4)	134 (21)	611 (44)	0.28 (0.04)	0.95 (0.1)	0.099 (0.01)	4.54 (0.05)	1.5 (0.4)	31.7 (3)
Branch 247	8.2 (0.5)	0.33 (0.02)	24.5 (0.2)	74 (7)	565 (28)	0.24 (0.03)	0.48 (0.06)	0.081 (0.004)	4.54 (0.03)	7.5 (0.4)	27.1 (0.5)
Klanawa	10.2 (0.1)	0.49 (0.006)	20.8 (0.1)	105 (7)	712 (25)	0.19 (0.02)	0.47 (0.06)	0.08 (0.009)	4.42 (0.03)	5.3 (0.7)	24.2 (0.06)
Branch 167	8.2 (0.3)	0.31 (0.006)	26.2 (0.9)	228 (30)	376 (9)	0.36 (0.08)	1.31 (0.4)	0.074 (0.01)	4.56 (0.02)	2.8 (0.3)	39.5 (2)
Mean	**9**	**0.39**	**23.3**	**124**	**508**	**0.27**	**0.75**	**0.015**	**4.56**	**3.7**	**29.6**
p value	0.23	0.55	0.63	0.71	0.46	0.27	0.46	0.74	0.07	0.49	0.79

Table 3. Mean soil anion and cation levels measured by PRS probes (µg nutrient/10 cm²/12 weeks) averaged over all plots of each tree species, with standard error in brackets (n=15). P values for species comparisons were calculated using a mixed linear model.

	NH_4^+	**NO_3^-**	**PO_4^-**	**Ca^{2+}**	**K^+**	**Mg^{2+}**
Spruce	2.4 (0.3)	3.1 (0.8)	0.7 (0.02)	606 (16)	252 (7.3)	102 (2.6)
Hemlock	4.8 (0.3)	2.1 (0.4)	0.9 (0.02)	527 (18)	310 (4.1)	71 (3.1)
***p* value**	*0.003*	*0.47*	*0.12*	*0.14*	0.008	*0.005*

2.3.2 Sporocarp nutrition across host and range

A total of 39 EMF species were sampled (Table S1, Appendix). Sporocarp nutrient concentrations and ratios did not differ significantly between fungal communities of the two host species (Table 4). On average, endemic EMF sporocarps displayed higher N, K and Mg concentrations than cosmopolitan species' sporocarps, as well as a lower C:N ratio (Table 4, Figure 1). The top 6 species with the highest sporocarp N were all endemic (Figure 2). A t-test of average nutrient concentrations per fungal species (Table S2), across treatments, supported the significantly higher N (28%), C:N (20%) and Mg (17%) concentrations in the endemic group (p= 0.003, p= 0.004, p=0.03, respectively, n=39). These results were replicated when species with fewer than two samples were removed from the data set (p= 0.02, p= 0.02, p=0.01, respectively, n=20; % difference in N= 32%, % difference in C:N= 23%, % difference in Mg=20). Sporocarp K concentration differed significantly between endemic and cosmopolitan fungi in the mixed model but not between these groupings for species averages (see Table S2).

Table 4. Mean (standard error) sporocarp N, P, Ca, K and Mg concentrations and C:N and N:P ratios, averaged by tree host species and fungal community ranges. P values are associated with the Chi^2 value of regressions, and the percent difference between endemic and cosmopolitan range means. Statistical significance of p values (a= 0.05) is indicated in bold.

	N(%)	P(%)	C:N (molar)	N:P (molar)	Ca (mg kg^{-1})	K (mg kg^{-1})	Mg (mg kg^{-1})
Host species							
W. hemlock (49)	4.4 (0.2)	0.46 (0.07)	12.4 (0.5)	21.5 (0.6)	177 (10)	3.7 (1.5)	962 (1.7)
Sitka spruce (51)	4.6 (0.1)	0.49 (0.01)	12.0 (0.5)	21.6 (0.8)	224 (8.7)	4.1 (1.4)	934 (1.4)
p value	0.35	0.38	0.30	0.31	0.06	0.1	0.86
Fungal range							
Cosmopolitan (36)	3.9 (0.1)	0.45 (0.02)	13.9 (0.1)	19.9 (0.6)	182 (18)	3.5 (0.1)	855 (24)
Endemic (64)	4.8 (0.1)	0.49 (0.01)	11.3 (0.3)	22.3 (0.6)	210 (13)	4.1 (0.1)	998 (24)
% difference in endemic	+24%	+8%	-19%	+10%	+15%	+16%	+17%
p value	**$p < 0.001$**	0.13	**$p < 0.001$**	0.07	0.20	**0.005**	**$p < 0.001$**
Host*range *p value*	0.38	0.31	0.33	.06	0.5	0.9	0.5

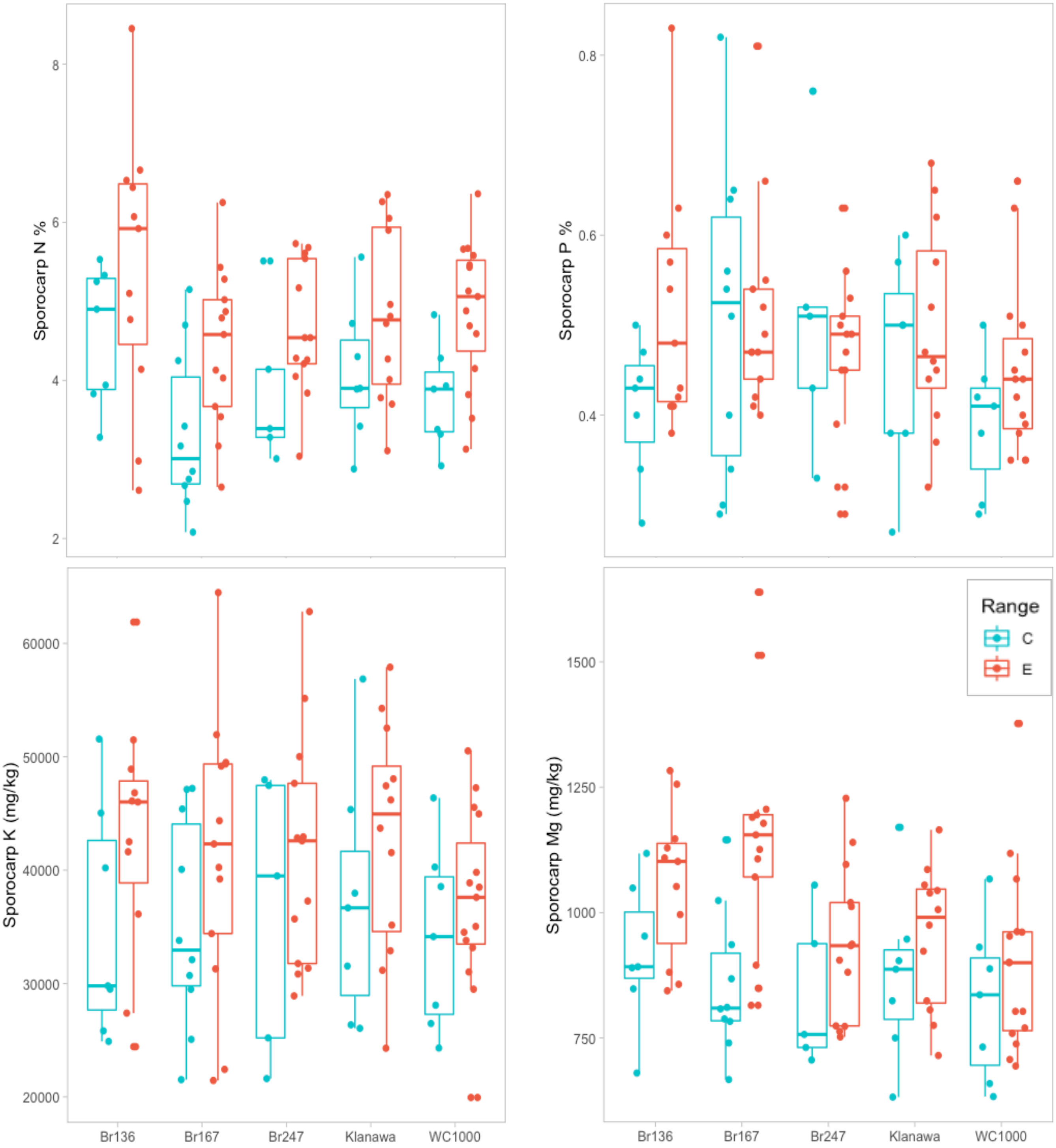

Figure 1. Average N, K, Mg and P concentrations of endemic (rust) and cosmopolitan (cyan) EMF sporocarps on five sampling sites (n= 100). Whiskers represent the 95% confidence intervals.

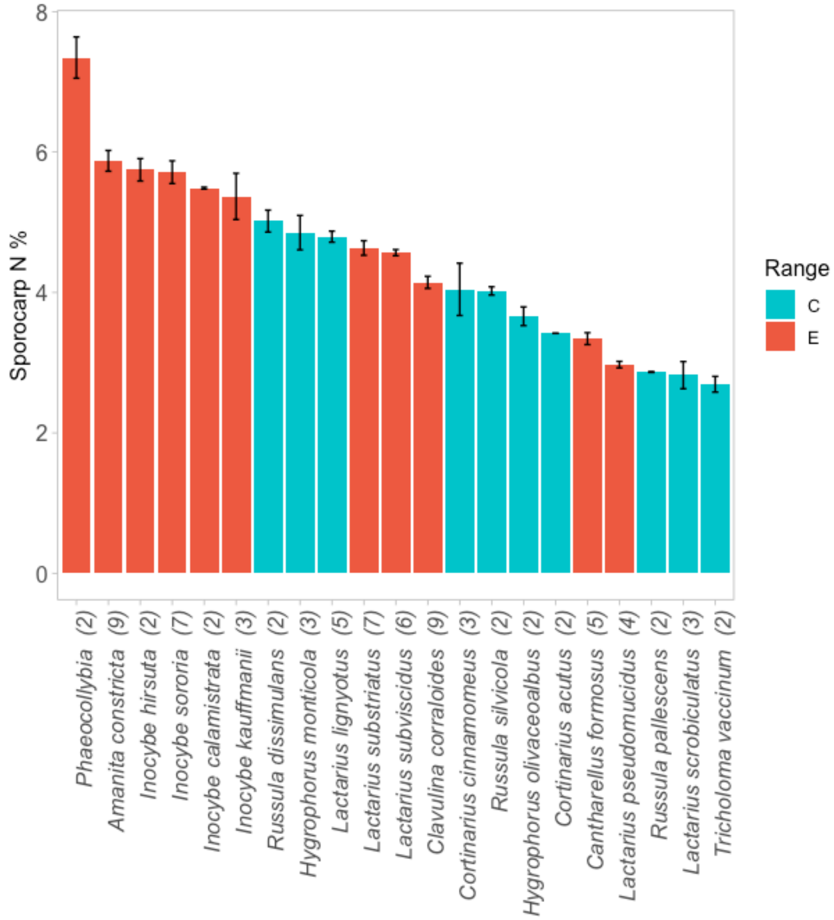

Figure 2. Mean and standard error of sporocarp N% for EMF species with two or more collections (number of observations per species in brackets).

2.3.3 Sporocarp nutrient correlations

A Pearson correlation analysis between pairs of nutrient concentrations for all sporocarp samples found significant correlations between most pairs of nutrients but the strongest and most significant coefficient was between N and P (cor= 0.52, p<0.001) (Table 5, Figure 3). For all significant pairs of nutrients, residual correlation was significant and high (cor=0.63, p <0.001) for models including site and species effect, indicating results are reliable for interpretation. A second Pearson correlation analysis, using species average sporocarp nutrient concentrations (for species with a minimum of two observations; n ≥2) found a significant correlation between N and P (p=0.32, p=0.04), but not between any other pair of nutrients. This relationship was plotted using a linear model, where sporocarp P% increased at approximately half the rate of N%, diverging from a constant stoichiometric ratio (Figure 4).

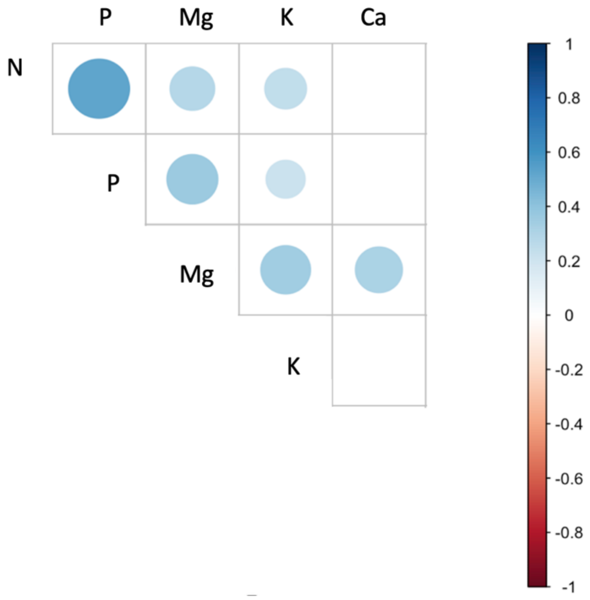

Figure 3. Significant Pearson correlations of sporocarp sample nutrient concentrations, including single sporocarp collections. Color represents the magnitude of correlation coefficients (r), and circle size is proportional to significance (a=0.05), with a larger circle signifying greater significance, and no circle when p> 0.05 (see Table 5).

Table 5. Pearson correlation coefficients (r) and significance for sporocarp nutrient concentrations across all samples (n=100, a=0.05). Significant correlations are bolded.

Nutrient pair		Correlation	p
N	P	0.52	**<0.001**
N	Ca	-0.17	0.09
P	Ca	-0.04	0.71
N	Mg	0.28	**0.004**
P	Mg	0.37	**<0.001**
Ca	Mg	0.31	**0.001**
N	K	0.25	0.01
P	K	0.21	0.03
Ca	K	0.04	0.71
Mg	K	0.35	**<0.001**

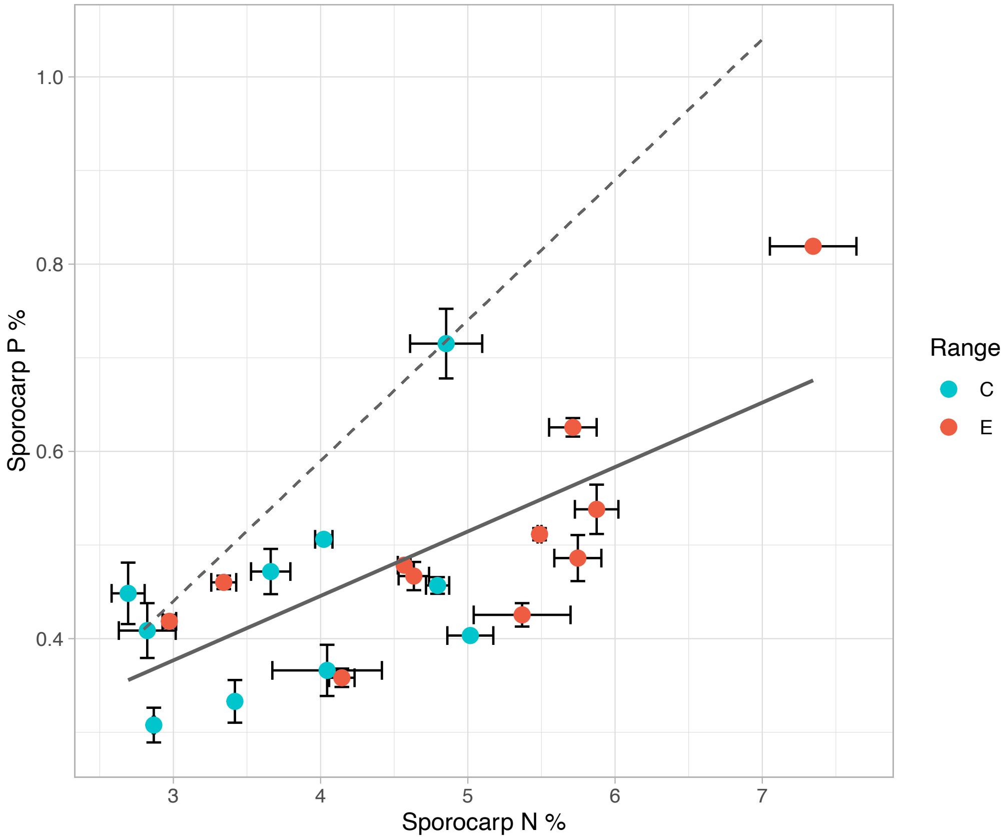

Figure 4. Relationship between the average sporocarp N and P concentrations for endemic (rust) and cosmopolitan (cyan) EMF species (n=21). The grey dotted line represents the theoretically balanced stoichiometry of 15 N:P molar ratio (Reich and Oleksyn 2004), while the black line represents the linear regression line [P%] = 0.17+ 0.07[N%]).

2.4 Discussion

This study examines the variation in sporocarp nutrition among 39 EMF species in a coastal temperate rainforest ecosystem. It was conducted to investigate how species range theory might apply to these symbiont communities; if species only observed in this ecosystem (endemics) might provide superior nutrition to host trees than those with ranges spanning across the globe (cosmopolitan). It was hypothesized that these cosmopolitan species, as generalists, would be less adapted to the specific soil niche of the PNE coast, and display lower sporocarp nutrient concentrations than the endemic group.

2.4.1 Sporocarp N

Many of the species collected had higher sporocarp N content than has been previously observed in EMF species, with values ranging between 3-7% N. Average values across all collections, however, were comparable to previous work in this region (Kranabetter and Hawkins 2019; Zhang and Elser 2017). Kranabetter and Hawkins (2019) observed EMF sporocarp N concentrations of seasonal and perhumid rainforest stands averaged 3.9% ± 1.0, with the highest N concentration in *Phaeocollybia* (6.9% ± 0.06 SD), a species found only in western BC and Oregon (Norvell 2002). Similarly, my study, conducted with twice the number of species, found an average of 4.5% N in sporocarp samples, with *Phaeocollybia* as the upper outlier (7.3% N ± 0.3 SD). The consistency of these results with previous work further underscores the reliability of EMF sporocarp nutrition, and its applicability as a species-specific indicator.

Of the species with the highest average N concentration, the top 9 of 12 were all endemic to the PNE coast. As EMF nutrition can be affected by site locations (Kranabetter 2019a; Zhang and Elser 2017), how endemism predicts sporocarp N% was examined across both sites and hosts.

EMF fungi endemic to the PNE coast had a significantly greater average N concentration than species with a cosmopolitan distribution, and the increase of 24%, on average, was more than for any of the other tested nutrients. This finding suggests that endemism was particularly successful in identifying a species group with superior capacity to exploit the local N sources on these sites.

As the selected study plots with low C:N ratios, would have high inorganic N availability (Kranabetter et al. 2021), it is logical that this difference in N uptake be attributed to an adaptation to exploiting this resource (Baar et al. 1997). It is known that the capacity for NH_4^+ use varies widely among EMF species. For example, for roots collected on the same sites, those colonized by *Cortinarius* species, typically found in higher abundance on dry, low inorganic N soils, took up three times less NH_4^+ than roots colonized by *Tomentella* species, which are characteristic of wetter, higher inorganic N soils (Kranabetter et al. 2015). While nitrification rates and concentrations of NO_3^- increase with declining C:N ratios in these rainforest ecosystems (Kranabetter et al. 2021), previous work has indicated the absence of an effect of NO_3^- in structuring EMF communities (Grenon et al. 2005; Kranabetter 2015). Soils with high NO_3^- : NH_4^+ demonstrate a microbial preference for NH_4^+, presumably because NH_4^+ assimilation is less costly (von Wirén et al. 2000; Chalot et al. 2011). Considering the comparable levels of NO_3^- and NH_4^+ in our plots, and the lack of consistent relationship of NO_3^- with community composition and range, it is likely that differences in N concentrations between EMF species range groups are driven primarily by NH_4^+ uptake capacity.

The physiological mechanism behind NH_4^+ exploitation is likely related to differential expression and regulation of NH_4^+ uptake and transport proteins. The kinetics of the NH_4^+

transport system have been shown to very significantly among EMF species, with K_m values ranging between 6 and 250 µM (Javelle et al. 1999). This transport system in fungi is composed of three known types of membrane proteins in the AMT family: two high affinity sensors and transporters (AMT1 and AMT2,) and a low affinity transporter (AMT3). Studies of these cloned genes in *Hebeloma cylindrosporum* showed the latter to be constitutively expressed, while AMT1 and AMT2 have demonstrated N level- dependent regulation (Javelle et al. 2003). However, AMT1 and orthologs in *Laccaria bicolor* demonstrated constitutive expression, independent of environmental NH_4^+ levels, suggesting that the regulation processes governing expression levels can vary with phylogeny (Lucic et al. 2008). Furthermore, excess NH_4^+ intake in some mycorrhizae species can be coupled with unique storage and transfer mechanisms, such as intracellular vesicle packaging and diffusion, permitting the achievement of high N status without toxicity (Chalot et al. 2006).

While further research is needed regarding the mechanism of superior inorganic N uptake, it is likely that the endemic species achieve higher N acquisition through adaptation to soils with high levels of Ni. They may have evolved to acquire NH_4^+ more readily through higher expression levels and less downregulation of AMT genes, and storage mechanisms to avoid toxicity. Together, this transport system would permit more rapid uptake of available NH_4^+ than in most cosmopolitan species. On upland, mesic sites, where inorganic N is less available and most N is bound up in organic matter (high C:N ratios), species specialized to inorganic N may have lower fitness and be less competitive. Lower investment or loss of organic N acquiring enzymes (Taylor et al. 2000) may cause inorganic N specialists to be outcompeted by species able to survive solely on organic N (Guidot et al. 2005; Plassard et al. 2000; Finlay et al. 1992), contributing to their geographic restriction (Pelletier and Zac 2021). Moreover, certain cosmopolitan species, such as *Hygrophorous monticola* and *Russula dissimulans*, demonstrated an acquisition of reasonably high N, highlighting how some species with widespread distributions can still be competitive in niche local conditions.

2.4.2 Sporocarp P

The sporocarp P concentrations reflected the limited P in these soils, with an average of 0.47% P ± 0.1 SD, the exact value reported in sporocarps under Douglas-fir on similar podzols in Kranabetter and Hawkins (2019). As found with sporocarp N, *Phaeocollybia* was a significant upper outlier, with 0.82% P ± 0.03 SD, comparable to the Kranabetter and Hawkins (2019) measurement of 0.75% P ± 0.01 SD, from different sites, with varying Pi and Po levels.

Since these heavily podzolized soils have little available Pi for direct absorption by organisms, and a proportionally higher Po: Pi than surrounding ecozones (Meeds et al. 2021; Lundstrom et al. 2000), I predicted that EMF endemic to the PNE coast would be better adapted to exploiting Po sources. Exoenzymes are a primary means by which EMF fungi acquire P directly from organic matter, the most common being acid phosphomonoesterases (APM) and phosphodiesterases (APD), which liberate phosphate from nucleic acids (Nannipieri et al. 2011). APDs have been shown to be a rate limiting step in soil P mobilization (Turner et al. 2005; Jarosch et al. 2019). They have been demonstrated to function optimally under the same pH range as the PNE temperate rainforest soils (4-5.5), highlighting their potential relevance to adaptation to this region (Leake and Miles 1996). While APM is considered widespread in EMF species and its activity does not vary dramatically in response to organic P (Nygren and Rosling

2009), the activity of APD enzymes has been shown to increase significantly with decreasing inorganic P and increasing phosphodiesters: orthophosphate ratios in the forest floor. APD activity levels varied 4-fold between species and APDs are more highly expressed in species exclusive to podzolized soils (Meeds et al. 2021). Given the evolutionary pressure to invest in these enzymes, in addition to the observation of their activity in this region, P levels were predicted to be significantly higher in species endemic to the PNE coast.

When sporocarp P was observed across sites and hosts, no difference between endemic and cosmopolitan EMF species was found, indicating that there was no difference in P acquisition. While this may suggest that the range grouping did not capture a difference in phosphatase expression, or the relative selection pressure on phosphatase enzymes may not be distinct enough in this region to demonstrate any difference. One reason for this could be related to the greater selection pressure on fungal APD enzyme activity in forests with high levels of organic matter, where phosphodiesters are significantly higher (Leake and Miles 1996; Condron et al. 1990). The presence of unmanaged forests, or varying forest management practices outside of the PNE coast may select for APD enzymes as well, dampening the effect of range designation. Alternatively, the lack of significant difference in P acquisition may be due to the dominance of phytate, or phosphonate in the organic P pool, both of which are abundant in humic podzols (Turner 2007). These forms have shown resistance to breakdown by EMF fungi, and the effect of enzyme activity on sporocarp P may be dampened by substrate limitation (Menezes-Blackburn et al. 2013; Louche et al. 2010; Jakobsen et al. 2005). In this case, sporocarp P may not capture a difference in phosphatase expression levels, even with greater investment in the endemics. The likelihood of recalcitrant P dominating the organic soil is supported by Meeds et al. (2021), who found phosphatase expression levels and EMF community turnover to be predicted by soil Pi, but not Po on the same sites as this study. The correspondence of sporocarp P measurements between this study and Kranabetter et al. (2015) further support that P acquisition is highly constrained in this ecosystem.

The N:P ratio of species can be a useful indicator of both P limitation as well as P acquisition investment by EMF species (Meeds et al. 2021; Zhang and Elser 2017). These values varied for the fungi found in this study, consistent with what has been reported in other studies (Meeds et al. 2021; Zhang and Elser 2017). Most values fell above the optimal N:P ratio of 15 (Reich and Oleksyn 2004), highlighting the P limitation of the community. As sporocarp N concentration increased, the deviation from stoichiometric balance followed, leading to a greater N:P ratio since P uptake was only half that of N. EMF have been known to fill requirements for the most limiting nutrient to achieve stoichiometric balance (Meeds et al. 2021; Johnson et al. 2010). However, coupling between nutrients has been shown to further increase the fungal N:P ratio when P must be acquired from Po. In soils where most of the phosphorous is bound in organic material, it is predominantly in the form of phosphoesters. Phosphatase enzymes (APD, AMP), required to mobilize it into Pi are rich in amino acids (Duff et al.1994), implying that N must be utilized to acquire limiting P. Fertilization trials have consistently supported this, demonstrating a linear increase in phosphatase activity in response to inorganic N fertilization (Marklein and Houlton 2012; Gress 2007). Similarly, Meeds et al. (2021) found that foliar N:P ratio was a strong positive predictor of the activity levels of all enzymes involved in P acquisition (APM, APD, LAC, NAG, GU), suggesting that species with high capacity for N uptake were better able to allocate resources towards P acquisition. While the greater N:P in the endemic EMF was only marginally significant (p= 0.07), a greater ability to exploit the inorganic N pool may allow endemic fungi to allocate more energy and a higher proportion of N towards more costly phosphatase enzymes.

2.4.3 Sporocarp cations

The increased concentration of Mg and K observed in endemic EMF in my study suggests an increased adaptation to regional soils, as effective cation exchange and base saturation of cations is comparatively low throughout the PNE coastal rainforest. To mobilize cations in such conditions, EMF weather minerals through both mechanical and chemical force, releasing protons and organic acids (Smits and Wallander 2017). They have been shown to target different minerals in response to varying nutrient stresses (Smits et al. 2012; van Scholl et al. 2006). This has also been shown to differ between species, suggesting specialization to different substrates. Scholl et al. (2012) found that muscovite (a K-rich phyllosilicate) was mined significantly more under K deficiency, but only by *Paxillus involutus*. Similarly, *Suillus* species demonstrated distinct differences in K acquisition under the same nutrient supply, mirrored by production of oxalate (Peng et al. 2020). This organic acid plays a dominant role in fungal mineral weathering, and the release of Mg, K and P (Robert et al. 1986). Furthermore, EMF production of oxalate can be influenced by N source. Paris et al. (1996) showed that the co-limitation of K and Mg did not increase oxalate production under NH_4^+ growth medium for *P. involutus*, but did for *P. tinctorus*. These species-specific differences in oxalate under changing NH_4^+ levels are thought to be connected to interspecific differences in the oxalate production pathway (Plassard and Franson 2009; Lapeyrie et al. 1987). The relevance of organic acid production to fungal nutrient acquisition, and its interspecific variation across nutrient regimes suggests the higher Mg and K concentrations in the endemic group indicate superior production of these acids, possibly aided by adaptation to soils with high NH_4^+.

The correlations observed between concentrations of P, N, Mg and K in sporocarps supports the co-transportation of these nutrients by EMF under P limitation, as observed by Jentschke et al. (2008) who demonstrated the translocation of P in EMF was coupled with significant increases in N, Mg^{2+} and K^+ fluxes. While it is not entirely clear why these are coupled, EMF are known to store phosphate as negatively charged polyphosphate in fungal vacuoles, and Mg^{2+} and K^+ ions found in association may serve a neutralizing function (Bücking and Heyser 1999; Orlovich and Ashford 1993). It should be noted that the Mg^{2+} and K^+ found in association with phosphate were proportionally transferred to tree hosts as well, suggesting that the uptake of these cations is relevant to tree nutrition (Jenschke et al. 2000). However, unlike that study, in which P was the sole limiting nutrient, cations were likely also limiting in our study due to the combination of acidity and leaching under high rainfall (Carpenter et al. 2014).

It is important to note that while the endemic and cosmopolitan EMF groupings captured differences in nutrient acquisition, they are likely subject to a degree of sampling bias. Some cosmopolitan species may have been identified in other countries in exotic tree plantations, not representing their natural distribution (Salomón 2018). Similarly, some endemic species may represent unsampled cosmopolitans; rare species in particular pose a higher risk of avoiding detection (Roth et al. 2018; Yang et al. 2013). Only two generic groups, endemic and cosmopolitan, were chosen to increase this certainty under the presently imperfect range resolution for fungi (Mueller et al. 2022). As the accuracy of the UNITE database grows, this study could be improved by using more specific geographic distributions. While the cosmopolitan group can be assured to capture a group of species with a wider niche breadth, the effect of the endemic group representing rainforest specialist is likely dampened by species that are less exclusive to the PNE rainforest. While the west of Vancouver Island is rainforest, the eastern side is a dry ecosystem, where selection pressures may be more comparable to forests in other parts of the world (Green and Klinka 1994). Some of our endemic species, such as *Lactarius pseudomucidus*, can be commonly observed in both these ecosystems, while others, like *Inocybe sororia*, are only reported on the west site. This nesting of niche space, not captured by the range designations used in this thesis, may thus lead to an underestimation of the nutritional quality of EMF fungi exclusive to the PNE rainforest (Lee-Yaw 2016)

Despite the described limitations, this study revealed that on average, EMF species endemic to the PNE coast display superior ability to acquire N, Mg and K than those with a cosmopolitan distribution. This demonstrates how species ranges can predict niche specialization and competitive ability, as endemic species appear better adapted to these soils. The results of this study are of interest to EMF conservation efforts, both by identifying a group of EMF that can provide trees with superior nutrition, as well as specific endemic species. In particular, a rare *Phaeocollybia sp*. had a higher sporocarp N and P concentration than any other species by a wide margin. While the species' name is not yet resolved, this genus has been reported to inhabit areas of high N availability in old growth forests on the west coast of North America (Trudell and Edmonds 2004). In British Columbia, 8/11 *Phaeocollybia* species are red listed (BC Conservation data centre, Nov 10th 2022). Based on the results of this study, conservation practices can expand beyond diversity indices of EMF by using the endemic status of fungi as a proxy for probable partnership quality for host trees.

3. Endemism and community assembly

3.1 Introduction

The wide variation among taxa observed in species' range sizes has long been of interest in better understanding the current ecology and evolutionary history of species (Brown et al. 1996). As conservation concerns increase with expanding land use and ongoing climate change, the determinants of species' ranges have received more attention, especially as small-range species become at greater risk of extinction (Kiessling and Aberhaun 2007). The range sizes of species are commonly understood through a filter model, which considers the non-exclusive roles of the evolutionary history of a lineage, present day ecological filtering (biotic and abiotic) and dispersal (Brown et al. 1996). While these processes have been well examined for many plants and animals, understanding the distribution of microorganisms is a comparatively new endeavour (Martiny et al. 2006). The range sizes of fungi and other microbes were originally thought to be determined solely by ecological constraints (niche incompatibility and biotic competition), as popularized by Baas-Becking (1934) who claimed "everything was everywhere" and the environment selects. It is now recognized that the distributions of some fungal species are limited more by dispersal effectiveness (via the abundance of spores and distance of effective spread), while others are primarily limited by habitat filtering. The differing roles of these respective constraints in different species can highlight different evolutionary strategies, from generalism, where species ranges are most limited by dispersal, to specialism, where species ranges are limited most by niche (DeVictor et al. 2010).

Regional endemism in fungal communities across the globe is well recognized in the literature (Kranabetter et al. 2018; Talbot et al. 2014; Meiser et al. 2013; Taylor et al. 2006). Studies seeking to explain the origins of endemism have explored the relative roles of habitat filtering and dispersal limitation by examining distance decay. For example, Talbot et al. (2014) tested the relative response of fungal communities to variation in soil properties and physical distance to determine the processes driving endemism. They compared the relative community response to environmental versus spatial gradients, concluding that low relative response to soil chemistry indicated that habitat filtering had a minor role in predicting fungal ranges. In contrast, Frier et al. (2009) found a strong deterministic role of habitat filtering predicting fungal ranges, suggesting endemism is driven primarily by niche. The difficulty with these approaches is that the possibility of distinct ecological strategies among species within the fungal community is not accounted for. By seeking to determine the effect size of various trends in the community, species groups with distinct assembly patterns can be masked. Discrepancies in reported trends could thus be biased by sampling effects, for example, an overrepresentation of species with generalist strategies, where habitat filtering plays a weaker role (Jiao and Lu 2020). Analogously, conflicting results are found regarding the role of host tree specificity in predicting EMF species distributions (Pickles et al. 2005; Bruns et al. 2002). It would seem critical to separate subcommunities of aligned species (e.g. specialists vs generalists) to better elucidate the processes giving rise to fungal endemism. For example, Jiao and Lu (2020) revealed distinct assembly processes between rare and abundant fungi within a region; the assembly of the former was constrained by habitat filtering, and the latter primarily by dispersal. It is not yet known if EMF range groupings capture such distinct assembly processes on a community scale.

Previous work across nutrient gradients have demonstrated that the availability of inorganic phosphorus (Pi) and nitrogen (N) has a distinct effect on EMF species community composition. Some species are reported to respond positively to available Pi and ammonium (NH_4^+), observed through greater abundance and incidence on root tips, while others respond negatively (Meeds et al. 2021; Zavisic et al. 2016; Kranabetter et al. 2015; Read et al. 2004;). If endemism in the Pacific northeast (PNE) is driven by habitat filtering, we might expect endemic species to hold a competitive advantage under the characteristic soils of this region where inorganic N is highly available, and intense podzolization has diminished the supply of cations and P (Kranabetter et al. 2020). The enhanced sporocarp N, Mg and K concentration found in endemic fungi (Chapter 2) supported this concept, demonstrating that on average, the range limitation captured species with traits better suited to obtain nutrients in these local conditions. However, nutrition alone may represent an incomplete definition of competitive advantage (see for example Smith et al. 2018). If endemic EMF represent a group that are limited to these unique soils by their niche, then their success on root tips should demonstrate this trend. If endemic species have a competitive advantage at soils with low P_i, high NH_4^+ and low cations, then we might expect them to become more dominant on root tips as these soil properties are more pronounced (DeVictor et al. 2010).

While the role of habitat filtering is often observed across distinct BEC zones, a significant degree of nutritional variation can occur within these zones. Such variation in N and P within BEC zones has been shown to relate to functional differences and phylogenetic turnover in EMF communities. In the boreal forests of BC, the abundance and occurrence of many EMF species have shown strong alignment with N availability gradients (Kranabetter et al. 2009a; Kranabetter et al. 2009b). While no work has yet been done observing the effect of N availability on EMF community composition within the CWHvm zone, EMF alignment with N availability has been observed within the C:N range seen in this region (Pelletier and Zac 2021). Analogously, a range of soil P content comparable to what can be observed in our study plots has been shown to predict changes in EMF community composition. He and Shin et al. (2017) observed that in tropical forest plots, EMF community composition responded significantly to the variation in total P within these plots, which ranged only between 150-250 mg/kg. Previous work on Vancouver Island found that the range of Pi within the podzolized soils of the PNE coast (30-240 mg/kg) had a strong effect on Po mobilizing enzyme activity when compared to the range of Pi within Brunisols (555-960 mg/kg) (Meeds et al. 2021). Although a negative relationship between soil Pi and Po mobilizing enzymes was observed, higher resolution data is needed to confirm if the range in P availability within the CWHvm zone is ecologically relevant to EMF community assembly. My objective is to examine the role of this abiotic filtering on the EMF community, and then examine if species range plays a role in predicting this response and see if cosmopolitan and endemic fungi respond differently to host and soil properties.

Analogously, host tree species is understood to have a strong role in filtering EMF species assemblages (Ishida et al. 2007). While both host species observed in this study Sitka spruce and western hemlock) are endemic to the PNE, the genus' have distinct geographic distributions: *Picea* being circumpolar and *Tsuga*, coastally restricted (Lockwood et al. 2013). I will examine how EMF species endemism to the PNE coast is affected by host tree species, predicting endemic fungi to have an advantage on Western hemlock plots, due to host phylogeographic fidelity to this coastline (Ishida et al. 2007). Results will elucidate if the geographic range of EMF species can predict community assembly at a local scale, and offer implications in regard to the role of niche governing range restriction (Brown et al. 1996).

Biogeographical studies of plants and animal ranges have found that while species ranges vary by orders of magnitude at higher levels of classification (e.g. family), phylogenetic signals for range size are reported at lower classification levels in taxa (Machac et al. 2011; Hunt et al. 2005; Blackburn et al. 2004; Freckleton et al. 2002; Jablonski 1987). This similarity in ranges for closely related species infers that range sizes are determined by specific biological traits reflecting fitness for the organism under contrasting habitats. Other studies have found no phylogenetic signal for species ranges (Carotenuto et al. 2010; Freckleton et al. 2002; Gaston and Blackburn 1997), concluding that biology was dominated by "history of place", referring to historical events such as colonization, speciation and extinction (Brown et al. 1996).

If PNE endemic fungi display traits related to habitat filtering, I predict endemism will show phylogenetic clustering within the community (Jiao and Lu 2020; Horner-Devine and Bohannan 2006). In other words, the extent of species endemism will be centred among key genera that possibly have a longer evolutionary history in environments similar to the perhumid rainforest. To test this, the distribution of range was examined against the phylogenetic structure of the EMF community dataset, using a method appropriate for a binary trait (Fritz and Purvis 2010). To adequately characterize the community, phylogeny and endemism status, sporocarp data alone is insufficient, often capturing less than half of the species in the community (Hui et al. 2011). Single molecule real time sequencing methods allow for longer and higher quality read depths than commonly used second-generation sequencing methods, such as Sanger sequencing. Such read depths will permit a more accurate identification of fungal species (Rhoads and Au 2015). I use this method to capture the EMF community present on root tips, and answer questions regarding the role endemism plays in community structure, and how it is dispersed across the phylogeny.

3.2. Methods

3.2.1 Root tip sampling of the EMF community

EMF communities on the tree roots were characterized by directly sampling root tips, followed by PacBio analysis. In June 2021, soil cores approximately 15x15cm in area and 10 cm in depth were removed with a knife at 15 random locations in each of the ten plots across five sites (see Chapter 2, section 1.1 for site and plot descriptions). The soil core included upper mineral soil and organic horizons. All roots in these cores were gently cleaned with water and examined under a compound microscope, and root tips with fresh vigorous EMF colonies were removed. Root tips from 3 microsites were composited into one vial, for a total of 5 samples per plot (n = 50 for the study). This process was conducted for 60 minutes per soil core. This time period was considered sufficient for comprehensively sampling the EMF community, and resulted in 7-10 mL of root tips per vial. The samples were freeze-dried and ground in a ball mill before DNA extraction.

3.2.2 Sequencing and bioinformatics

DNA was extracted from 20 mg of the ground roots from each of the 50 samples using the QIAGEN protocol (Cat No. 12888, Hilden, Germany) and underwent PCR amplification to test for quality before sequencing. The full length ITS region was sequenced using PacBio amplicon sequencing at an integrated microbiome resource lab at Dalhousie University. Sequences were filtered using the Dada2, ShortRead, and Biostrings packages in R-programmer (Callahan et al. 2016). Sequences less than 200 base pairs were discarded, and chimeras were removed. OUT's were clustered using the lulu package at a 98.5 % cut off, and taxonomy was assigned using the UNITE reference database. From there, following the species hypothesis (SH) designation algorithm of Nillson et al. (2019), samples with <99% match to their assigned SH on UNITE were compared with the other samples assigned that same SH using USEARCH (Edgar 2010). If a sample matched a minimum of one member of the SH by >99%, it was included. Any species that did not match was considered a new species. Similarly, if a sample matched another two different species hypotheses by >99%, than these were combined into one SH, following the algorithm of Nillson et al. (2019). From there, non-EMF clades were removed manually, using the UNITE description of EMF lineages. The community abundance data was normalized using the median of ratios method (Anders and Huber (2010) in using Excel, before being converted to proportional abundance. This method is effective in comparison to rarefying, as it divides each plot by the geometric mean of all relative species abundances at that plot as opposed to total reads. This reduces the problem of misrepresenting the abundance of some taxa while rarefying (McMurdie and Holmes 2014).

3.2.3 Effect of tree host species on community assembly

An analysis of similarity (*anosim* function) was used to test for the effect of tree host on the EMF community. This was conducted with both incidence and abundance community data. The Bray-Curtis index (Bray and Curtis 1957) was used to calculate pairwise community dissimilarity between sites with regard to species abundance, and the Jaccard index was similarly used for species incidence. EMF communities for each tree species host were checked to ensure community dispersions were not significantly different prior to analysis (*betadisper* function). All analyses were conducted in R package vegan (Okansen et al. 2013).

3.2.4 Analysis of EMF community assembly in response to soil properties

The effect of site properties on EMF community composition were examined using a partial mantel test (*mante*l function in R package *ecodist* (Goslee and Urban 2007)). Host effect was held constant as an intercept. All analyses were conducted on both incidence and abundance data.

3.2.5 The interaction between EMF endemism and community assembly processes

Endemism and host effect

To examine covariation between tree host and EMF range, a fourth-corner analysis was conducted (*fourthcorner* function, ade4 package)(Dray and Dufour 2007; Legendre et al. 1997). Model 1 was used in this analysis, as is considered appropriate for examining environmental control over a fixed set of species (Legendre et al. 1997). This model holds fixed the total number of sites occupied by each species, while permuting traits (range values) independently within site columns. Using 4999 permutations, the probability of achieving the test statistic under the null hypothesis that species associations are random, and not influenced by an environmental variable, is calculated. The "G" stat was used to calculate the global F statistic between the host and range, and the significance of the fourth corner statistic "d" was interpreted as a post-hoc test. This analysis was conducted using both species incidence and abundance data. P values were adjusted using Holm's (1979) method to reduce the likelihood of type I error.

To improve accuracy in interpreting the significance of the post hoc test, a generalised linear mixed model was used to test the effect of host species. This was conducted using per-site proportional endemic abundance and incidence (*lmer* function, lme4 package).

To identify species with significantly more abundance and incidence on each host, an indicator species analysis was conducted using the *multipatt* function in the *indicspecies* package (De Caceres and Legendre 2009).

Endemism and site properties

Site properties that showed a correlation with community composition were analyzed with respect to EMF species endemism. This relationship between species traits and site properties was also examined using a fourth-corner analysis. Species endemism status was permuted within sites, and the "D2" statistic was used to identify the direction and significance of Pearson correlations between soil property values and traits, as is appropriate for this type of data (Legendre et al. 1997).

Post hoc tests of the relationship between site properties and EMF endemism were conducted using per-site values of proportional endemic abundance and incidence. Generalized linear models examined the effect of soil properties on these variables (selected through AIC model comparison).

3.2.6 The distribution of endemism across community phylogeny

A maximum likelihood phylogenetic tree was constructed for all species found in the data set using phylogeny.fr. ITS sequences were aligned using MUSCLE, PhyML was used for tree building, and Tree Dyn for rendering (Dereeper et al. 2008). The phylogenetic signal of species range was calculated to see if endemism was more likely to occur in closely related species. Range was converted to a binary variable, and the D statistic was calculating using the "phylo.d" function in the caper package (Orme et al. 2013). The significance of this statistics' distance from 1 (representing range being completely dispersed through the phylogeny) and 0 (representing range being completely Brownian phylogenetic clustering (Felsenstein 1985)) was calculated using 1000 permutations (Fritz and Purvis 2010).

3.3 Results

3.3.1 Community composition and host effect

The PacBio method detected 203 different EMF species in the sampling area, spanning a diverse phylogeny (Figure S3). Russulaceae and Thelephoraceae were the most abundant families, together accounting for 61% of total reads (Figure 5, Table 6).

Sitka spruce plots hosted a total of 140 EMF species, with average of 64.4 (±1.63 SE) species per plot while western hemlock plots hosted a total of 162 EMF species, with an average of 69.2 per plot (±8.4 SE). An analysis of similarity found the fungal communities did not differ significantly in response to site ($p > 0.1$) but differed significantly in response to host species. This effect was stronger when observing relative abundance ($R=0.81$, $p= 0<0.01$) (Figure 6, Figure S2) than species incidence ($R=0.27$, $p < 0.05$).

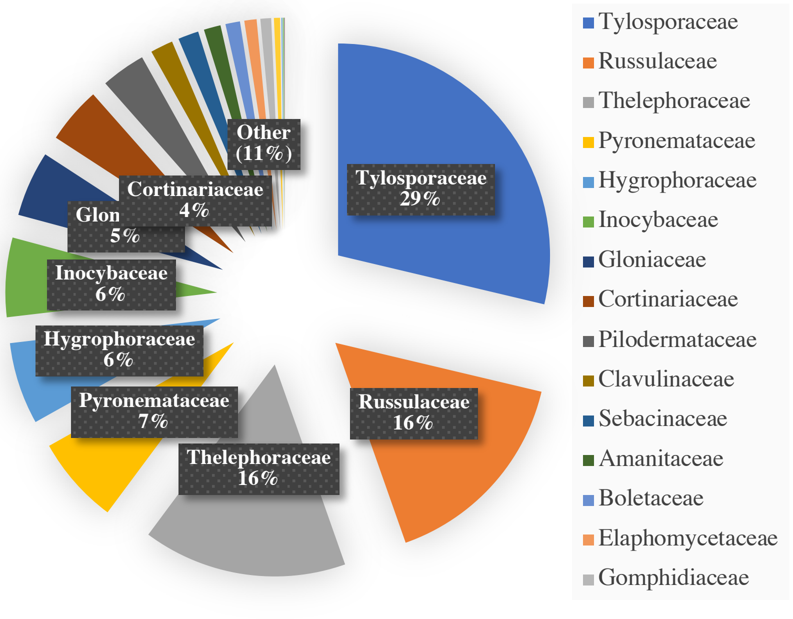

Figure 5. Abundance of ITS sequence reads by EMF family

Table 6. Table 7. Top twelve of the most abundant species in the data set and their UNITE species hypothesis (SH) and range (C = cosmopolitan, E = endemic). Total abundance represents the total percent abundance of the ten plots sampled, where 10 would mean a species was on 100% of the total root tips sampled.

Species (SH)	**Range**	**Total abundance**
Tylospora fibrillosa (SH1571571.08FU)	C	0.56
Amphinema sp. (SH1648322.08FU)	C	0.48
Cenococcum geophillum (SH1639590.08FU)	C	0.72
Atheliaceae sp. (SH1571572.08FU)	E	0.48
Thelephoraceae sp. (SH1503413.08FU)	E	0.40
Lactarius sp. (SH1679722.08FU)	E	0.42
Rhodoscypha sp. (SH1546041.08FU)	E	0.31
Tylospora sp. (SH1571570.08FU)	C	0.36
Lactarius sp. (SH1679693.08FU)	E	0.25
Thelephoraceae sp. (SH1502195.08FU)	C	0.29
Trichophaea sp. (SH1557658.08FU)	C	0.24

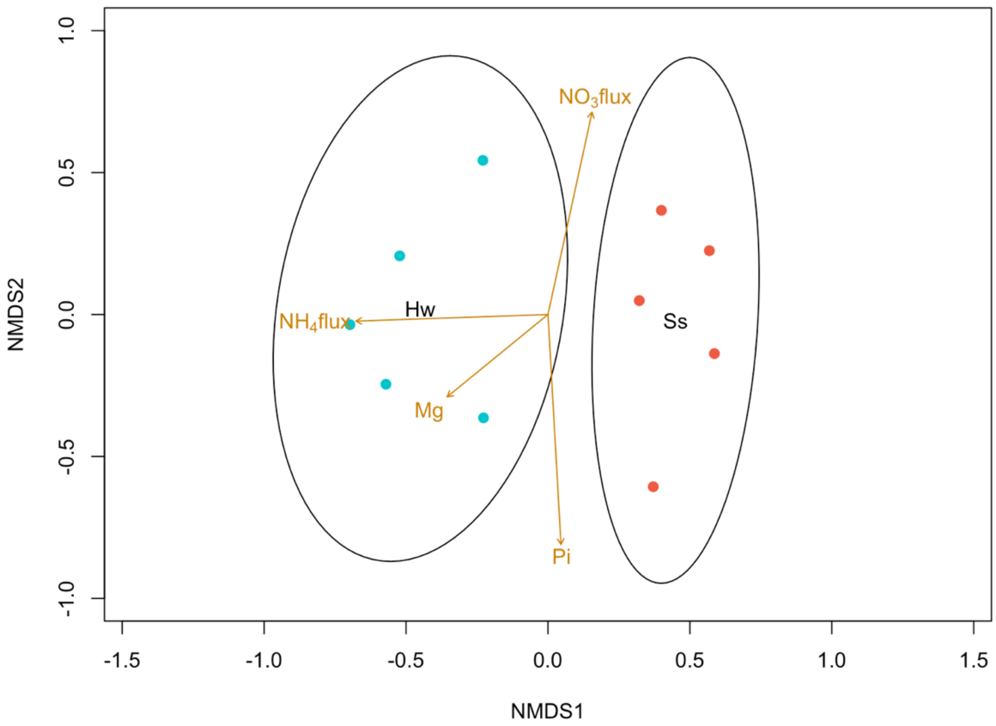

Figure 6. NMDS of EMF community abundance dissimilarity, fitted with significant environmental vectors (inorganic N flux, and mineral soil Mg and Pi). 99% confidence intervals are mapped around each host species, with cyan points representing western hemlock plots and rust representing Sitka spruce plots. Stress= 0.1.

3.3.2 Community composition across soil gradient

Mineral soil Pi similarity covaried significantly with community similarity in both species' occurrence and abundance. In addition, mineral soil C:N and NH_4^+ flux from PRS probes covaried significantly only with community occurrence similarity, and Mg and Ca only with community abundance similarity (Table 7).

Table 8. Partial Mantel test results of community dissimilarity in response to site properties, with host species effect held constant. "r" represents the Mantel test statistic, a Pearson product-moment correlation coefficient, ranging from -1 to 1.

	Occurrence		**Abundance**	
N	r=-0.12	p=0.36	r=-0.27	p=0.07
Pi	r=0.61	p=0.007	r=0.34	p= 0.007
Po	r=-0.03	p=0.8	r=0.07	p=0.7
C:N	r=0.33	p=0.03	r=0.22	p=0.13
Ca^{2+}	r=0.46	p=0.11	r=0.37	p=0.001
Mg^{2+}	r=0.47	p=0.11	r=0.36	p=0.001
K^+	r=-0.05	p=0.85	r=-0.02	p=0.89
pH	r=-0.18	p=0.34	r=0.14	p=0.32
NO_3^- flux	r=0.12	p=0.62	r=0.15	p=0.313
NH_4^+ flux	r=0.48	p=0.04	r=0.09	p=0.5

3.2.3 Host species and endemism

Overall, 97 of the EMF species identified (48%) were endemic to the PNE coast. Endemic fungi occurrences were skewed 60% on western hemlock, while cosmopolitan species occurrences were 50% on each host. A fourth corner analysis revealed endemic fungi were significantly more likely to occur on hemlock than spruce, while cosmopolitan species occurrence was not predicted by the host (Table 8, Figure 7).

Prior to rarefication, EMF total abundance reads were higher on spruce plots (22,327 reads/subsample) than hemlock (14,675 reads/ subsample), but species accumulated at a higher rate on hemlock with respect to the number of reads, indicating a more diverse community (Figure S1). Of total endemic fungal reads, 58% were on hemlock plots, while 58% of cosmopolitan species were found on spruce plots. Range type showed marginal global significance between species ranges and hosts for root tip abundance, but pairwise comparisons within range groups indicated that they were not significantly more abundant on either host than would be expected at random (Legendre et al. 1997) (Table 8).

Generalized linear mixed model analysis supported these results and found hemlock to be a significant predictor of greater proportional incidence of endemic fungi per site (p=0.046, m=0.08) but not their proportional abundance (p=0.12, m= -0.16). The three EMF species that were significantly more likely to be found on hemlock were all endemic, while the two that were more likely to occur on spruce were cosmopolitan (Table 9). This trend was not apparent for abundance data (Table S3).

Table 9. Fourth corner statistic d (measure of within group homogeneity) between host species and fungal range, with sign (+/-) signifying if the statistic is below or above the expected value under a null hypothesis of random distribution. Statistics represent the association between the entire categorical variables, and listed p-values were adjusted by the Holm-Bonferroni method to account for multiple tests (Holm 1979).

		Endemic	**Cosmopolitan**
	G=3.82, p=0.01		
Incidence	Spruce	d= -136, p=0.03	d= +179, p=0.83
	Hemlock	d= +168, p=0.03	d=-168, p=0.83
	G=2.10, p=0.05		
	Spruce	d= -1.96, p=0.46	d=3.03, p=0.46
Abundance	Hemlock	d= 2.80, p=0.46	d=-2.19, p=0.46

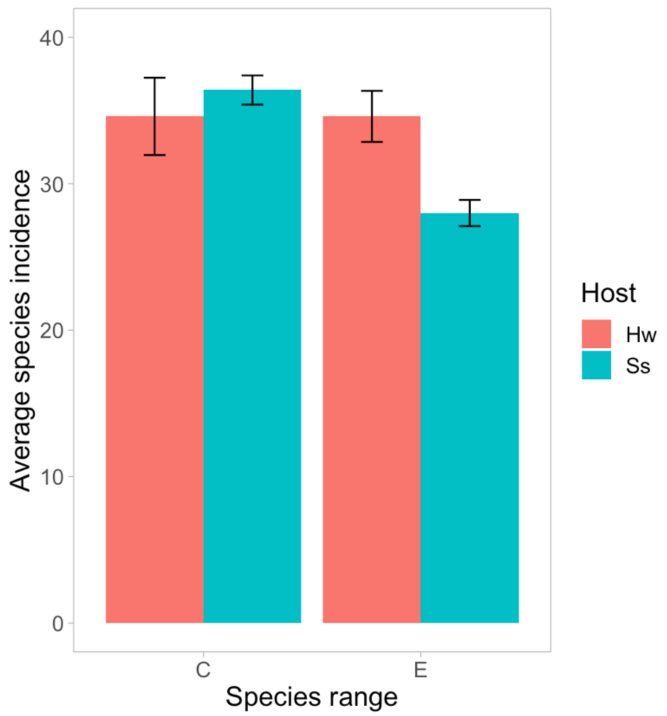

Figure 7. Mean cosmopolitan (C), and endemic (E) EMF species incidence per plot for each tree species plot (Hw=western hemlock, Ss=Sitka spruce) (n=10). Error bars represent the standard error of the mean.

Table 10. EMF indicator species analysis for Sitka spruce and western hemlock plots (n=10) using UNITE species hypotheses (SH) and EMF incidence data. Stat values represent the indicator value, calculated as the product of relative frequency in host species clusters, where 1 = present in every plot (Dufrene and Legendre 1997), and p values represent the likelihood of this statistic occurring under species' random distribution across hosts, where $p < 0.05$ indicates the EMF species is unlikely to be randomly distributed.

SH (98.5)	Putative Species ID	Range	Stat	p-value
Western Hemlock				
SH1564296.08FU	*Pseudotomentella sp*.	E	1.0	0.015
SH1561892.09FU	*Sebacina sp*.	E	0.91	0.025
SH1538903.08FU	*Russula sp*.	E	0.89	0.035
Sitka spruce				
SH1528420.08FU	*Thelephoraceae sp*.	C	0.89	0.035
SH1542731.08FU	*Hygrophorous sp*.	C	0.69	0.035

3.3.4 Endemism and species response to soil properties

NH_4^+ flux and Pi covaried significantly with endemism by tree host species in predicting the occurrence of EMF species. Pi also covaried with endemism by tree host in predicting species' relative abundance, but NH_4^+ flux did not. None of the other soil properties tested covaried with endemism by tree host in explaining EMF community composition (Table 10). Proportional richness of endemics demonstrated a significant negative relationship with mineral soil Pi (p=0.017, m=- 9.7e-04) (Figure 8), and a positive relationship with $NH_4^=$ flux (p=0.041, m=0.22). However, the collinearity between NH_4^+ flux and host obscures this relationship (Figure 9). Proportional endemic abundance showed a marginally significant negative relationship with Pi (p=0.052, m=-0.002).

Table 11. Fourth corner analysis of correlation between EMF endemism and soil attributes in predicting variation in EMF species' occurrence and abundance. P values represent the likelihood of obtaining the correlation the value (r) from a randomly organized environment and species range status (standard r) after the Holm correction (Holm 1979).

		r	**p**
Occurrence	NH_4 flux	0.07	0.04
	NO_3 flux	0.04	0.56
	C:N	-0.02	0.91
	Inorganic P	-0.08	0.01
Abundance	NH_4 flux	0.17	0.12
	NO_3 flux	0.18	0.09
	Ca	-0.03	0.74
	Mg	-0.03	0.78
	Inorganic P	-0.2	0.05

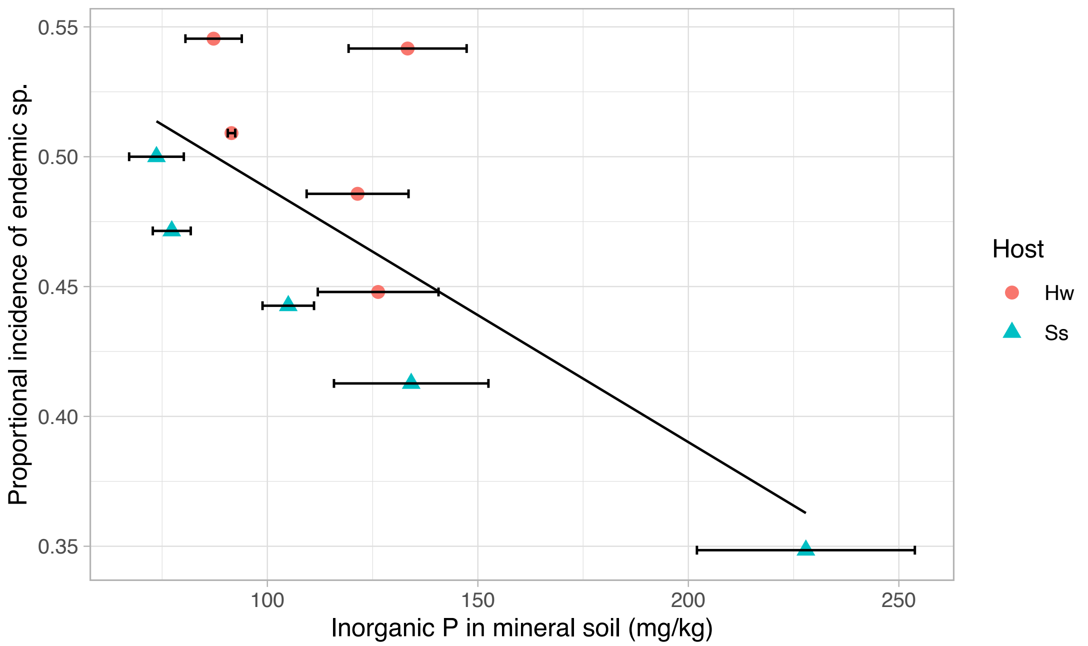

Figure 8. Proportional incidence of endemic species with respect to average mineral soil Pi per plot (n=30). Regression line was calculated using a univariate generalized linear model, and the colour of the data points represents plots of different host tree species (Hw = western hemlock, Ss = Sitka spruce). Error bars represent the standard error of the mean (n = 3).

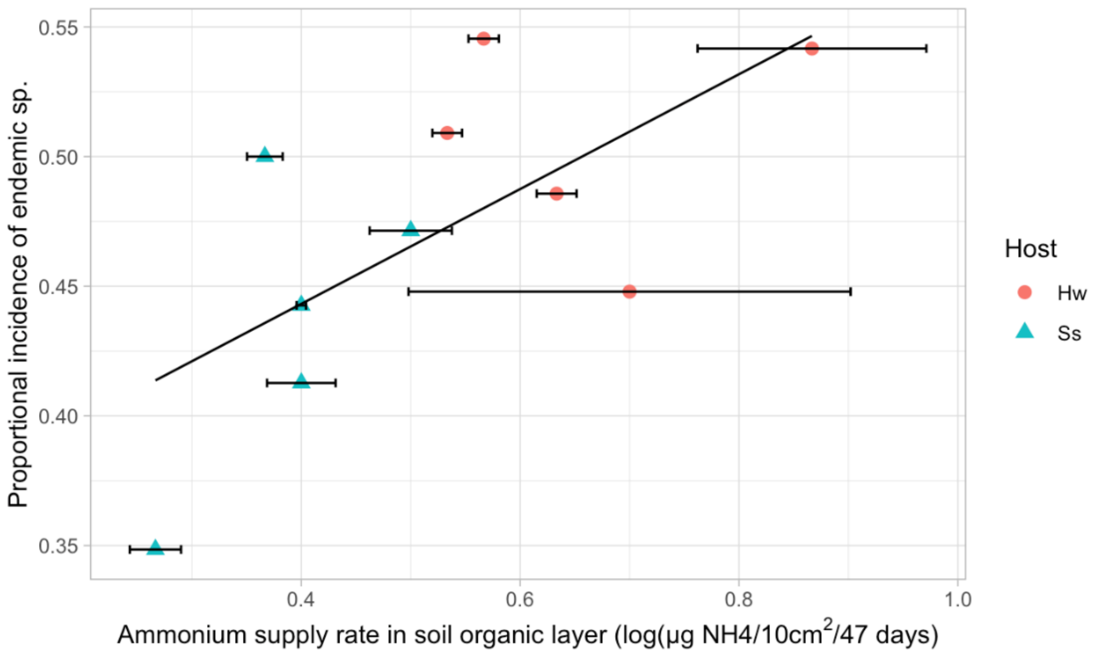

Figure 9. Proportional incidence of endemic species with respect to average NH4+ flux rate per plot (n=3). Regression line is the univariate generalized linear model, and the colour of data points represents plots of different host tree species (n = 10) (Hw = western hemlock, Ss = Sitka spruce). Error bars represent the standard error of the mean (n = 3).

3.3.5 The distribution of endemism across community phylogeny

The EMF community spanned a diverse phylogeny, with of 35 genera (Figure S3). The Inocybaceae family held the greatest percentage of endemic species (88%), and second greatest number of endemics (15) after Thelephoraceae, which held 25 endemics, making up 58% of species in the family. The greatest number (25) and proportion (83%) of cosmopolitan species were found in the Cortinariaceae family (Figure 10).

The estimated D statistic for the phylogenetic structure of species range was 0.76, suggesting moderate dispersion. The probability of it being entirely random (D=1) across the phylogeny was 0.01, and the probability of it being significantly clumped (D=0) was <0.001 (Figure 11, Figure 12).

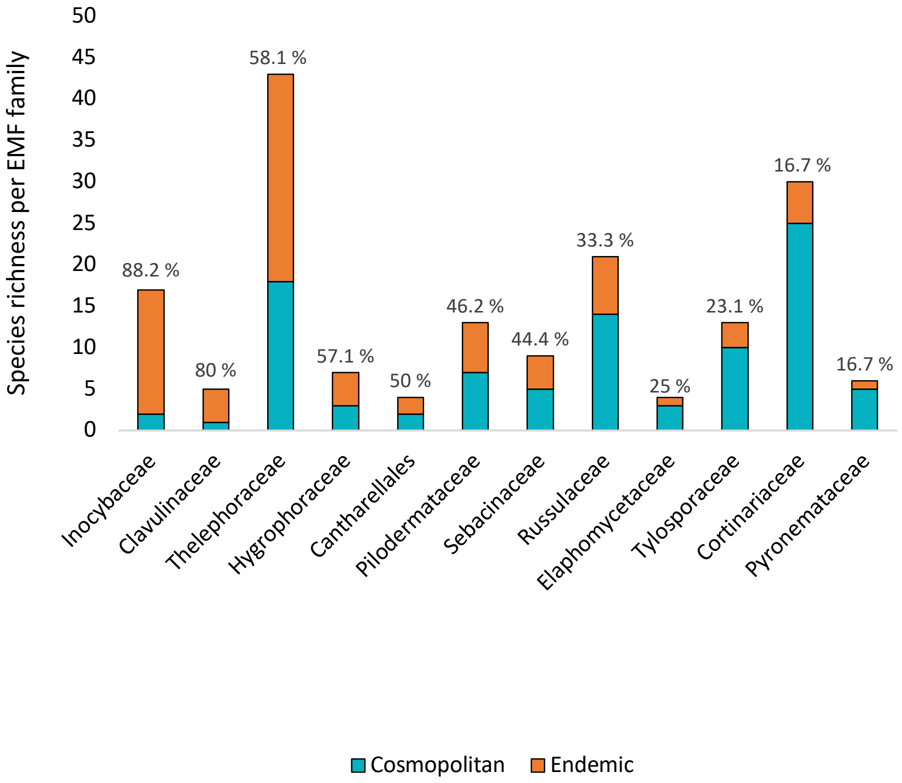

Figure 10. EMF species richness (number of species) for cosmopolitan (cyan) and endemic (rust) species within EMF families (n=172). Percentages represent the percentage of the endemism in the family.

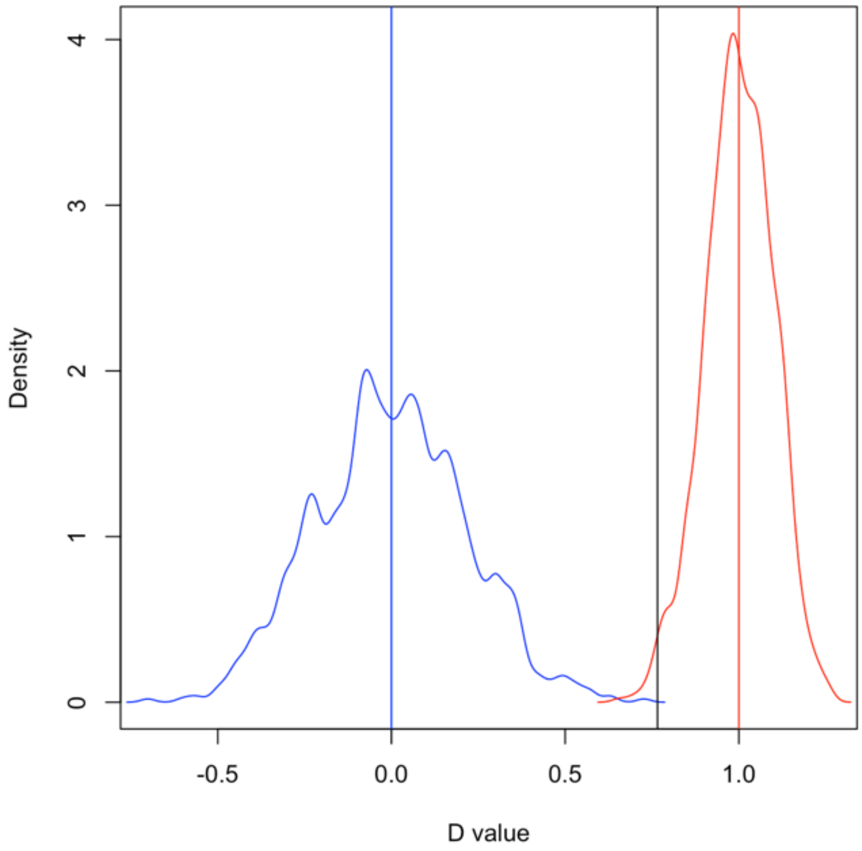

Figure 11. Density of D statistic, calculating the dispersion of species ranges across the phylogeny. The blue line indicates the expected distribution of the D statistic if range was phylogenetically clumped, the red line represents the expected distribution of D if range was randomly dispersed, while the black vertical line represents the observed D statistic.

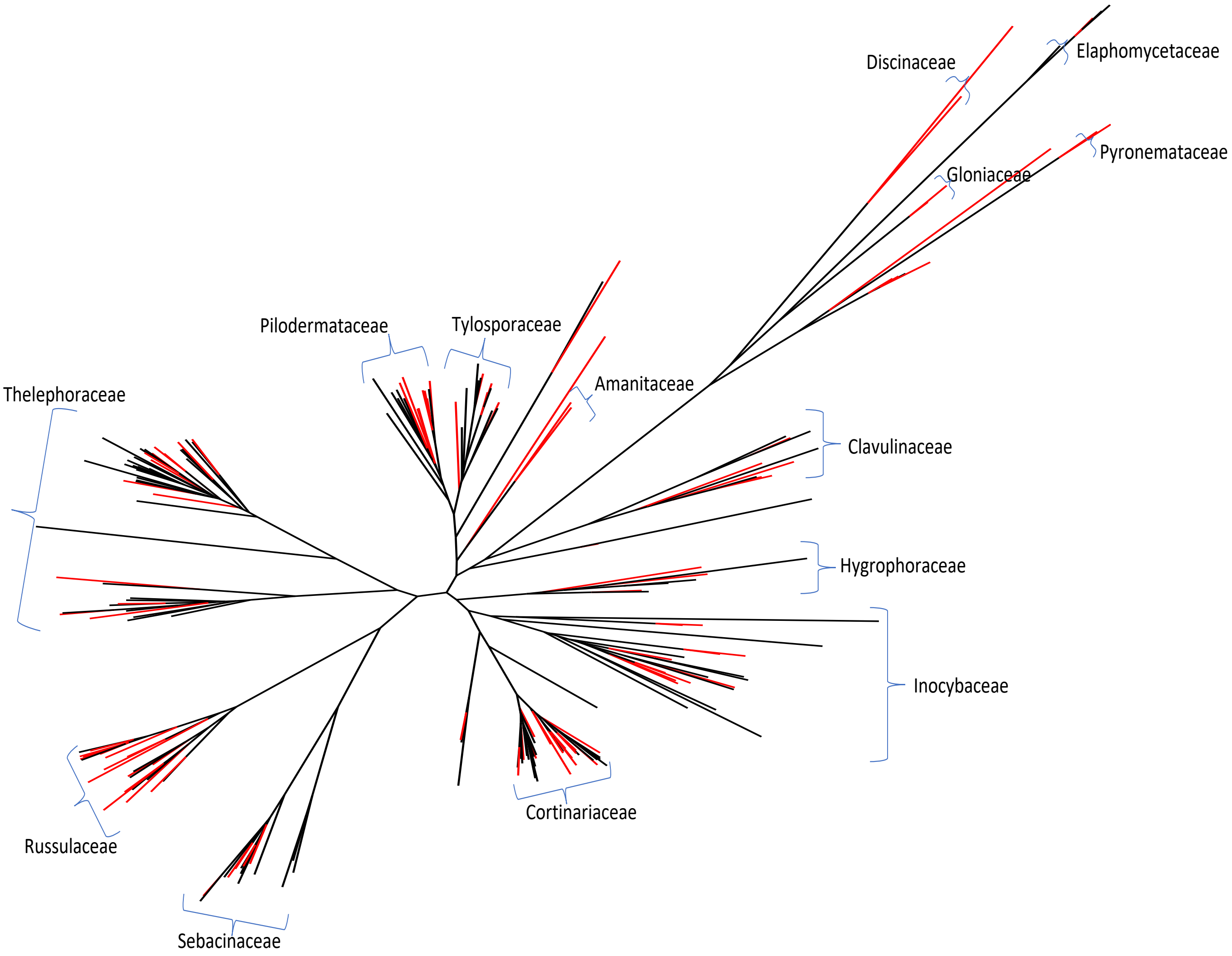

Figure 12. Distribution of endemism (plotted as red nodes) across the phylogeny of EMF species found in the community

3.4 Discussion

3.4.1 Endemism and community assembly

This study addressed how endemic and cosmopolitan EMF species respond to variation in host species and soil nutrition at the scale of the ecozone. Overall, mineral soil Pi, Mg, NH_4^+ availability and tree host were the most important factors affecting community composition, supporting existing literature showing that both edaphic factors and host species affect EMF species turnover (Tedersoo et al. 2012). Interestingly, the endemic and cosmopolitan range groupings captured distinct responses to Pi and inorganic N availability, as well as host species.

Endemic and cosmopolitan EMF species diverged in their response to Pi, suggesting endemic species hold a competitive advantage over the cosmopolitan group when P is less available. Moreover, the availability of NH_4^+ in the soil solution was also a strong predictor of EMF endemism. The relatively high availability of inorganic N in the soil solution may have offered a competitive advantage to endemic species better able to exploit it. As high NH_4^+ availability and low Pi are characteristic of these unique soils on the Pacific northeastern coast, these divergent responses further support the results from Chapter 2, indicating that endemism reflects superior local adaptation. On a broader scale, it also supports the role of habitat filtering in structuring species ranges in EMF (Frier et al. 2009). These results are consistent with Jiao and Lu (2020), who found higher NH_4^+ availability and warmer climate, as well as low available P predicted the abundance of rare symbiotic fungal species.

It should be noted that these multivariate trait-environment analyses were weakened by limited replication of soil data, thus restricting the J dimension of the R, and L matrices in the fourth corner analysis (see Legendre et al. 1997). The use of weighted correlations, while more statistically reliable than commonly used methods of testing quantitative trait-environment associations (Miller et al. 2019), can be subject to type 1 error if sites are not permutated in addition to species endemism status (Dray and Legendre 2008). The *fourthcorner* function would not hold adequate statistical power if sites as well as traits were permuted in this analysis. Future work should consider a higher resolution of sampling units. For example, individual microsites within a plot could be tested for EMF community and soil properties, rather than averaging these attributes at the plot level. In this study, p-value corrections go some way towards addressing this concern (Holm 1979).

I examined how the EMF community varied between host species, and the role endemism played in this variation. Few EMF species were strict specialists to either host, supporting the existing evidence that the EMF community is conserved at the family level of the tree host (Tedersoo and Smith 2013). The EMF community varied in incidence and abundance between the two hosts, indicating host preference and differential fitness. This distinction in EMF communities observed between hosts is well reported in the literature and can be driven by both distinct abiotic environments surrounding hosts, as well as phylogenetic relatedness between hosts (Essene et al. 2017; Ishida et al. 2007). Interestingly, some endemic EMF species were more pervasive under hemlock, while cosmopolitan species occurrence was not predicted by host. One explanation for this could be differences in the phylogeographic distribution of the host, which has been shown to be a strong predictor of EMF community similarity in conifers (Ishida et al. 2007). *Tsuga*, native to mild, wet climates, is only present in disjunct populations of temperate North America and eastern Asia. *Picea*, however, is circumpolar, occupying a wide variety of drier landscapes, and may thus be more likely to associate with cosmopolitan EMF species (Lockwood et al. 2013). Moreover, the greater availability of inorganic N in the soil solution on hemlock plots, possibly due to the relatively lower rooting density of this species, compared to spruce (Mercer et al. 2022; Butler et al. 2010), may have offered a competitive advantage to endemic species better able to exploit the available N. Due to the collinearity between host and NH_4^+ availability however, it was not possible to separate the relative effects of these variables on EMF community composition in this study. It is reasonable to believe that both inherent host effects and soil N availability contributed to processes governing community assembly.

EMF species occurrence data was consistently more affected by soil properties than was abundance data, due to lower overall variation in incidence. It is logical that constraining this variation would produce clearer results with limited sampling sites. Caution should be exercised in the interpretation of fungal abundance data, as amplification bias can exist at numerous steps in PCR (Bellemain et al. 2010). Species such as *Cantherellus formosus*, for example, with consistently present fruiting bodies (Chapter 2), were absent from the root tip community data. Undoubtedly some portion of the interspecific variation in abundance can be attributed to this bias, thus a larger sampling effort may be needed to observe and model community abundance in response to soil properties, particularly when considering low effect sizes.

3.4.2 Distribution and prevalence of endemism in the EMF community

A number of studies predict phylogenetic clustering when habitat filtering plays a significant role in determining EMF community assembly (Jablonski 1987; Freckleton et al. 2002; Blackburn et al. 2004). This was tested across all 201 EMF species found in the study area. However, I found that endemism did not show significant clustering across the phylogeny, indicating that range groups could not, overall, be predicted by the degree of EMF species relatedness. This lack of overall phylogenetic clumping across the phylogeny contrasted with other results from this study, such as superior nutrition and distinct response to unique site properties in the endemic group, both of which indicated a role of habitat filtering in determining range groupings.

While it is not possible to reconcile these discrepancies in the influence of host species versus site properties on EMF community with certainty, it should be noted that the hypothesis that habitat filtering predicts phylogenetic clustering has not been tested with fungi or other microbes. Niche conservatism, the idea that closely related taxa are more ecologically similar, underpins the interpretation of these statistics. While presence or absence of phylogenetic clustering has been interpreted as an indicator of phylogenetic niche conservation in microbial ecology literature (Horner-Devine and Bohannan 2006), the concept is based on data from macroflora and fauna (Harvey and Pagel 1991). Ackerly (2009) examined the relationship between phylogenetic signal and diversification rates in different tree species, and found a significant negative relationship, suggesting that niche conservatism relies on low diversification rates. In addition to microbes having naturally higher diversification rates, many EMF lineages are thought to have undergone adaptive radiations, where speciation ratse were unusually high due to novel ecological opportunities (Lutzoni et al. 2018; Sanchez-Ramırez et al 2015). Moreover, a meta-analysis of species range sizes with respect to phylogeny showed them to be more closely correlated under spatially and temporally stable environments (Zacai et al. 2017), consistent with predictions by Futumaya and Moreno (1988). As the time period and climate during EMF diversification varies by lineage (Ryberg et al. 2022), niche conservatism may follow. Future work should explore range conservation in EMF using more complete, monophyletic lineages, as certain families shown an overrepresentation of one range grouping.

The distribution of endemism across the phylogeny was not what would be predicted under random assortment. Certain range groupings were overrepresented in some EMF, supporting the role of lineage diversification history in predicting species range groupings in certain clades. The Inocybaceae family, 88% of which were endemic to the PNE coast, originated in the paleotropics, and underwent four separate radiations upon migrating to northern temperate regions of North America (70-75 Mya), when members of the Pinaceae, such as *Tsuga*, were diversifying (Wang et al. 2000; Matheny et al. 2009). The novel ecological opportunity of conifer species adapted to the mild wet climate during that time period may have fostered these adaptive radiations in this group (Halling 2001). Analogously, *Cortinarius* sect. *Cortinarius* (83 % of which were cosmopolitan), diversified 10 Mya in Australasia with Fagaceous hosts, namely *Quercus*, tolerant of drier conditions, and are thought to migrate through long distance dispersal and host generalism (Harrower et al. 2015). The prevalence of this genus in boreal forests that experience dry conditions, may reflect this phylogeographic history (Liimatainen et al. 2022).

As the phylogeography of most EMF clades remains unresolved (Ryberg et al. 2022), the origins of endemic species are speculative, but relevant to understanding the relationship between PNE range limitation and ecological specialisation. The spatial separation of the PNE coastal ecosystem from other ecozones, in combination with its moist, mild and stable climate could have created suitable conditions for speciation by local adaptation, giving rise to endemic species (Tedersoo et al. 2022; Brown et al. 2014; Kassen 2002). The ecosystem was established in this region 1.5 Mya, and the rainforest species underwent periodic range restrictions and expansions after that. Thus, endemic species may have recently speciated through adaptation to the novel niche presented by this ecosystem (Ryberg and Matthew 2012), or alternatively, be populations of generalist species bottlenecked in refugia during the last glaciation event (Holliday et al. 2010). Both these possible origins are supported by the similarity of many of our endemic species to other species (99% similarity in ITS region), although the competitive advantages observed in this study lends more support to the hypothesis of adaptation to a novel niche. In comparison, many cosmopolitan species may have originated during the range expansion of the boreal forest (20-40 Mya), when a cooling climate placed selection pressures on EMF lineages adapted to the previously mild, wet climate (Bruns et al. 1998; Ryberg and Matthew 2012).

While superior sporocarp nutrition and distinct response to the soil profile observed in endemic species supports the hypothesis that ranges are driven by niche breadth, it is important to note there was still considerable variation in both these responses within each range group. This variation observed within the endemic group may reflect species originating only from reproductive isolation, unrelated to adaptation. Moreover, the variation observed within the cosmopolitan group may reflect genetic plasticity in traits related to nutrient acquisition (Sheiner 1993). Overall, these results showed that PNE coast endemic EMF species span most lineages and makes up close to half the total fungi in the community. This group, on average, displayed greater local adaptation to the unique regional soil nutrition, and a slight competitive advantage on a more phylogeographically isolated host.

4. Conclusion and future directions

Overall, the EMF species endemic to the PNE coast displayed a superior acquisition of nutrients to the cosmopolitan group. Yet, the cosmopolitan group are present in comparable numbers to the endemic species. Considering a lifestyle in which carbon and nutrients are the primary limiting resources and source of competition, it first appears counter-intuitive that a species group of lower competitive rank could have similar success to a higher one.

The competition colonization trade-off model would explain such coexistence by emphasizing that the fitness of a species has multiple axes, one of which is spatial (Durrett and Levin 1998). A cosmopolitan species, with an inferior ability to compete for local nutrients, might invest more heavily in dispersal, persisting in a new location through priority effects. McPolin and Kranabetter (2020) found that cosmopolitan species displayed less clustering in their spatial assembly, consistent with such a strategy. Studies have suggested that 55- 65 years after disturbance, the EMF of a forest stand community remains consistent in species composition, with some increase in abundance in late seral EMF taxa (ie. *Russula*) and a decrease in pioneer species abundance (i.e. *Cenococcum geophilum*) in the older stand (Ma et al. 2012; Twieg et al. 2007). Durrett and Levin (1998) consider the long-term spatial dynamics between an EMF species with higher competitive ability, and one with higher dispersal ability under a stable, uniform environment. They predict a steady outgrowth of clusters of the more competitive species, which eventually outcompete early seral strategists. While the applicability of this model to a forest ecosystem is dampened by soil heterogeneity, the decline of root-tip turnover disturbance with stand age around 100 years would theoretically lead to the exclusion of early seral species in older forests (Yuan and Chen 2010; Raymond and Mackenzie 2013). Though research on the EMF community succession in forests older than 100 years is minimal, stands in the studied ecosystem can persist for many centuries. Future research can examine if the more competitive endemic fungal species characterized in this study become increasingly dominant as forests age.

The conclusions from this study are relevant to current forest management efforts to conserve the diversity of the EMF community. The endemic fungi represent a target group for conservation, as they provide superior nutrition to trees, and display distinct dispersal behaviour (McPolin and Kranabetter 2021). Given the likely delay in re-establishment of endemics in regenerating stands, traditional forest operations (multiple, short-term rotations) may deplete the endemic species pool over time. In showing greater specialisation to the ecosystem, this group may require more connectivity to older stands than generalist species. Methods such as retention forestry or more conservation areas may be needed for endemic EMF species to populate regenerating forests nearby (Sverdrup-Thygeson et al. 2017; Devictor et al. 2008; Kranabetter et al. 2013; Varenius et al. 2016; Jones et al. 2008). As the climate changes, specialist species are at a disproportionate risk of extinction (Reed and Tosh 2019). It is my hope that this research will help to inform EMF conservation research to move beyond diversity indices and prioritize ecologically important species.

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Appendix 1.

Table 12. Average sporocarp nutrient concentration per species. Standard error for species with multiple occurrences is listed in brackets.

Species	Range	C	N	P	Ca	Mg	K	C:N	N:P
Amanita constricta (9)	E	45.7 (0.34)	5.86 (0.14)	0.53 (0.02)	193.6 (26.22)	1041 (38.67)	45358 (1313.58)	7.8 (0.2)	11 (0.94)
Cantharellus formosus (5)	E	43.8 (0.44)	3.31 (0.23)	0.46 (0.02)	360.1 (27.29)	1150 (31.8)	48892 (1014.01)	13.2 (0.87)	7.2 (1.35)
Catethelasma ventricosa (1)	C	42.7 (0)	4.25 (0)	0.65 (0)	135.7 (0)	783 (0)	33797 (0)	10 (0)	6.5 (0)
Clavulina corraloides (9)	E	45 (0.25)	4.13 (0.13)	0.36 (0.01)	218.6 (28.64)	1041 (27.81)	46609 (935.4)	10.9 (0.33)	11.6 (0.99)
Cortinarius acutus (2)	C	40.2 (0.3)	3.42 (0)	0.33 (0.05)	228.4 (23.81)	806 (17.76)	51809 (4824.56)	11.8 (0.09)	10.4 (3.17)
Cortinarius cinnamomeus (3)	C	43.1 (0.23)	3.92 (0.74)	0.36 (0.05)	180.8 (27.6)	782 (79.99)	44735 (3756.68)	11 (1.79)	10.9 (1.4)
Cortinarius glaucocephalus (1)	E	42.6 (0)	5.67 (0)	0.4 (0)	81.7 (0)	770 (0)	19945 (0)	7.5 (0)	14.3 (0)
Cortinarius piceidisjungendus (1)	C	42.3 (0)	3.89 (0)	0.3 (0)	126.2 (0)	1067 (0)	46368 (0)	10.9 (0)	12.8 (0)
Cortinarius substriatus (1)	C	41.8 (0)	3.83 (0)	0.28 (0)	113.4 (0)	1118 (0)	40197 (0)	10.9 (0)	13.5 (0)
Cortinarius variosimilis (1)	C	41 (0)	2.75 (0)	0.56 (0)	70.5 (0)	811 (0)	47119 (0)	14.9 (0)	4.9 (0)
Hygrophorus monticola (3)	C	44.2 (0.37)	4.8 (0.49)	0.71 (0.07)	95.4 (11.98)	897 (80.85)	43561 (2996.11)	9.2 (1.11)	6.8 (0.6)
Hygrophorus olivaceoalbus (2)	C	42.4 (0.23)	3.65 (0.27)	0.47 (0.05)	271.3 (56.47)	682 (23.44)	39875 (390.59)	11.6 (0.91)	7.8 (3.05)
Inocybe calamistrata (2)	E	43.1 (0.12)	5.49 (0.05)	0.51 (0.03)	69.6 (2.3)	832 (60.79)	39713 (2518.63)	7.8 (0.05)	10.7 (1.44)
Inocybe hirsuta (2)	E	43.8 (0.71)	5.74 (0.32)	0.48 (0.05)	59.2 (2.63)	857 (128.98)	40224 (1371.87)	7.6 (0.3)	11.9 (1.21)
Inocybe kauffmanii (3)	E	43.1 (1.1)	5.32 (0.54)	0.42 (0.02)	161.9 (63.73)	908 (79.43)	44361 (2502.44)	8.1 (0.56)	12.5 (3.51)
Inocybe melleiconica (1)	E	43.1 (0)	4.96 (0)	0.46 (0)	241.3 (0)	1006 (0)	35149 (0)	8.7 (0)	10.8 (0)
Inocybe olympiana (1)	E	45.3 (0)	3.17 (0)	0.47 (0)	393.4 (0)	1107 (0)	39218 (0)	14.3 (0)	6.7 (0)
Inocybe sauveolens (1)	C	39.5 (0)	5.56 (0)	0.38 (0)	467.9 (0)	947 (0)	31536 (0)	7.1 (0)	14.5 (0)
Inocybe sororia (7)	E	42.3 (0.25)	5.69 (0.21)	0.62 (0.01)	118.3 (31.56)	1098 (38.11)	57944 (1940.32)	7.4 (0.23)	9.1 (0.78)
Inocybe sp (1)	E	42.9 (0)	4.88 (0)	0.35 (0)	339.3 (0)	953 (0)	38495 (0)	8.8 (0)	13.9 (0)
Laccaria bicolor (1)	C	41.6 (0)	4.3 (0)	0.6 (0)	108.8 (0)	1170 (0)	45346 (0)	9.7 (0)	7.1 (0)
Lactarius kauffmanii (1)	C	46.2 (0)	3.94 (0)	0.44 (0)	184 (0)	953 (0)	24892 (0)	11.7 (0)	8.9 (0)
Lactarius lignyotus (5)	C	45.2 (0.43)	4.78 (0.16)	0.46 (0.02)	186.6 (39.4)	930 (30.17)	26755 (903.45)	9.4 (0.23)	10.5 (1.02)
Lactarius olivaceoumbrinus (1)	C	44.2 (0)	3.28 (0)	0.33 (0)	60.5 (0)	731 (0)	25202 (0)	13.5 (0)	9.9 (0)
Lactarius pallescens (1)	C	44.3 (0)	3.17 (0)	0.54 (0)	137.6 (0)	1145 (0)	29489 (0)	14 (0)	5.9 (0)
Lactarius pseudomucidus (4)	E	43.8 (0.81)	2.96 (0.12)	0.42 (0.02)	213.8 (11.5)	892 (28.43)	26671 (1403.19)	14.8 (0.59)	7.1 (0.86)
Lactarius scrobiculatus (3)	C	44.6 (0.55)	2.77 (0.39)	0.4 (0.06)	220.6 (22.33)	776 (29.52)	23090 (1635.33)	16.1 (2.3)	6.9 (0.14)

Lactarius substriatus (6)	E	44.7 (0.45)	4.62 (0.17)	0.46 (0.02)	185.7 (29.39)	767 (13.95)	34240 (1748.54)	9.7 (0.31)	10 (1.86)
Lactarius subviscidus (7)	E	44.4 (0.3)	4.56 (0.08)	0.48 (0.01)	202.9 (19.42)	799 (23.95)	33018 (795.27)	9.7 (0.21)	9.6 (0.75)
Otidea leporina (1)	C	43.5 (0)	2.67 (0)	0.64 (0)	569.1 (0)	936 (0)	40063 (0)	16.3 (0)	4.1 (0)
Phaeocollybia (2)	E	45.6 (4.17)	7.26 (1.1)	0.82 (0.01)	268.8 (100.22)	1217 (63.85)	24784 (2481.63)	6.3 (0.37)	8.9 (2.69)
Ramaria cyanaigrosa (1)	E	42.9 (0)	6.36 (0)	0.66 (0)	76.1 (0)	1377 (0)	34518 (0)	6.7 (0)	9.6 (0)
Ramaria gelatiniaurantia (1)	E	41.6 (0)	3.67 (0)	0.44 (0)	534.4 (0)	1639 (0)	21445 (0)	11.3 (0)	8.4 (0)
Ramaria leptoformosa (1)	E	43.2 (0)	4.58 (0)	0.47 (0)	116.6 (0)	1513 (0)	34404 (0)	9.4 (0)	9.8 (0)
Russula dissimulans (2)	C	46.4 (2.45)	5.01 (0.31)	0.4 (0)	69.1 (8.19)	741 (63.77)	30245 (456.1)	9.3 (1.07)	12.4 (1.72)
Russula pallescens (2)	C	47.2 (0.43)	2.87 (0.01)	0.31 (0.04)	132.4 (9.06)	649 (17.83)	25555 (488.44)	16.5 (0.08)	9.4 (2.61)
Russula silvicola (2)	C	45.2 (0.1)	4.02 (0.12)	0.51 (0.01)	259.1 (66.76)	967 (84.27)	41724 (5395.78)	11.2 (0.35)	7.9 (0.21)
Tricholoma saponaceum (1)	E	41.9 (0)	3.52 (0)	0.38 (0)	70.7 (0)	707 (0)	35029 (0)	11.9 (0)	9.2 (0)
Tricholoma vaccinum (2)	C	42.7 (0.11)	2.69 (0.22)	0.45 (0.07)	123.7 (23.44)	800 (67.98)	33114 (1020.59)	16 (1.29)	13.8 (3.12)

Table 13. Results for a two way t-test of nutrient concentration between the endemic and cosmopolitan EMF community

All species

	N	P	Ca	Mg	K	C:N	N:P
P value	0.003	0.36	0.69	0.034	0.89	0.004	0.19
Endemic average	4.83	0.48	206	1035	36843	9.58	10.12
Cosmo average	3.78	0.46	187	885	36224	12	9.25
% difference	27.8	6.02	9.9	17.1	1.71	-21	9.45

n > 1

P value	0.023	0.16	0.93	0.01	0.35	0.02	0.69
Endemic average	4.99	0.51	187	964	40165	9.4	9.96
Cosmo average	3.79	0.44	177	803	36046	12.2	9.68
% difference	31.7	15.5	5.58	20	11.4	-23	2.88

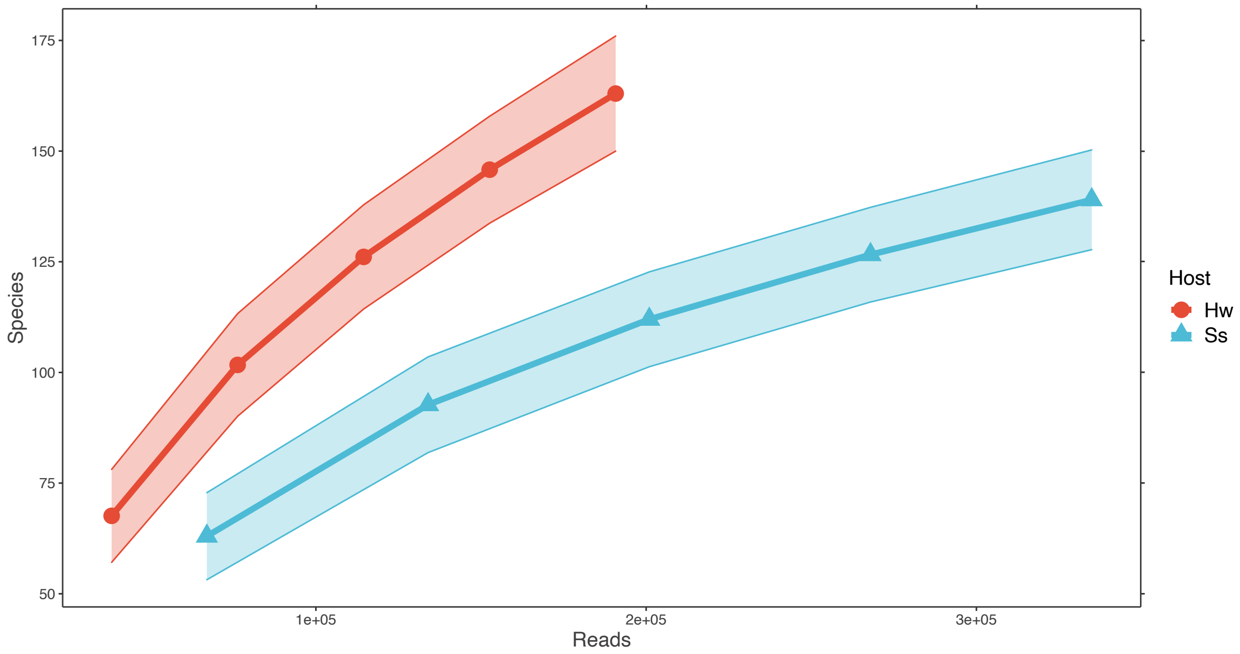

Figure S1. Species accumulation per number of reads, compared between Western hemlock and spruce plots (n=10).

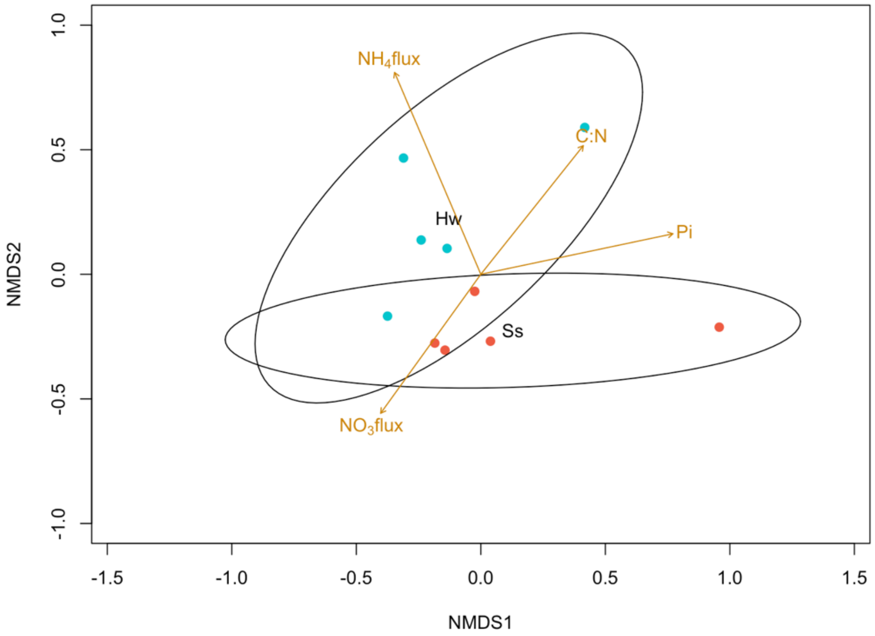

Figure S2. NMDS of EMF community incidence dissimilarity, fitted with significant environmental vectors (Ni flux, and mineral soil Ca, Mg and Pi). 99% confidence intervals are mapped around each host species, with green points representing hemlock plots and blue representing spruce plots. Stress=0.09.

Table 14. Indicator species analysis for spruce and hemlock using EMF abundance data.

SH (98.5)	**Common name**	**Rang**	**Stat**	**p-value**
Western Hemlock				
SH1564296.08FU	*Pseudotomentella*	E	1.00	0.02
SH1519114.08FU	Lactarius *pseudomucidus*	E	0.99	0.02
SH1544812.08FU	*Piloderma*	E	0.99	0.02
SH1639626.08FU	Cenococcum	E	0.99	0.02
SH1561892.08FU	Sebacina	E	0.99	0.04
SH1509052.08FU	Russula	C	0.89	0.05
SH1503717.08FU	Cortinarius	C	0.89	0.05
Sitka spruce				
SH1557658.08FU	Trichophaea	C	0.99	0.02
SH1557661.08FU	Trichophaea	E	0.99	0.05
SH1648320.08FU	Amphinema	C	0.98	0.02

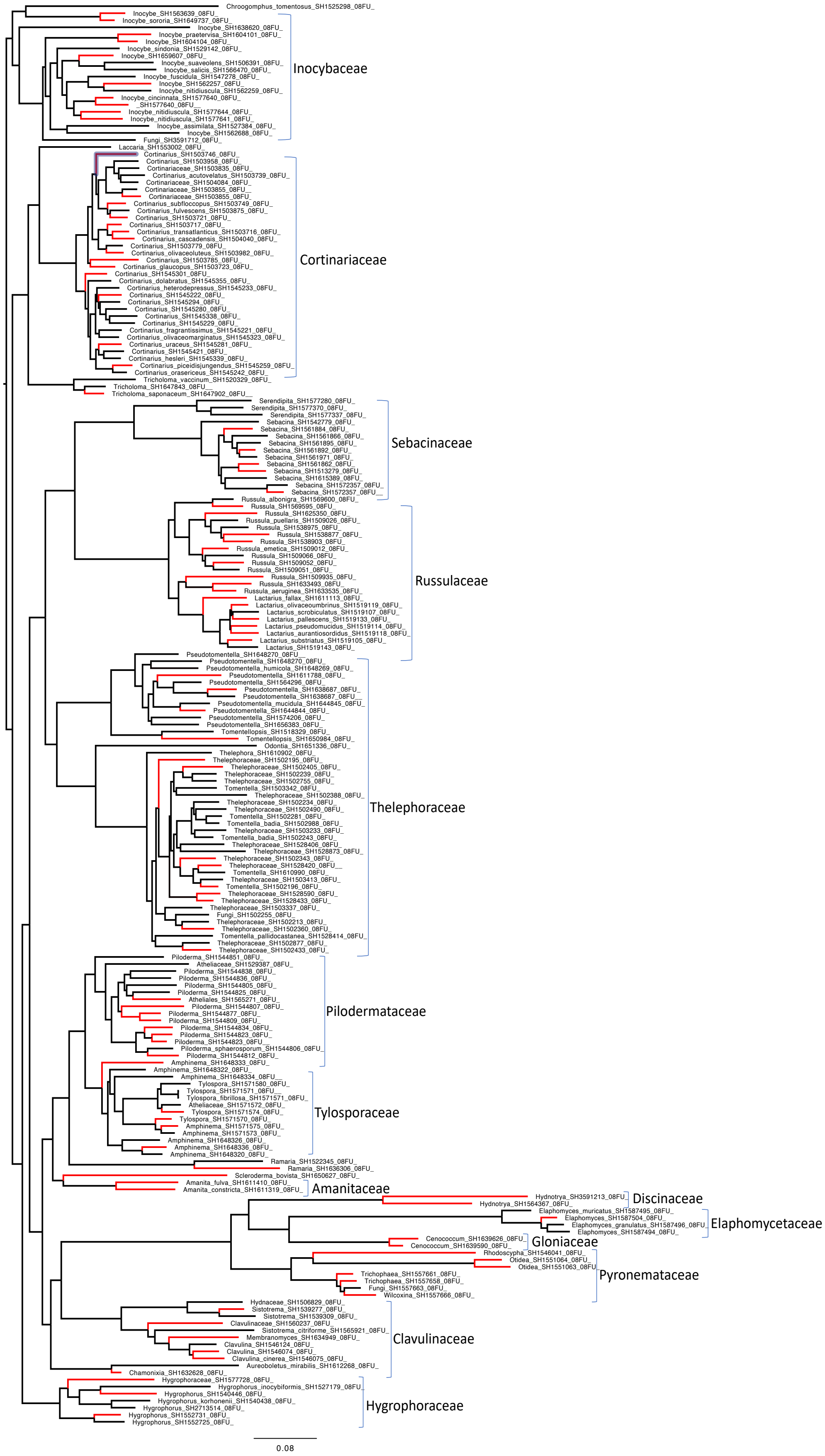

Figure S3. Maximum likelihood tree, constructed using the ITS sequences of all EMF species found in community dataset.