# Effects of Soil Chemistry, Niche Partitioning, and Competitive Exclusion on the Ectomycorrhizal Community Composition of Red Alder (*Alnus rubra*)

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## Abstract

Effects of Soil Chemistry, Niche Partitioning, and Competitive Exclusion on the Ectomycorrhizal Community Composition of Red Alder (*Alnus rubra*)

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There have been few studies of ectomycorrhizal fungal (EMF) communities in alder forests. The effects of spatial variability, competition, and environmental gradients on EMF community composition have seldom been investigated in sufficient depth to tease out more subtle impacts, particularly in field settings. In an exploratory field-based study, I analyzed how soil chemistry gradients influence EMF interspecific interactions and overall community composition of EMF species associated with an 85-year-old western Washington *Alnus rubra* stand. From May to September 2011, 150 root cores, soil variables (soil moisture, total C and N, available P, soil pH) and *Frankia* nodule presence data were assessed. Root tip morphology along with DNA analyses of root tip and sporocarp sequences revealed 22 EMF species, including 14 new associates with *A. rubra*. Spatial analysis of the community revealed a random pattern, indicating no spatial

autocorrelation of samples. Compositional analysis identified two large groups of root cores due to the spatial separation of two dominant species: *Cortinarius* cf. *alboviolaceous* and *Naucoria escharoides.* Month of sampling and *Frankia* nodule presence ($p<0.05$) explained 16% of the variation of the EMF community. Soil chemistry variables (moisture, pH, phosphorous, total N) impacted community composition at finer scales; EMF species varied in abundance and tolerance for each variable. The use of multivariate techniques and intensive sampling effort provided the tools necessary to form a more complete picture of *Alnus rubra* ectomycorrhizal community composition than was previously described. Niche partitioning relative to soil chemistry gradients and competitive exclusion are inferred for species of the same genus and between *Lactarius, Tomentella, Naucoria,* and *Cortinarius* species. Further research incorporating ecological concepts of distribution in ectomycorrhizal community composition is essential for understanding the ecology of EMF in natural environments.

## Introduction

Forest plant communities vary with physiography (Whittaker 1956), stand initiating disturbance event, time since disturbance, biological legacies and seed availability (Franklin et al. 2002), and the relative environmental tolerances and competitive interactions of the plant species present (Grime 1979, Tilman 1994). The importance of environmental gradients in abiotic stresses, facilitation, niche partitioning, and competition in plant communities is complex as these factors and their interactions vary spatially and temporally (Callaway and Walker 1997). In spite of the multiple variables that can alter plant community composition and structure, repeatable plant communities (i.e., vegetation associations) can be described (Bray and Curtis 1957) and there is a history of describing changes in plant associations at multiple spatial and temporal scales (Clements 1936, White 1979).

The role of soil communities in influencing plant community dynamics is also important (Bever et al. 1997), and both positive and negative plant-soil interactions potentially influence plant community composition (Revilla et al. 2012). Plant-rhizosphere interactions include: nodule forming nitrogen-fixing bacteria and mycorrhizal fungi which form important symbiotic relationships that influence nutrient cycling and uptake by plants—often affecting plant competition (Smith and Read 2008, Wolfe et al. 2009). Soil pathogens also alter plant communities and changes in rhizosphere communities may promote plant diversity by altering plant competition (Bradley et al. 2008). The production, diversity and stability of plant communities may be related to mycorrhizal communities (van der Heijden et al. 1998, Schultz et al. 2001), and therefore understanding mycorrhizal communities is important for understanding plant communities.

The ecological principles that determine plant community distribution may also influence ectomycorrhizal fungal (EMF) composition (Smith et al. 2002, Smith and Read 2008, Wolfe et al. 2009, Kennedy 2010, Johnson et al. 2012). The influence of environmental gradients on EMF community composition has been increasingly acknowledged. Environmental gradients (moisture, nutrients, temperature) have been shown to mediate EMF diversity and interspecific interactions, influencing the overall community composition in both greenhouse and field studies (Smith and Read 1998, Kennedy 2010). The spatial and temporal patterns of EMF community composition often reflect interactions between the EMF community and the soil environment (Lilleskov et al. 2004, Izzo et al. 2005, Kennedy 2010, Johnson et al. 2012).

The analysis of patterns in EMF community structure over spatial and temporal scales provides information on overall EMF community composition (Peter et al. 2001b, Lilleskov et al. 2004, Koide et al. 2007). EMF communities tend to have a clumped spatial distribution at small scales (< 3m on roots, <8m as sporocarps), with random spatial patterns at larger scales (Peter et al. 2001b, Lilleskov et al. 2004, Izzo et al. 2005). Interestingly, these spatial relationships remain constant over temporal scales, even with fluctuations in EMF diversity (Izzo et al. 2005). Determining the overall spatial distribution of EMF communities is an important first step in community composition analysis and must be assessed prior to analyzing the impacts of competition and environmental gradients on species distribution (Peter et al. 2001b, Lilleskov et al. 2004).

The spatial structure of EMF communities is determined by interspecific interactions within the community such as niche partitioning and competitive exclusion (Taylor and Bruns 1999, Kennedy and Bruns 2005; Kennedy et al. 2007, 2011). Competitive interactions tend to be density dependent, with the timing of root tip colonization influencing overall competitive

outcomes (Kennedy and Bruns 2005, Kennedy et al. 2007, Kennedy 2010, Kennedy et al. 2011). Laboratory and field based EMF competition experiments have opposing outcomes of competition due to the significant influence of environmental gradients on competition (Kennedy et al. 2007, Kennedy 2010). Inclusion of environmental gradients in assessing community composition also reveal EMF niche partitioning, which are differences in EMF species resource and habitat preferences and root colonization strategies (Taylor and Bruns 1999, Geml et al. 2010). The complexity of interspecific interactions between EMF species within a community is relatively unknown as experimental manipulations of many common EMF species are difficult because they cannot be cultured (Horton and Bruns 2001) and experimental design is complicated by the high EMF diversity in natural systems (Smith et al. 2002, Smith and Read 2008). The outcome of interspecific interactions in terms of the overall structure of EMF communities may be entirely reliant on local environmental variables (Horton and Bruns 2001, Kennedy et al. 2007), and has yet to be intensively examined.

Soil chemistry gradients have been shown to affect EMF diversity, abundance, and community composition across plant communities (Worely and Hacskaylo 1959, Harvey et al. 1978, Ostonen et al. 2009, Akata et al. 2012). Nitrogen gradients influence the diversity and presence of EMF communities in a variety of systems (Smith and Read, 2008). In boreal forests, nitrogen gradients are the primary determinant for the type of ectomycorrhizal species present (Giesler et al. 1998, Nilsson et al. 2005, Toljander et al. 2006, Kranabetter et al. 2009, Kjoller et al. 2012). EMF diversity in stands is generally mediated by total nitrogen availability (e.g., C:N ratios) regardless of phosphorous levels applied (Alvarez et al. 2012).

Soil moisture levels also significantly influence the composition of EMF (Worley and Hacskaylo 1959, Baar et al. 2002, Becerra et al. 2005, Smith and Read 2008). For both water

limited and saturated soil moisture conditions, the level of soil saturation selects for EMF species with different stress tolerances and physical abilities to either manage high levels of saturation, or have extensive extracellular hyphae that can bring water to the tree from greater distances than other ectomycorrhizal fungi in moisture limited systems (Baar et al. 2000, 2002; Smith and Read 2008). Changes in EMF community composition can occur within a site based on subtle temporal differences in soil moisture content, as substrates with varying amounts of humus, decayed wood and mineral soil have different moisture retaining capabilities, impacting overall EMF colonization and biomass (Harvey et al. 1978). Within plant communities, the temporal variation of soil moisture and its impact on nutrient availability has been established (Fogel and Cromack 1977), but how temporal fluctuations of the soil environment mediates EMF community composition has only recently been examined (Pritsch et al. 1997ab, Becerra 2005ab, Izzo et al. 2005, Kennedy and Peay 2007, Koide et al. 2007, Bahram et al. 2012).

Difficulties obtaining larger spatial or temporal sample sizes have led to a gap in field-based analysis of EMF community composition and the ecological principles outlined above. The number of root cores needed to capture EMF diversity in most forested systems is high because of the large number of EMF species (Smith and Read 2008, Smith et al. 2009), and sampling is usually limited by budget and time constraints (Horton and Bruns 2001). Approximately 50 species of EMF are known to associate with the genus *Alnus*, some of which are considered "host specific" as they have only been reported to occur with *Alnus* species (Molina 1979, 1981, Miller et al. 1992, Pritsch et al. 1997ab). In the Pacific Northwest, red alder (*Alnus rubra* Bong.) aged from 10 – 70 y, have been shown to associate with 11 to 14 EMF species (Miller et al. 1992, Kennedy and Hill 2010). The relatively small EMF community that

associates with *Alnus* (Betulaceae) is well suited to provide a natural laboratory for study of EMF community dynamics.

*Alnus rubra* forests are a potential model system to describe the effects of soil fertility and moisture on EMF communities, as *A. rubra* occupy a variety of soil moisture conditions (Baar 2002, Deal and Harrington 2006) and can colonize soils with a wide range of nitrogen levels (Harrington 1984). On lower nitrogen sites, alders form root nodules containing nitrogen-fixing *Frankia* bacteria (Molina et al. 1994). *Frankia* bacteria, a genus of Actinomycetes filamentous soil bacteria, actively convert atmospheric nitrogen ($N_2$) to ammonium ($NH_4^+$) in the nodules. *Frankia* activity increases foliar nitrogen levels, which increases soil nitrogen content (3-6 mg/kg) due to the decomposition of *A. rubra* leaves (Edmonds et al. 1986, Cole et al. 1990). With higher soil nitrogen, stimulation of nitrification occurs, which is a strong acidifying process that can displace nutrient bases such as phosphorous from the exchange complex (Cole et al. 1990). Over time, therefore, alder stands tend to have soils with low pH (4.5-4.9) and limited levels of phosphorous (< 7.0 mg/Kg) (Cole et al. 1990). A change in soil chemistry as stands age affects EMF community composition (Becerra et al. 2005a, b, Smith and Read 2008, Tedersoo et al. 2009) and causes a decrease in *Frankia* nodulation (Smolander and Sundman 1987, Martin et al. 2003, Yamanaka et al. 2003). *Frankia* nodules may indirectly impact EMF composition as alders excrete genes promoting *Frankia* nodulation of new root tips that also act as a fungicide to reduce EMF colonization (Guan et al. 2006).

No research has described EMF communities in mature (75+ years) *A. rubra* stands, nor has work focused on EMF community composition in red alder stands. Decreased nutrient availability (Compton et al. 1997) and performance (Cole et al. 1995) of regenerating *A. rubra* has been observed in current red alder stands. The result implies that red alder has a negative

effect on its own ability to persist on a site perhaps due to the negative impacts of low phosphorous availability and high levels of aluminum that are mobilized due to nitrification (Cole et al. 1990). This negative impact of *A. rubra* on subsequent regeneration could negatively impact EMF diversity and composition in older *A. rubra* stands, as pH, phosphorous and nitrogen may be variable in relation to surrounding Pacific Northwest stands (Fogel and Cromack 1977, Edmonds et al. 1986, Cole et al. 1990). The *A. rubra* EMF community composition may be explained by variations in the soil environment and interactions within the community may be based on species tolerances in relation to resource preferences.

In this study, I describe ectomycorrhizal species in a mature *A. rubra* stand and assess the temporal and spatial influences of soil moisture, C:N ratio, *Frankia* presence, available phosphorous, and soil pH on EMF community composition. I also infer the role of niche partitioning and competition exclusion on community composition. Examining EMF interactions in the context of soil chemistry gradients over a season allows a comprehensive view of EMF community composition and provides a framework for future research on EMF interspecific interactions.

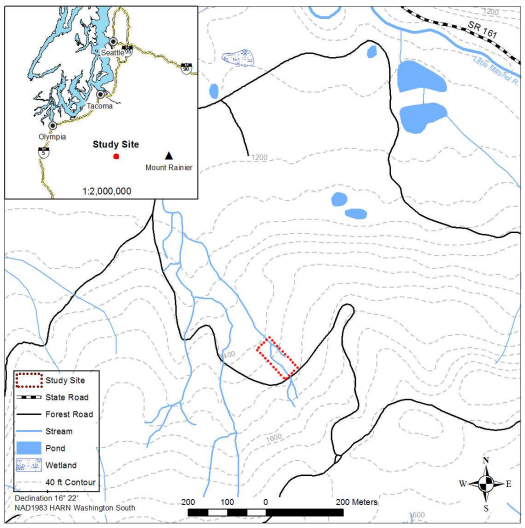

Figure 1: The study site is in the Charles L. Pack Experimental Forest, owned by the University of Washington, in western Washington.


Figure 2: Photograph of the dominant vegetation in the 0.45ha study site, located 427 – 457m above sea level on a slope between 10-20% grade.

## Methods

*Study Site*

A 0.45 ha plot was established at the Charles Lathrop Pack Experimental Forest, owned by the University of Washington, in western Washington (Figure 1). The stand originated after a fire in 1926 that killed all standing vegetation (Swanson 2006). *Alnus rubra* quickly colonized the site resulting in a stand dominated by 75 to 80-year-old *A. rubra* trees at the time of data collection. This stand has an average basal area (BA) of 15$m^2$/ha alder (28 $m^2$/ha total BA). The stand also contains a few large *Populus balsamifera* L. ssp. *trichocarpa* (Torrey & A. Gray)(BA = 11 $m^2$/ha) with *Tsuga heterophylla* (Raf.) Sarg interspersed in the understory and *Pseudotsuga menziesii* (Mirb.) Franco surrounding the stand. The dominant understory vegetation includes *Rubus spectabilis* Pursch, *Polystichum munitum* (Kaulf.) C. Presl, and *Ribes lacustre* (Pers.) Poir, with dense herbaceous ground cover of various species including *Carex obnupta* L.H. Bailey, *Galium* L., and *Ranunculus* L. (Figure 2).

The site characteristics are shaped by the general topography and climatic influences common to the Pacific Northwest region. The soils are Wilkeson series, described as fine-loamy, isotic, mesic Vitrandic Haploxeralfs (National Cooperative Soil Survey 2011). The site has varying topography due mainly to the inputs of two ephemeral springs that run through the site creating a shallow bowl-shape, and the presence of decaying large woody debris that adds overall variation. Seasonal variation in moisture and large woody debris may also influence the variation in moisture and soil chemistry variables on site. Cool, wet winters and summers with an extended drought describe the climate of the region (Franklin and Dyrness 1988). Average January and July temperatures are 3.9 and 18.3˚C, respectively. Annual precipitation averages 97.8 cm, with 88% of this falling between September and May (Swanson 2006).

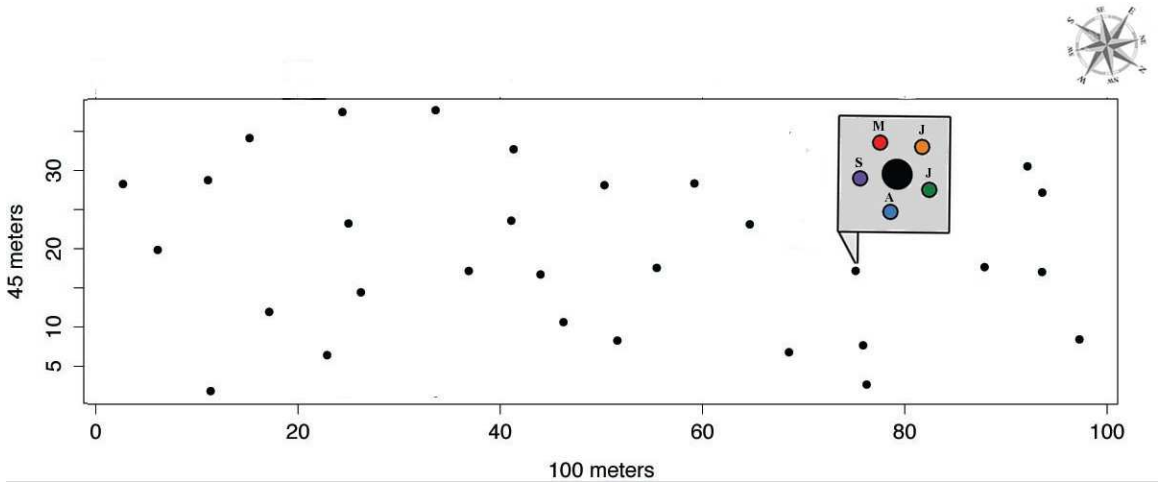

Figure 3: Map of study site. Site is 100 m (x-axis) by 45 m (y-axis). Thirty red alder (*Alnus rubra*) trees were randomly sampled within the study site. Around each tree, root cores samples were taken May (red), June (orange), July (green), August (blue) and September (purple) following a clockwise pattern around each of the 30 trees. All root cores were sampled within 2 meters of the tree.

*Field Sampling*

Thirty randomly dispersed *Alnus rubra* trees were selected (similar in age, DBH and height) and tagged within the 0.45ha plot (Figure 3). The *Alnus rubra* ectomycorrhizal community was assessed by extracting root cores within 2 meters around each tree (Figure 3). Each root core was 10 $cm^3$ (10 x 10 x 10 cm; after Lilleskov et al. 2004, Kennedy and Hill 2010) and each tree was sampled once a month from May to September 2011(5 cores per tree; 30 cores per month; 150 cores total). The location of root cores was systematically assigned prior to sampling to ensure the cores were spaced evenly around each tree to reduce spatial autocorrelation (Lilleskov et al. 2004), though close enough to each *A. rubra* tree for accurate root sampling (Figure 3). Cores were transported back to the laboratory on ice and kept cool until processing.

Soil chemistry variables were collected to relate to the potential variation of the EMF dataset. Soil moisture (volumetric water content) levels were taken in the center of each root core prior to coring (n=150) using a portable time domain reflectometer fitted with a 12cm probe (HydroSense, Campbell Scientific, Australia) and values were relativized (0 to 100) over the

sampling period. Soil pH, total carbon (%), total inorganic nitrogen (%), and available phosphorous ($PO_4$-P) were analyzed (Analytical Service Center, University of Washington, WA) from the top 10cm mineral soil immediately adjacent to the root cores taken in August 2011 (Table 1). The presence or absence of *Frankia* nodules for each root core was also recorded.

Table 1: Mean values for each soil variable for each month. Soil pH, available phosphorous, total C and N are from 30 soil cores taken in August 2011, while soil moisture and ectomycorrhizal fungal (EMF) colonization variables are from 150 root cores sampled May to September 2011. ±SE: standard error.

| | May | June | July | August | September |
|---|---|---|---|---|---|
| Soil Moisture (%) | 61±0.03 | 50±0.04 | 39±0.03 | 17±0.02 | 22±0.02 |
| EMF Colonization (%) | 64±0.05 | 63±0.04 | 75±0.03 | 68±0.05 | 87±0.02 |
| Soil pH | - | - | - | 5.15±0.1 | - |
| Total C (%) | - | - | - | 9.53±1.37 | - |
| Total N (%) | - | - | - | 0.49±0.06 | - |
| Phosphorous (mg/Kg) | - | - | - | 10.62±0.91 | - |

*Morphological analysis*

Morphotyping based upon the physical characteristics of EMF species on root tips, was used to group potential EMF species within each root core for DNA analysis. Within 30 days of sampling, each core was washed over a 1mm sieve to identify *A. rubra* roots. Using a 40x-dissecting microscope, 60 root tips were randomly selected and sorted into categories of mycorrhizal, non-mycorrhizal and necrotic (Cline 2004). EMF root tips were identified by distinguishing physical characteristics of EMF colonization, such as coloration, swelling, branching, and hyphal presence (Agerer 1991), while non-mycorrhizal root tips were distinguished by the presence of dense root hairs which are absent if roots are colonized. Desiccated or partially decayed roots were considered to be necrotic. From the 60 root tips collected from each of the root cores, up to 30 tips on average were ectomycorrhizal and sorted into morphotypes (Appendix I). The proportion of EMF colonization for each root core was recorded and was used in EMF compositional analyses.

*DNA Sequencing*

DNA analysis of one root tip per morphotype per root core was performed to determine the EMF species associated with the *Alnus rubra*. The DNA of EMF sporocarps, collected in October 2011 and oven dried at 60˚C for 12 hours, was also analyzed to refine DNA analysis of EMF root tips. Over 4,000 EMF root tips were morphotyped and 658 tips were extracted. The CTAB method with chloroform/isoamyl extraction (24:1 ratio) was performed on each root tip and sporocarp sample to extract DNA (Garnes and Bruns 1993, Cline 2004), and extracted samples were stored in TE (10 mM Tris, 1 mM EDTA, pH 8.0) at -20˚C until PCR amplification. Fungal DNA was amplified using fungal primers ITS-1f and ITS-4 (White et. al 1990) and Taq polymerase enzyme (Promega Inc.). The amplified DNA was subsequently sequenced using the Sanger method (High Throughput Genomics Center, University of Washington, WA). EMF DNA sequences were compared to the GenBank and UNITE databases using the "massBLASTer" function (unite.ut.ee) to identify taxa to the genus (≥95% similarity) or to the species level (at ≥97% match), a threshold shown to distinguish the majority of EMF species (Horton and Bruns 2001).

*DNA Analysis*

DNA analysis of EMF root tips and sporocarps was performed by first analyzing overall sequence quality, followed by comparison of taxa using phylogenetic trees. Out of the 658 root tips sequenced, only 157 samples produced sequences of adequate base-pair length (>470) and matched 95% and above to ITS sequences on GenBank and UNITE databases. Many samples had either shorter sequences from amplification (<470) or were mixed DNA samples of EMF species with either entophytes or saprophytic fungal species on the root, results characteristic of environmental analyses (White et al. 1990). Genetic relatedness of species within a genus were

analyzed by generating phylogenetic trees to examine genetic differences at the base pair level using MEGA5 software (Tamura et al. 2011). Within MEGA5, ClustalW alignment was used for each genus. Nearest neighbor trees with 500 bootstrap permutations and the maximum composite likelihood method were generated, treating gaps as pairwise deletions to determine the most parsimonious tree. An EMF taxa database was then organized using phylogenetic tree results and morphotypes (Appendix I).

*Visualization and Statistical Analyses*

Data analyses and visualization techniques were performed using R statistical software (R Development Core Team, 2011, version 2.14.1), in the VEGAN package (Oksanen et al. 2011), unless otherwise specified. For most compositional analyses, EMF species abundance data were transformed to frequency values and rare species occurring in <5% of root cores were removed resulting in a matrix of 150 root cores by 13 species for statistical analysis. The Bray-Curtis (BC) distance measure was used to examine differences among cores as it permits the inclusion of zeros (Bray and Curtis 1957, McCune and Grace 2002) for analyses requiring dissimilarity matrices.

To verify that EMF species diversity and abundance were captured by collecting 30 root cores each month (N=150), species richness curves and Simpson and Shannon diversity indices were produced. Species richness curves (specaccum in R 2.14.1) were generated using the random method and 100 permutations to equally weigh the presence of rare species to common species and to ensure the sample size for each month ($n$=30) captured EMF diversity on site using the full dataset with 22 species (Appendix IV). Simpson and Shannon diversity indices were generated for each sample to examine if there were differences in species diversity across months sampled.

The structure of the *A. rubra* EMF community was determined by examining spatial patterns and overall composition of EMF species within the site. Spatial patterns were determined by comparing the root core location for each month (n=30) and for the sampling period (N=150) to the presence of EMF species on site using Mantel tests applying the Pearson method and 10,000 permutations. The organization of EMF species within the community was examined by first grouping EMF species in the 150 root cores using cluster analysis and then determining representative species for each group using an Indicator Species Analysis. A hierarchical polythetic agglomerative cluster analysis was generated using Ward's minimum variance method (hclust in R.14.1) (Ward 1963). A plot of fusion distances against number of clusters was used to choose the preferred number of groups based on evidence of an elbow (change in amount of information explained as a function of number of clusters). Indicator Species Analysis (indval in R.14.1) with 1,000 random permutations determined Indicator Values (Indval) for each EMF species for the two groups formed in cluster analysis, which are the product of the relative frequency and relative average abundance of each species in each cluster (Dufrene and Legendre 1997, Roberts 2012). Significant indicator species distinguished clustered root cores from one another, revealing how species may be influencing EMF community composition.

The influence of soil conditions on EMF community composition was tested using: 1) Permutational multivariate analysis of variance (PERMANOVA) and non-metric multidimensional scaling (NMDS) ordinations for analyzing the overall EMF community response, and 2) Threshold Indicator Taxa ANalyses (TITAN) to examine species-level variation to the soil environment. PERMANOVA tests using 10,000 permutations, compared soil variables, EMF colonization (%) and month sampled to the EMF frequency dataset (adonis in

R.14.1; Anderson 2001). NMDS ordination of the root cores visualized fungal composition in two dimensions (metaMDS in R.14.1). Groups identified from cluster analysis were overlaid on the NMDS ordination (ordihull in R.14.1). Soil variables were visually displayed as vectors on the ordination space, to detect linear relationships between soil variables and the NMDS ordination axes (ordisurf in R.14.1).

Individual EMF species responses to the environmental gradients were tested using TITAN (Baker and King 2010). TITAN identifies the optimal value of an environmental variable distinguishing two groups with maximal difference in Indicator Species Analysis for each EMF species (Baker and King 2010). The Indicator Value (IndVal) scores for the low or high groups reflect whether a taxon shows greater association with either side of the gradient. TITAN analyses were performed to examine the EMF community composition at the species level in relation to soil moisture (N=150; sampled monthly) and other soil chemistry variables (soil pH, available phosphorous, total nitrogen) (N=30; sampled only in August) using the mvpart package (De'Ath 2002) and code provided (see Appendix S3, Baker and King 2010). Confidence intervals (90%) for each IndVal were generated using 500 permutations of the data. The EMF abundance dataset was log10(x+1) transformed prior to analysis to reduce the influence of highly variable taxa for the indicator species analyses (Baker and King 2010). Rare species (in <5 root cores) were removed from analysis and Bray-Curtis distance matrices were used for the TITAN analyses. Graphs of the TITAN results were constrained by cutoff values of purity (80%), which is how representative the indicator value for each species was of either the high or low group over 500 permutations. Significance levels of $\alpha$= 0.05 for the soil moisture gradient and $\alpha$= 0.10 for the other soil variables were used (Appendix III, IV). Reliability of each IndVal, which is the proportion of bootstrap replicates whose max IndVal was significant at $p \leq 0.05$ and $p \leq 0.01$,

was set (rel 0.05 ≥75% and rel 0.01 ≥50% respectively) for soil moisture and (rel 0.05 ≥ 45% and rel 0.01 ≥10%) for the other soil variables. Visualization of frequency of species occurrence in relation to the soil gradients was plotted. Species frequency of occurrence relative to root cores was overlaid on the NMDS ordination to visualize potential niche partitioning and competitive interactions between EMF species from TITAN analyses.

Table 2: *Alnus rubra* ectomycorrhizal (EMF) DNA sequences. EMF species labeled "*sensu* KH 2010" matched closest to species in Kennedy and Hill (2010). DNA sequences over 450 base pairs were matched to GenBank and UNITE databases results using the "massBLASTer" feature on the UNITE webpage (unite.ut.ee).

| | GenBank/UNITE Match | GenBank UNITE ID | # bases | % match |
|---|---|---|---|---|
| *Clavulina* sp 1 | Uncultured *Clavulina* | JX198537 | 665/669 | 99 |
| *Clavulina* sp 2 | Uncultured *Clavulina* | JX198537 | 609/617 | 99 |
| *Cortinarius* cf. *alboviolaceus* | *Cortinarius alboviolaceus* | GQ159793 | 471/471 | 100 |
| *Cortinarius* sp 2 (*sensu* KH 2010) | Uncultured *Cortinarius* | GQ398244 | 561/568 | 98 |
| *Entoloma* cf. *alpicola* | *Entoloma* cf. *alpicola* | HQ445607 | 215/217 | 99 |
| *Inocybe rimosa* var. *rimosa* | *Inocybe* rimosa var. rimosa | HQ604624 | 689/695 | 99 |
| *Inocybe* sp 1 | *Inocybe fuscidula* var. *fuscidula* | HQ604301 | 605/698 | 96 |
| *Lactarius* cf. *obscuratus* (*sensu KH 2010)* | *Lactarius* cf. *obscuratus* | GQ398246 | 710/713 | 98 |
| *Lactarius* cf. *brunneohepaticus* | *Lactarius brunneohepaticus* | HQ714773 | 642/642 | 99 |
| *Naucoria escharoides* | *Naucoria escharoides* | GQ398240, AY900081 | 671/671 | 100 |
| *Naucoria* sp 2 | *Alnicola* cf. *umbrina* *Alnicola inculta* | JN943991, AY900066 | 649/662 | 98 |
| *Naucoria* sp 3 | *Alnicola alnetorum* | JN943951, AY277276 | 671/671 | 100 |
| *Tarzetta* sp | *Tarzetta* sp | AJ969614 | 554/555 | 100 |
| *Tomentella* sp 1 (*sensu* KH 2010) | Uncultured *Tomentella* | GQ398252 | 645/657 | 98 |
| *Tomentella* sp 2 | Uncultured EM fungus | FM993178 | 596/601 | 99 |
| *Tomentella sublilacina* (*Tomentella* sp 2 *sensu* KH 2010) | Uncultured *Tomentella* | GQ398248 | 644/646 | 99 |
| *Tomentella* sp 3 (*sensu* KH 2010) | Uncultured *Tomentella* | GQ398249 | 606/606 | 100 |
| *Tomentella* sp 4 (*sensu* KH 2010) | Uncultured *Tomentella* | GQ398250 | 642/643 | 99 |
| *Tomentella* sp 5 (*sensu* KH 2010) | Uncultured *Tomentella* | GQ398251 | 610/614 | 97 |
| *Tomentella* sp 6 | Uncultured *Tomentella* | FR852201 | 597/625 | 95 |
| *Tomentella* sp 7 | Uncultured EM fungus | FM993226 | 627/635 | 98 |
| *Tuber* sp | Uncultured EM (*Tuber*) | AY634172 | 592/598 | 96 |

## Results

DNA analysis of EMF root tips and sporocarps revealed 22 ectomycorrhizal fungal species associated with *Alnus rubra* (Table 2, Appendix I, II). Eight species matched closely to previously sequenced *A. rubra* EMF species in Kennedy and Hill (2010) and their names are

consistent with that study ("*sensu* KH 2010") (Table 2). Fourteen new species to associate with *A. rubra* were identified, most of which are from genera (*Clavulina*, *Entoloma*, *Tarzetta*, and *Tuber*) known to associate with other species of Betulaceae. Phylogenetic analysis and EMF sporocarp collections determined 3 *Naucoria* species, although the placement of one of these, "*Naucoria* sp 3" was uncertain due to the relatively long horizontal distance on the phylogenetic tree (Appendix I, II). *Cortinarius* cf. *alboviolaceous* has not been described on *A. rubra* before (Appendix I, II; Table 3).

Table 3: Frequency of occurrence (%) of the 22 *Alnus rubra* associated ectomycorrhizal fungi (EMF) over the sampling period (May to September 2011).

| | Frequency of Occurrence (%) |
|---|---|
| *Clavulina* sp 1 | 2.7 |
| *Clavulina* sp 2 | 1.3 |
| *Cortinarius* cf. *alboviolaceous* | 56 |
| *Cortinarius* sp 2 (*sensu* KH 2010) | 4.7 |
| *Entoloma* cf. *alpicola* | 0.7 |
| *Inocybe rimosa* var. *rimosa* | 4 |
| *Inocybe* sp 1 | 2.7 |
| *Lactarius* cf. *obscuratus* | 21.3 |
| *Lactarius* cf. *brunneohepaticus* | 12 |
| *Naucoria escharoides* | 43.3 |
| *Naucoria* sp 2 | 22 |
| *Naucoria* sp 3 | 22 |
| *Tarzetta* sp | 2 |
| *Tomentella* sp 1 (*sensu* KH 2010) | 17.3 |
| *Tomentella* sp 2 | 3.3 |
| *Tomentella sublilacina* | 27.3 |
| *Tomentella* sp 3 (*sensu* KH 2010) | 16.7 |
| *Tomentella* sp 4 (*sensu* KH 2010) | 16.7 |
| *Tomentella* sp 5 (*sensu* KH 2010) | 20 |
| *Tomentella* sp 6 | 2 |
| *Tomentella* sp 7 | 8 |
| *Tuber* sp | 6 |

EMF species varied in their frequency of occurrence (Table 3). Eleven species were rare (*Clavulina* sp 1, 2; *Cortinarius* sp 2 (s*ensu* KH 2010); *Entoloma* cf. *alpicola*; *Inocybe rimosa* var. *rimosa*, *Inocybe* sp 1; *Tarzetta* sp; *Tomentella* sp 2, 6, 7; and *Tuber* sp). Eight species were found in <25% of cores (*Lactarius* cf. *obscuratus*; *Lactarius* cf. *brunneohepaticus*; *Naucoria* sp 2, 3; *Tomentella* sp 1,3,4,5 (*sensu* KH 2010)). Three species were dominant throughout the

sampling period: *Cortinarius* cf. *alboviolaceus* was found in 56% of samples; *Naucoria escharoides* was found in 43% of samples; and *Tomentella sublilacina* was recorded in 27% of root cores (Table 3).

Table 4: Average species richness and diversity of *Alnus rubra* associated ectomycorrhizal fungi (EMF) over the sampling period (May to September 2011).

| | May | June | July | August | September |
|---|---|---|---|---|---|
| Species Richness | 2.77 | 2.9 | 2.9 | 3.37 | 3.67 |
| Shannon *H* | 0.87 | 0.99 | 0.98 | 1.11 | 1.25 |
| Simpson λ | 0.59 | 0.63 | 0.59 | 0.64 | 0.70 |

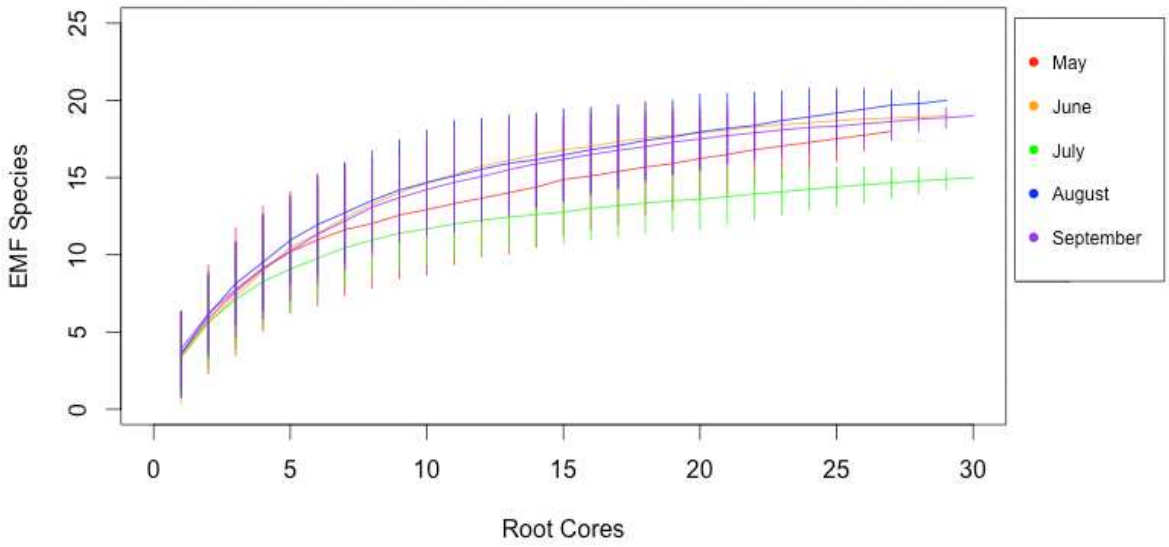


Figure 4: The mean species richness curves of ectomycorrhizal (EMF) species per number of root cores for each month with confidence intervals (vertical bars) visualizing variation of EMF species captured. Most of the EMF diversity in this study site was captured within the 30 root cores sampled each month.

EMF species richness and diversity remained stable throughout the sampling period (Figure 4, Table 4). Species richness curves begin to plateau around 10 – 20 root cores, suggesting that at 30 cores, the EMF species present were captured with the sample size taken in this study (Figure 4). Shannon and Simpson diversity indices remained relatively stable across the sampling period with a slight increase in diversity in August and September (Table 4). This trend is a result of an increase in EMF colonization from 64% tips colonized in May to 87% in September (Table 1).

Soil chemistry was spatially and temporally variable (Table 1). Across the 30 sample trees, soil pH ranged from 3.5 to 6.0, phosphorous from 5.0 to 28.0 mg/kg, total carbon had a large range of 3.78 to 36.11%, and total nitrogen ranged from 0.212 to 0.946%. The soil moisture decreased over the course of the study from a mean value of 61% in May to 22% in September (Table 1), corresponding to seasonal precipitation in the Pacific Northwest.

The spatial pattern of the EMF community was randomly distributed throughout the site for all months individually analyzed ($r < 0.0777$, $p > 0.07$) and for the entire sampling period ($r = 0.0148$, $p = 0.21$) (Table 5). EMF presence in root cores in May had a slight clustered pattern, though not significant ($r = 0.078$, $p = 0.07$). A lack of spatial autocorrelation of root core samples allowed additional analyses without consideration of the spatial pattern.

Table 5: Mantel statistic (*r*) between the ectomycorrhizal (EMF) frequency dataset and locations of the root cores within the 0.45ha plot. No spatial pattern of EMF species was found monthly (n=30) or for the entire sampling period (N=150).

| | Mantel's *r* | *p* value |
|---|---|---|
| May | 0.0777 | 0.0788 |
| June | 0.0026 | 0.4688 |
| July | 0.0645 | 0.1543 |
| August | 0.0542 | 0.1427 |
| September | 0.0392 | 0.2676 |
| Total | 0.0148 | 0.2153 |

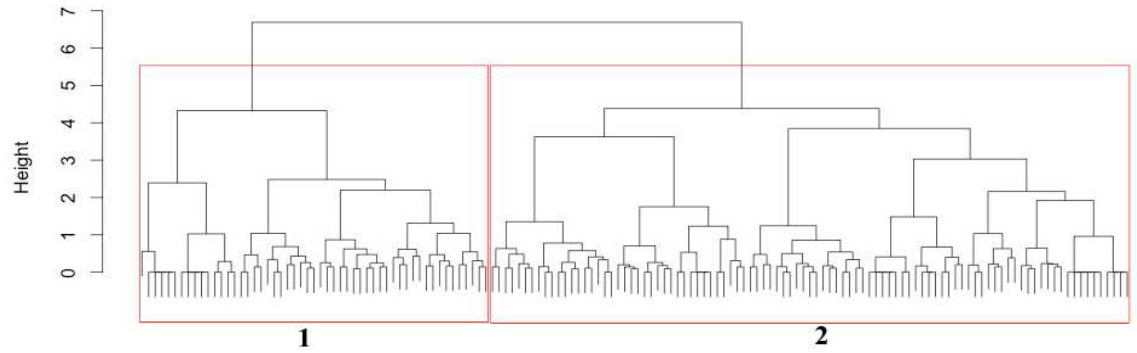


Figure 5: Cluster analysis of root cores of the EMF frequency data excluding rare species (in <5% of root cores) is depicted. Two groups were chosen which are outlined in red. Cluster 1 is on the left, with cluster 2 on the right.

The composition of the EMF community was in part explained by the presence of dominant EMF species (Figure 5, 7; Table 6). A scree plot revealed two main groups of root cores in the cluster analysis (Figure 5). Indicator Species Analysis clustered two groups. *Cortinarius* cf. *alboviolaceus* was a significant indicator species for cluster 1 ($p = 0.001$), while seven different species, including *Naucoria escharoides*, were indicators of cluster 2 ($p < 0.03$) (Table 6). Cluster results were mirrored when *C. alboviolaceous* or *Naucoria escharoides* occurrence in root cores were labeled and overlaid in the NMDS ordination (Figure 7). *C. alboviolaceous* and *N. escharoides* separate in the ordination space, as seen in the cluster analysis (Figure 6).

Table 6: Results from the Indicator Species Analyses, where indicator values were calculated to see if ectomycorrhizal (EMF) species significantly distinguish the two groups identified in the cluster analysis.

| | Cluster | Indicator Value | *p* value |
|---|---|---|---|
| *Cortinarius* cf. *alboviolaceous* | 1 | 61% | 0.001 |
| *Lactarius* cf. *obscuratus* | 2 | 54% | 0.001 |
| *Naucoria escharoides* | 2 | 35% | 0.034 |
| *Tomentella* sp 1 (*sensu* KH 2010) | 2 | 30% | 0.001 |
| *Tomentella sublilacina* | 2 | 26% | 0.026 |
| *Naucoria* sp 3 | 2 | 23% | 0.024 |
| *Tomentella* sp 4 (*sensu* KH 2010) | 2 | 22% | 0.011 |
| *Lactarius* cf. *brunneohepaticus* | 2 | 17% | 0.023 |

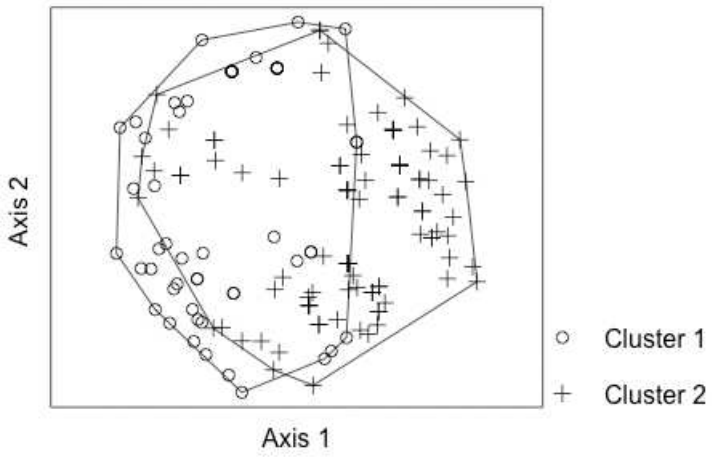


Figure 6: Non-metric MultiDimensional Scaling (NMDS) ordination visualizing ectomycorrhizal fungal (EMF) groupings of root cores (N=150) in two dimensions is displayed with groups from cluster analysis overlaid, with cluster 1 (○) and cluster 2 (+).

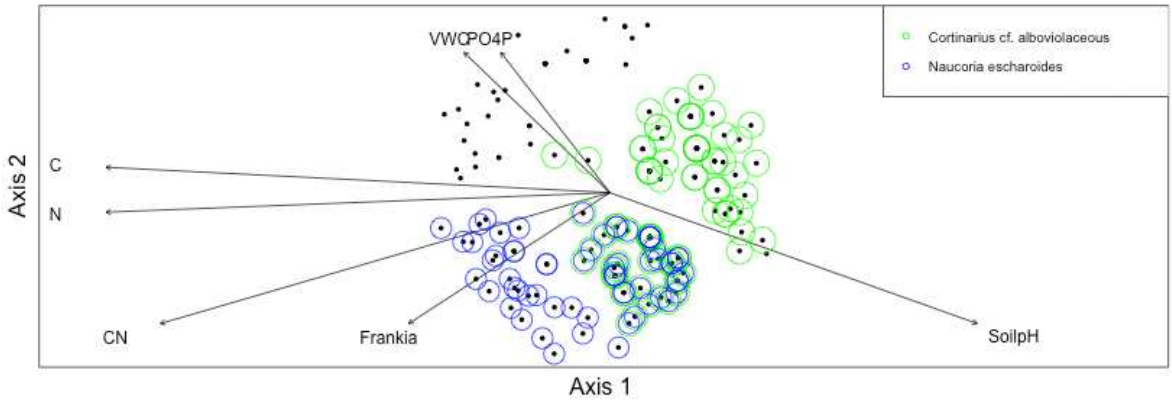


Figure 7: Non-metric MultiDimensional Scaling (NMDS) for visualizing ectomycorrhizal fungal (EMF) groupings of root cores (N=150) in two dimensions. *Cortinarius* cf. *alboviolaceous* (○) and *Naucoria escharoides* (Δ), species with high frequency of occurrence (Table 3a), are shown. The relationship between the NMDS ordination and soil variables (C= % carbon, N=% total inorganic N, CN= C:N ratio, *Frankia* presence, soil pH, available phosphorous ($PO_4$-P), VWC= soil moisture) are shown as vectors, where the length and direction of the arrow shows the linear relationship of that variable to the EMF dataset. Overall grouping of root cores in the ordination space is described by the presence of *C. alboviolaceous* and *N. escharoides.*

The influence of month sampled, *Frankia* nodule presence, and soil chemistry explained a portion of the variation of the EMF community overall, with no one factor explaining >10% of the variation ($p$ <0.05) (Table 7). Month significantly explained 10% of variation in EMF species ($p$ = 0.0001) (Table 7) representing the composition change of the EMF community over time (Figure 9). An increase in EMF colonization over the months sampled (Table 1) also explained 6% of the variation of the dataset ($p$ = 0.0001). The presence of *Frankia* nodules explained 5%

Table 7: PERMANOVA results comparing soil variables (*Frankia* presence, soil pH, C:N ratio, available P ($PO_4$-P), soil moisture, total C and total N), tree sampled (n=30), month (May – September 2011) , and ectomycorrhizal fungal (EMF) colonization separately to the EMF frequency dataset. All soil variables significantly explained variation in EMF composition at $p$ = 0.05. Degrees of freedom = df.

| | df | SS | MS | *F*.Model | $R^2$ | *p* value |
|---|---|---|---|---|---|---|
| Tree ID | 29 | 4.631 | 0.160 | 1.077 | 0.206 | 0.305 |
| Month | 4 | 2.257 | 0.564 | 4.057 | 0.101 | 0.000 |
| EMF Col (%) | 1 | 1.314 | 1.313 | 9.205 | 0.059 | 0.000 |
| *Frankia* | 1 | 1.086 | 1.086 | 7.531 | 0.048 | 0.000 |
| Soil pH | 1 | 0.554 | 0.554 | 3.747 | 0.025 | 0.003 |
| C:N ratio | 1 | 0.462 | 0.462 | 3.111 | 0.021 | 0.009 |
| $PO_4$-P (mg/Kg) | 1 | 0.436 | 0.436 | 2.956 | 0.019 | 0.013 |
| Soil Moisture (%) | 1 | 0.403 | 0.403 | 2.734 | 0.018 | 0.025 |
| C (%) | 1 | 0.396 | 0.396 | 2.660 | 0.018 | 0.025 |
| N (%) | 1 | 0.380 | 0.380 | 2.548 | 0.017 | 0.031 |
| Residuals | 108 | 10.512 | 0.130 | | 0.468 | |
| Total | 149 | 22.431 | | | 1.000 | |

of the variation ($p$ = 0.0001) (Table 7). Grouping of root cores in the ordination space revealed that *Frankia* nodule presence along with decrease in nitrogen availability (an increase in the C:N ratio and total C), separated root cores into two diagonal groups (Figure 8). There was also temporal variation of *Frankia* presence; more root cores contained *Frankia* nodules in May and September (Figure 9a) than in June and July (Figure 9b). In addition to the influence of nitrogen on EMF community composition, all other soil chemistry variables (soil moisture, pH, available phosphorous) were significantly correlated with EMF community composition ($p$<0.05) (Table 7, Figure 8).

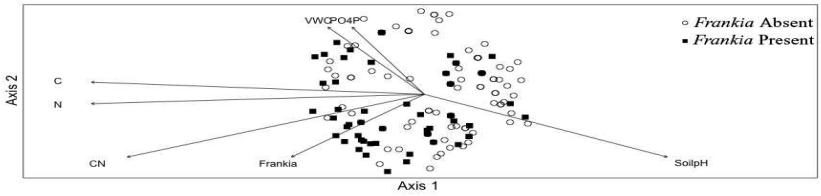


Figure 8: Non-metric MultiDimensional Scaling (NMDS) for visualizing ectomycorrhizal fungal (EMF) groupings of root cores in two dimensions. For each root core (N=150), *Frankia* nodules are shown based on whether they were present (■) or absent (○) in each core. Cores with *Frankia* present and higher C:N ratios separate from cores with more available nitrogen diagonally from left to right.

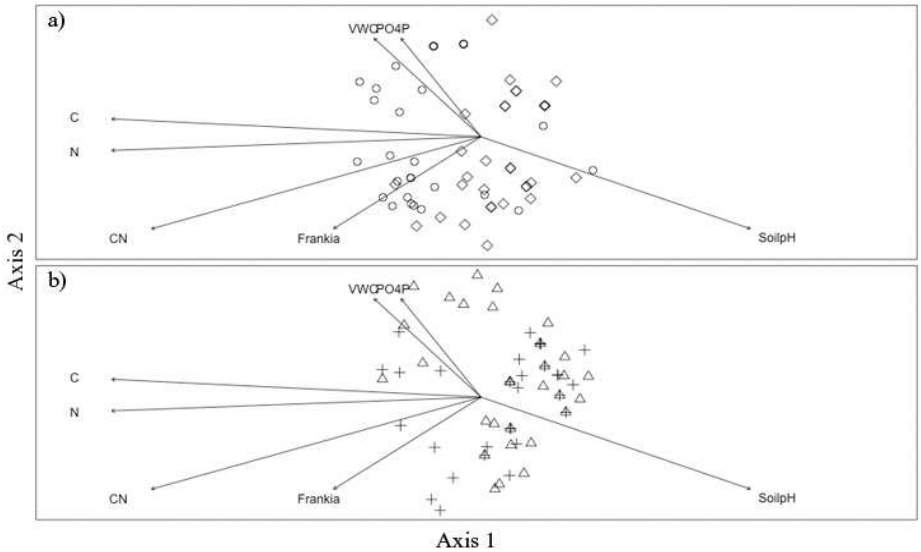


Figure 9: Non-metric MultiDimensional Scaling (NMDS) for visualizing ectomycorrhizal fungal (EMF) groupings of root cores in two dimensions displayed by the month sampled: a) May (○) and September (◊) and b) June (Δ) and July (+). More root cores have *Frankia* present in May and September (a) compared to June and July (b), showing the temporal variation of *Frankia* nodules within the site.

Species-level variation in EMF abundance and tolerance were found in relation to soil moisture, soil pH, available phosphorous, and total nitrogen (Figure 10a-d, Table 8, see Appendix III for complete analyses). *Tomentella* sp 3 and 5 (*sensu* KH 2010) and *Lactarius* cf. *obscuratus* had significant Indicator Values for low soil moisture ranges while *Lactarius* cf. *brunneohepaticus*, *Tuber* sp, *Tomentella* sp 1 (sensu KH 2010), and *Tomentella sublilacina* indicative of root cores with a higher soil moisture (Figure 10a, Table 8). *Naucoria escharoides, Tomentella* sp 5 (*sensu* KH 2010) and *Naucoria* sp 3 were prevalent at lower pH levels (5.19, 4.84 and 4.64, respectively), and *T. sublilacina*, *Tomentella* sp 1 and 3 (*sensu* KH 2010) at higher pH ranges (Figure 10b, Table 8). *Naucoria* sp 2, *Cortinarius* cf. *alboviolaceous*, and *Tomentella* sp 1 (*sensu* KH 2010) are associated with low phosphorous levels (below 13 mg/Kg), while *Naucoria* sp 3, *Tomentella* sp 5 (*sensu* KH 2010), and *L. obscuratus* were associated with higher phosphorous levels (Figure 10c, Table 8). Total nitrogen had only two species with lower values (*C. alboviolaceous* and *Naucoria* sp 2) with *Naucoria* sp 3, *L. obscuratus*, *T. sublilacina* and *N. escharoides* occupying higher nitrogen ranges (Figure 10d).

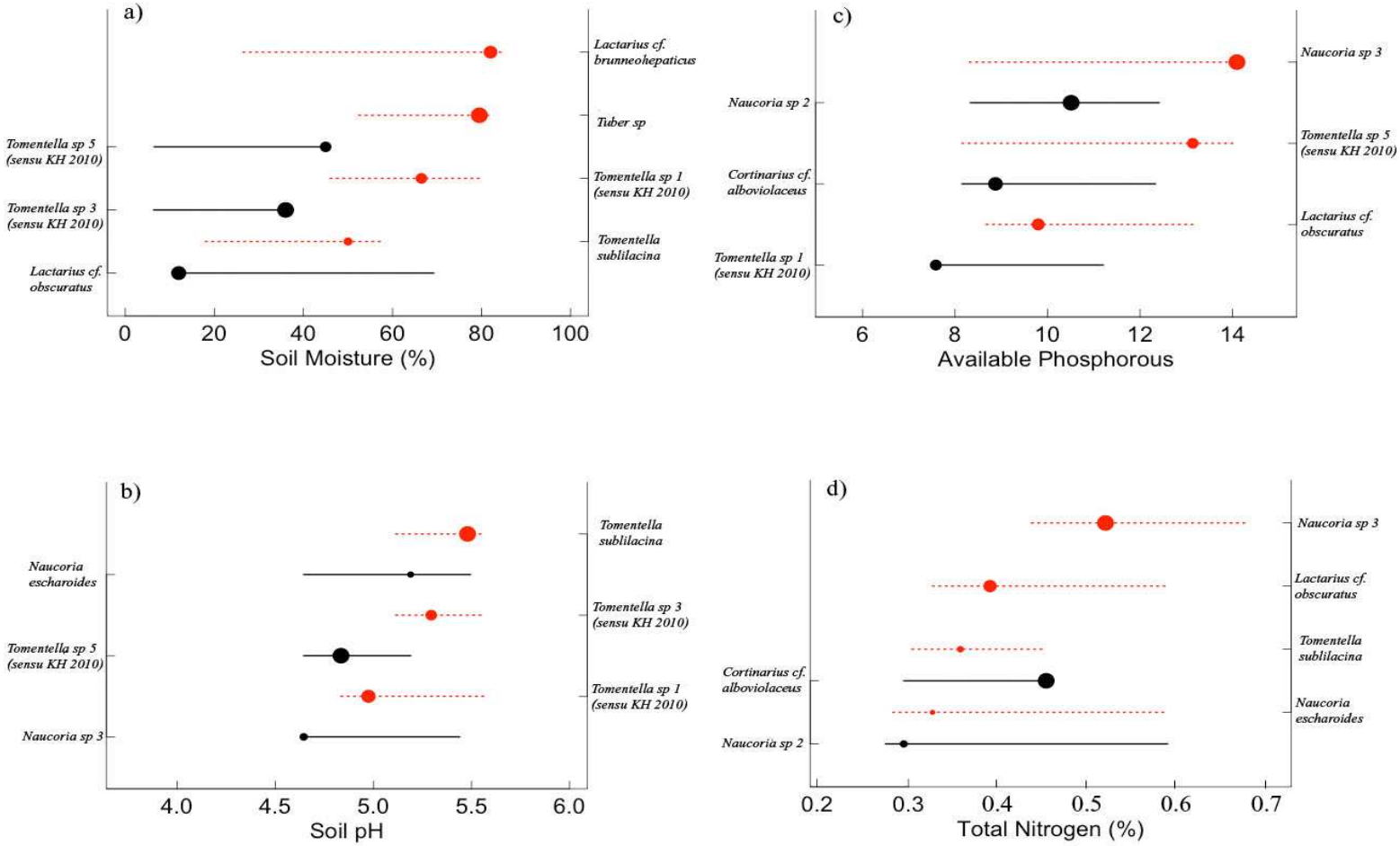


Figure 10: TITAN analyses for a) soil moisture, b) soil pH, c) available phosphorous, and d) total nitrogen. A midpoint was identified across each soil chemistry gradient to form two groups: low (black) and high (red) to calculate Indicator Values for each group using Indicator Species Analysis. The relative magnitude of IndVal scores for the low or high groups is reflected in the dot size along each environmental gradient. Confidence intervals (5-95%) were generated (horizontal lines) over 500 permutations. Only species with purity $\geq$ 80%, which is how representative the Indicator Value for each species was over 500 permutations, at significance levels of $\alpha$= 0.05 for the soil moisture gradient and $\alpha$= 0.10 for the other soil variables are shown.

Table 8: Threshold Indicator Taxa ANalysis (TITAN) results from Figure 10. The table lists: z-scores, Indicator Value (IndVal) scores for the low (-) or high (+) groups, which reflect an association for each taxon with either side of the gradient, and associated optimal value (Obs), 90% confidence intervals, *p* values, purity of the taxa for each z score classification and reliability *p* values ≤ 0.05 or 0.01 over 500 bootstrap permutations.

| | | | | Change Point | | | | | Reliability | |
|---|---|---|---|---|---|---|---|---|---|---|
| Species | +/- | IndVal | z | Obs | 5% | 95% | *p* value | Purity | ≤0.05 | ≤0.01 |
| **Soil Moisture** | | | | | | | | | | |
| *Lactarius* cf. *obscuratus* | z- | 31.55 | 3.71 | 12.0 | 10.0 | 84.5 | 0.008 | 0.83 | 0.77 | 0.54 |
| *Lactarius* cf. *brunneohepaticus* | z+ | 41.76 | 5.46 | 82.0 | 23.0 | 84.5 | 0.004 | 1.00 | 1.00 | 0.98 |
| *Tomentella* sp 1 *(sensu* KH 2010) | z+ | 31.7 | 4.49 | 66.5 | 31.0 | 84.5 | 0.004 | 0.98 | 0.94 | 0.74 |
| *Tomentella sublilacina* | z+ | 26.87 | 3.27 | 50.0 | 15.0 | 64.6 | 0.012 | 0.96 | 0.88 | 0.59 |
| *Tomentella* sp 3 (*sensu* KH 2010) | z- | 20.67 | 4.2 | 36.0 | 5.0 | 39.5 | 0.008 | 0.96 | 0.95 | 0.83 |
| *Tomentella* sp 5 (*sensu* KH 2010) | z- | 20.01 | 2.72 | 45.0 | 5.0 | 52.0 | 0.016 | 0.94 | 0.86 | 0.60 |
| *Tuber* sp | z+ | 33.25 | 6.86 | 79.5 | 20.5 | 84.5 | 0.008 | 0.92 | 0.87 | 0.69 |
| **Soil pH** | | | | | | | | | | |
| *Naucoria escharoides* | z- | 49.64 | 2.9 | 5.19 | 4.65 | 5.54 | 0.020 | 0.94 | 0.74 | 0.30 |
| *Naucoria* sp 3 | z- | 57.69 | 3.72 | 4.65 | 4.65 | 5.46 | 0.012 | 0.94 | 0.78 | 0.52 |
| *Tomentella* sp 1 *(sensu* KH 2010) | z+ | 45 | 2.13 | 4.98 | 4.84 | 5.57 | 0.032 | 0.90 | 0.63 | 0.18 |
| *Tomentella sublilacina* | z+ | 43.26 | 2.58 | 5.48 | 4.98 | 5.57 | 0.020 | 0.88 | 0.59 | 0.30 |
| *Tomentella* sp 3 (*sensu* KH 2010) | z+ | 38.41 | 1.68 | 5.30 | 4.98 | 5.57 | 0.088 | 0.86 | 0.57 | 0.29 |
| *Tomentella* sp 5 (*sensu* KH 2010) | z- | 83.69 | 8.37 | 4.84 | 4.65 | 5.25 | 0.004 | 1.00 | 1.00 | 0.97 |
| **Available Phosphorous** | | | | | | | | | | |
| *Cortinarius* cf. *alboviolaceous* | z- | 49.88 | 2.62 | 8.88 | 7.59 | 13.14 | 0.028 | 0.86 | 0.68 | 0.41 |
| *Lactarius* cf. *obscuratus* | z+ | 42.29 | 2.21 | 9.80 | 8.15 | 13.19 | 0.052 | 0.89 | 0.67 | 0.36 |
| *Naucoria* sp 2 | z- | 53.06 | 3.05 | 10.51 | 8.00 | 13.19 | 0.016 | 0.91 | 0.81 | 0.49 |
| *Naucoria* sp 3 | z+ | 50.72 | 2.98 | 14.10 | 8.00 | 14.10 | 0.028 | 0.84 | 0.58 | 0.33 |
| *Tomentella* sp 1 *(sensu* KH 2010) | z- | 47.46 | 2.05 | 7.59 | 7.59 | 12.34 | 0.036 | 0.86 | 0.68 | 0.35 |
| *Tomentella* sp 5 (*sensu* KH 2010) | z+ | 42.37 | 1.96 | 13.14 | 8.15 | 14.10 | 0.028 | 0.92 | 0.63 | 0.29 |
| **Total Nitrogen** | | | | | | | | | | |
| *Cortinarius* cf. *alboviolaceous* | z- | 63.16 | 3.7 | 0.45 | 0.28 | 0.47 | 0.008 | 1.00 | 0.98 | 0.86 |
| *Lactarius* cf. *obscuratus* | z+ | 57.41 | 4.17 | 0.39 | 0.31 | 0.67 | 0.004 | 1.00 | 0.97 | 0.81 |
| *Naucoria escharoides* | z+ | 38.87 | 1.42 | 0.33 | 0.28 | 0.67 | 0.100 | 0.80 | 0.46 | 0.19 |
| *Naucoria* sp 2 | z- | 42.71 | 1.56 | 0.30 | 0.28 | 0.67 | 0.080 | 0.89 | 0.61 | 0.28 |
| *Naucoria* sp 3 | z+ | 67.36 | 5.42 | 0.52 | 0.41 | 0.67 | 0.004 | 0.99 | 0.96 | 0.83 |
| *Tomentella sublilacina* | z+ | 34.36 | 1.91 | 0.36 | 0.28 | 0.52 | 0.048 | 0.78 | 0.47 | 0.18 |

## Discussion

### *Ectomycorrhizal fungus diversity*

Twenty-two species of ectomycorrhizal fungi were found to associate with *Alnus rubra*, 14 more than previously recorded (Miller et al. 1992; Kennedy and Hill 2010) (Table 2, Appendix I). The most common species, *Cortinarius alboviolaceus*, was documented to associate with *A. rubra* for the first time in this study (Table 2, Appendix I, II), although other *Cortinarius* species have been previously reported (Kennedy and Hill, 2010). Three *Naucoria*

species including: *Naucoria escharoides*, one new *Naucoria* species (*Naucoria* sp 2), and a third genetically distinct *Naucoria* species (*Naucoria* sp 3) that may represent a new genus of fungi (Pierre-Arthur Moreau, personal comm.) (Appendix I, II) were described. *Naucoria* species were common (Table 3a) and diverse, consistent with the idea that *Naucoria* is a significant component of older *A. rubra* forests (Kennedy and Hill 2010). No *Alpova* or *Xerocomus* species as described in Kennedy and Hill (2010) were found in this study. However, two *Clavulina* species new to *A. rubra,* were found, supporting the association of this genus with *Alnus*; the genus had been previously described on Mexican *Alnus* species (Kennedy et al. 2011). Alder *Clavulina* species appear to represent a novel group within *Clavulina*, potentially representing a new species, yet to be described (Appendix I, II). The majority of new species recorded were rare, but were an important contribution to the overall diversity (Table 3, 4). Higher diversity in this study compared to other studies in alder forests, could be attributed to the relatively large sample size, site characteristics including stand age and topographic complexity of the site.

The 150 root cores (30 per sampling period) compared to 20 per site taken in previous studies (Kennedy and Hill 2010) captured more rare species, and may be the biggest driver in the higher diversity of EMF species found in this study (Horton and Bruns 2001). Species richness curves illustrate that increasing the number of root core samples allowed more rare species to be captured with a higher certainty (Figure 4). This study also sampled over the course of a Pacific Northwest growing season, capturing "ephemeral" EMF species (Izzo et al. 2005) that may have been missed in other *A. rubra* studies that had only one sampling event (e.g. Miller et al. 1992, Kennedy and Hill 2010).

The site characteristics, including tree age and lack of recent disturbance, may also account for high EMF diversity found, a trend seen in other forest types (Smith et al. 2002, Smith

and Read 2008). The *A. rubra* host trees are 75-80 years old, near the species' age limit of about 100 years old (Franklin and Dyrness 1988). Some genera of EMF fungi, such as *Naucoria*, are more prevalent in older *Alnus* stands; a trend documented across age gradients in *Pseudotsuga* forests (Smith et al. 2002). The site has been free of large-scale disturbances as it is protected from prevailing winds, and this may also explain the high EMF diversity; other studies have shown dramatic decreases in diversity of the EMF community in response to disturbance (Cline et al. 2005, Wolfe et al. 2009).

The site was selected for its environmental complexity and therefore higher EMF diversity was expected. The shallow bowl-shaped topography, with two seasonal streams, places the *Alnus rubra* stand within 50 m of *Pseudotsuga menziesii* and *Tsuga heterophylla*. EMF species within the genera *Clavulina, Cortinarius, Inocybe, Lactarius, Tomentella,* and *Tuber* that occurred on root tips of *A. rubra* are known to colonize *Pseudotsuga menziesii* (Cline 2007), and *Cortinarius, Lactarius,* and *Tuber* also associate with *Tsuga heterophylla* roots (Kranabetter and Kroeger 2001). The rare genera observed in this study compared to the *A. rubra* EMF species found by Kennedy and Hill (2010) may be related to site location; additional species were mainly in the *Clavulina, Entoloma, Tarzetta, Tomentella,* and *Tuber* genera. The surrounding *P. menziesii* and *T. heterophylla* hosts near sampled *A. rubra* trees may account for increased diversity (Kranabetter and Kroeger 2001, Cline 2005).

Further research using a large sampling effort of various *A. rubra* stand ages might determine the relative importance of stand age, disturbance regime, and proximity to potential alternate EMF hosts on the high EMF diversity observed. Sampling *A. rubra* stands with different disturbance regimes would show how the type and severity of disturbance influences alder-associated EMF diversity. Systematic sampling along *Alnus rubra*/conifer ecoclines could

determine the relative importance of alternate EMF hosts on *Alnus rubra* EMF diversity. This study suggests the EMF diversity and community composition respond to differences in soil moisture and chemistry, but these results should be replicated on additional sites.

*Alnus rubra Ectomycorrhizal Community Composition*

EMF community composition was influenced by the environmental gradients and *Frankia* nodule presence and not tree-specific or spatial autocorrelation. Spatial patterns were not explicit for monthly sampling events or over the entire study (Table 5), probably due to the sampling design (i.e., systematic sampling around each tree [Figure 3]). Randomly dispersed EMF species on root tips have been reported at scales seen in this study in other forests, suggesting random spatial pattern may be representative for EMF communities (Peter et al. 2001ab, Lilleskov et al. 2004, Izzo et al. 2005). The random pattern of EMF species may also represent the response of the entire community to randomly patterned soil conditions.

The overall EMF community composition was influenced by the presence of the dominant species *Cortinarius* cf. *alboviolaceous* and *Naucoria escharoides* (Table 3). Indicator Species Analyses of the two groups from the cluster analysis revealed *C. alboviolaceous* as a significant indicator species of cluster 1, with *N. escharoides* and 6 other EMF species indicative of the second cluster (Figure 5, Table 5). The presence of these two species in root cores explained differences in EMF species composition across the sampling period. *C. alboviolaceous* and *N. escharoides* occurrence overlaid on the ordination space visualize the cluster analysis groups (Figure 7), showing relatively minimal overlap in occurrence. *C. alboviolaceous* dominates cores with higher N availability and lower occurrence of *Frankia*; in contrast *N. escharoides* is more prevalent with decreasing nitrogen availability and increasing *Frankia*. These indictor species may be associated with different soil variables, partitioning the

colonization of root tips depending on soil chemistry (i.e. niche partitioning), or through competitive exclusion of other EMF by *C. alboviolaceous*, *N. escharoides*, and *Frankia*, or a combination of these community interactions.

The temporal variation of *Frankia* nodulation and the relationship of *Frankia* to the EMF community structure are interesting findings of this study (Figure 8). The ordination space shows association between available N, C:N ratio, and presence of *Frankia* and these factors are likely correlated, although each explains a small amount of the EMF colonization (Table 7). *Frankia* nodulation significantly ($p = 0.0001$) explained EMF variation due to seasonal differences in the presence of nodules (Table 7, Figure 8, 9ab). *Frankia* infectivity in the soil has been shown to have seasonal patterns (Myrold and Huss-Danell 1994) and appears to be influenced by microsite variation in the C:N ratio, carbon and total N gradients (Figure 8). It can be inferred that variation in EMF species colonizing *A. rubra* root tips is related to nitrogen availability, supporting previous findings (Giesler et al. 1998, Nilsson et al. 2005, Toljander et al. 2006, Lindahl et al. 2007, Kranabetter et al. 2009, Kjoller et al. 2012). Cores with *Frankia* nodules present tended to have a higher C:N ratio (Figure 8). *Alnus rubra* trees induce *Frankia* nodulation in areas with low total N (Myrold and Huss-Danell 1994, Martin et al. 2003). *Frankia* nodulation also showed a seasonal pattern (Figure 7a-c), where nodulation varied across the months sampled. From the NMDS ordination, *Frankia* impacted the spatial and temporal distribution of the EMF species depending on nodule presence.

The variation of EMF composition significantly explained by the other soil variables was relatively small (Table 7). The soil chemistry variables (phosphorous, pH, N, C) explained a low amount of variation in the EMF dataset potentially because they were sampled in August (n=30). Measuring the soil chemistry adjacent to every root core (N=150) may have may have explained

a larger portion of the EMF variation. While soil moisture and soil chemistry variables explained EMF community variation (11.7%; $p<0.05$), the significant relationship was not well visualized in the ordination due to the high level of stress (0.271) (Table 7, Figure 7-9). High stress levels make interpretation of the ordination difficult, creating the potential for decreased visualization of significant relationships (Clark 1993). The amount of variation (19%) explained by the soil variables in this study is consistent with other ectomycorrhizal ecology studies (Ostonen et al. 2009). The difficulty in explaining EMF community structure using multivariate techniques such as ordination, cluster analysis, indicator species analysis, and PERMANOVA may be a reflection of a poor fit of models underlying these analyses to EMF datasets. The application of TITAN analysis and associated visualization techniques, allowed better description of species-level responses and variation in relation to the soil chemistry gradients.

*Niche Partitioning and Competitive Exclusion*

The EMF community structure in this *Alnus rubra* stand appears to be influenced by both niche partitioning and competitive exclusion. TITAN analyses revealed individual species' responses to soil moisture, pH, available phosphorous, and total nitrogen gradients (Appendix III, Table 8, Figure 10). The *Tomentella* genus has species that occupy both higher and lower ends of soil moisture, pH and phosphorous, with a low number of Indicator Values for total nitrogen (Figure 10 – 14, Table 8). The variation of abundance along each of the soil chemistry gradients among *Tomentella* species shows that they are specialized spatially and temporally. Niche partitioning of root cores is suggested by *Tomentella* sp 1 (*sensu* KH 2010) and *Tomentella* sp 5 (*sensu* KH 2010) for soil moisture, pH, and phosphorous (Figure 10-13, Table 8). The plotting of occurrence in the ordination space shows the species co-occur in only one root core (Figure 15a). *Tomentella* sp 1 (*sensu* KH 2010) occupies the root cores along the outer

edge of the ordination space, with *Tomentella* sp 5 present in the more central cores in the ordination space (Figure 15a). Indication of variation in species response to the soil environment, even within one genus such as *Tomentella*, leads to further questions of species tolerances and strategies, and challenges the assumptions that members of a genus behave similarly (Geml et al. 2010).

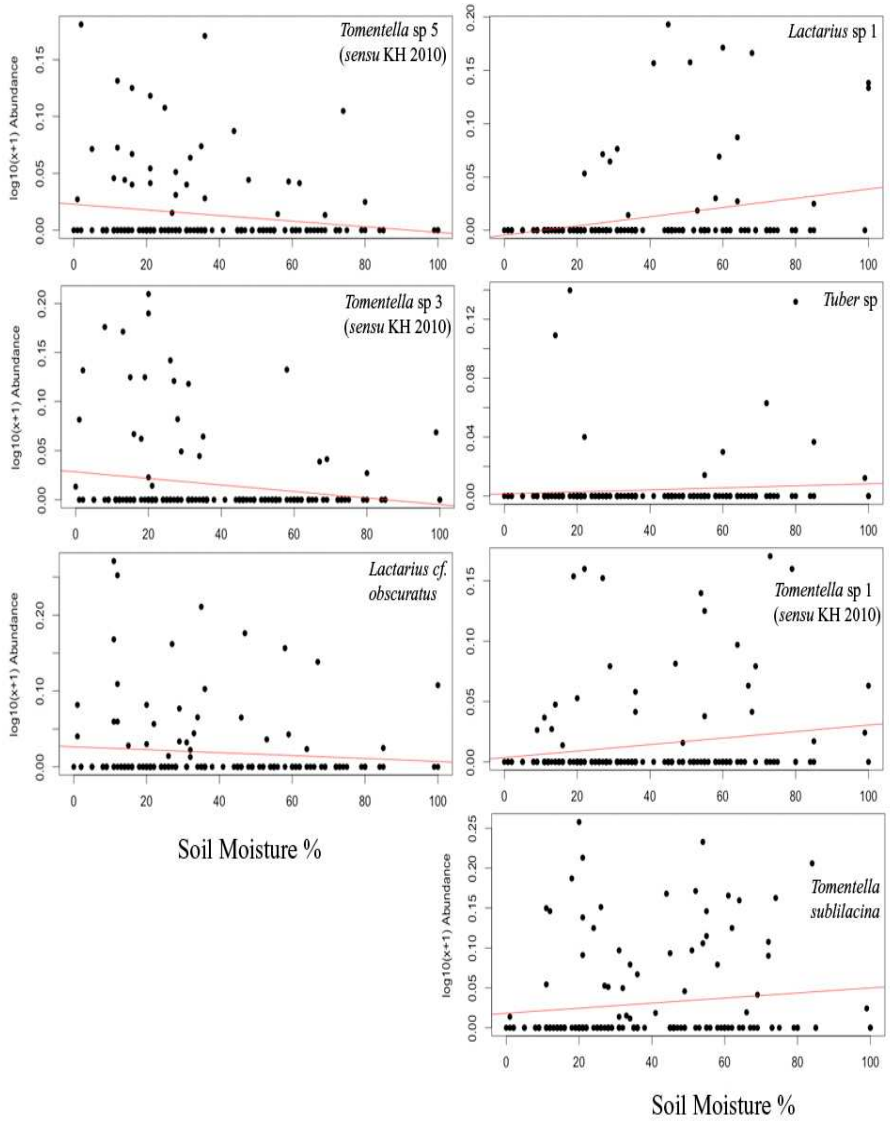


Figure 11: Plot of ectomycorrhizal fungal (EMF) species abundance (log10(x+1) transformed) (N=150) relative to the soil moisture (%). Species depicted are from TITAN (Figure 10a) and trend lines (abline in R.14.1) were applied to further visualize species abundance across the soil moisture gradient.

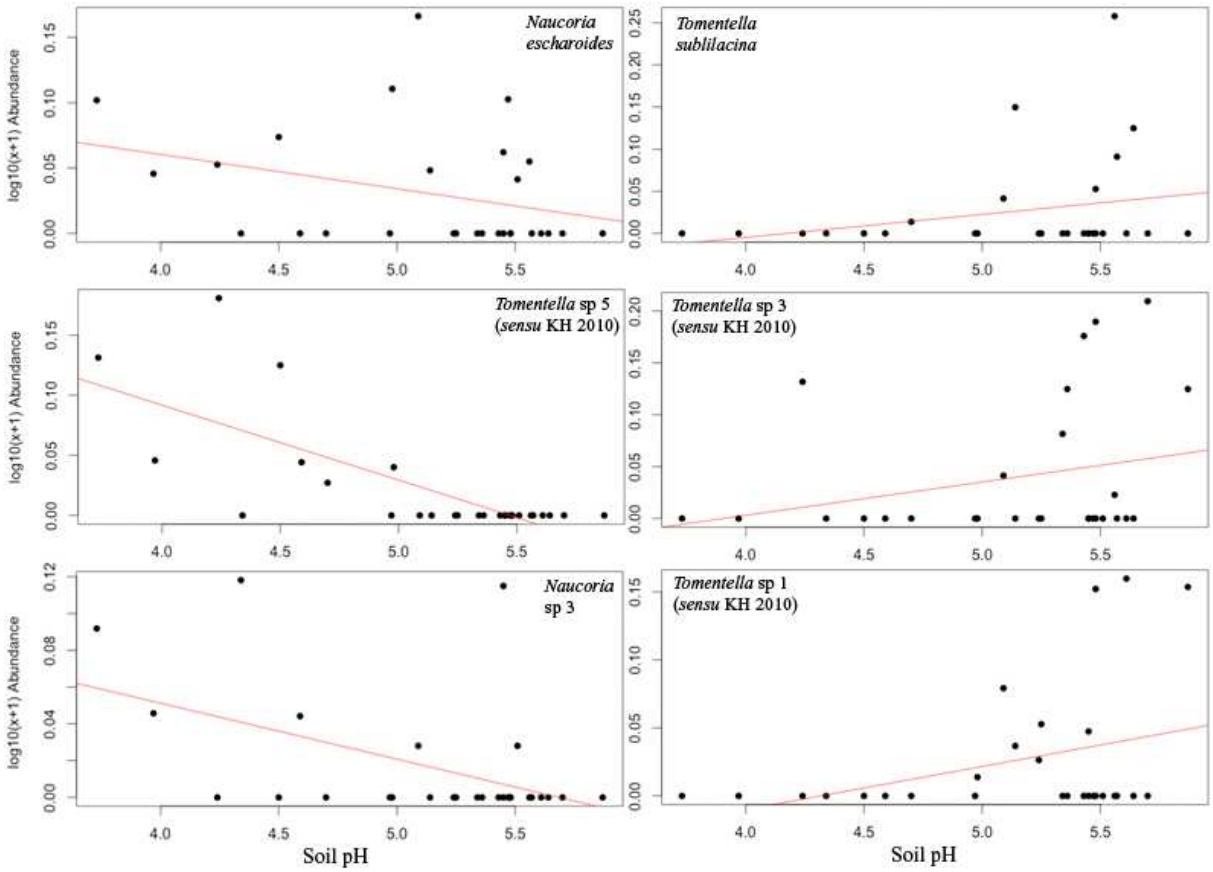


Figure 12: Plot of ectomycorrhizal fungal (EMF) species abundance (log10(x+1) transformed) (n = 30) relative to the soil pH. Species depicted are from TITAN (Figure 10a) and trend lines (abline in R.14.1) were applied to further visualize species abundance across the soil pH gradient.

*Cortinarius* and *Lactarius* also show possible niche partitioning along the moisture, phosphorous and nitrogen gradients (Figure 10 14, Table 8). For example, the abundance of *Lactarius* cf. *obscuratus* and *Cortinarius* cf. *alboviolaceous* in different levels of total nitrogen and available phosphorous from the TITAN results suggests niche partitioning (Figure 10c – d, Table 7). A slight overlap of these species can be seen in the scatterplots at mid-phosphorous levels and soils with lower total N (Figure 13 – 14). The overall occurrence of each species is more prevalent in separate root cores than together, with 11 cores sharing both species, and there is a noticeable split in the ordination space of species due to the phosphorous gradient and perhaps other gradients described by the ordination space (Figure 15b).

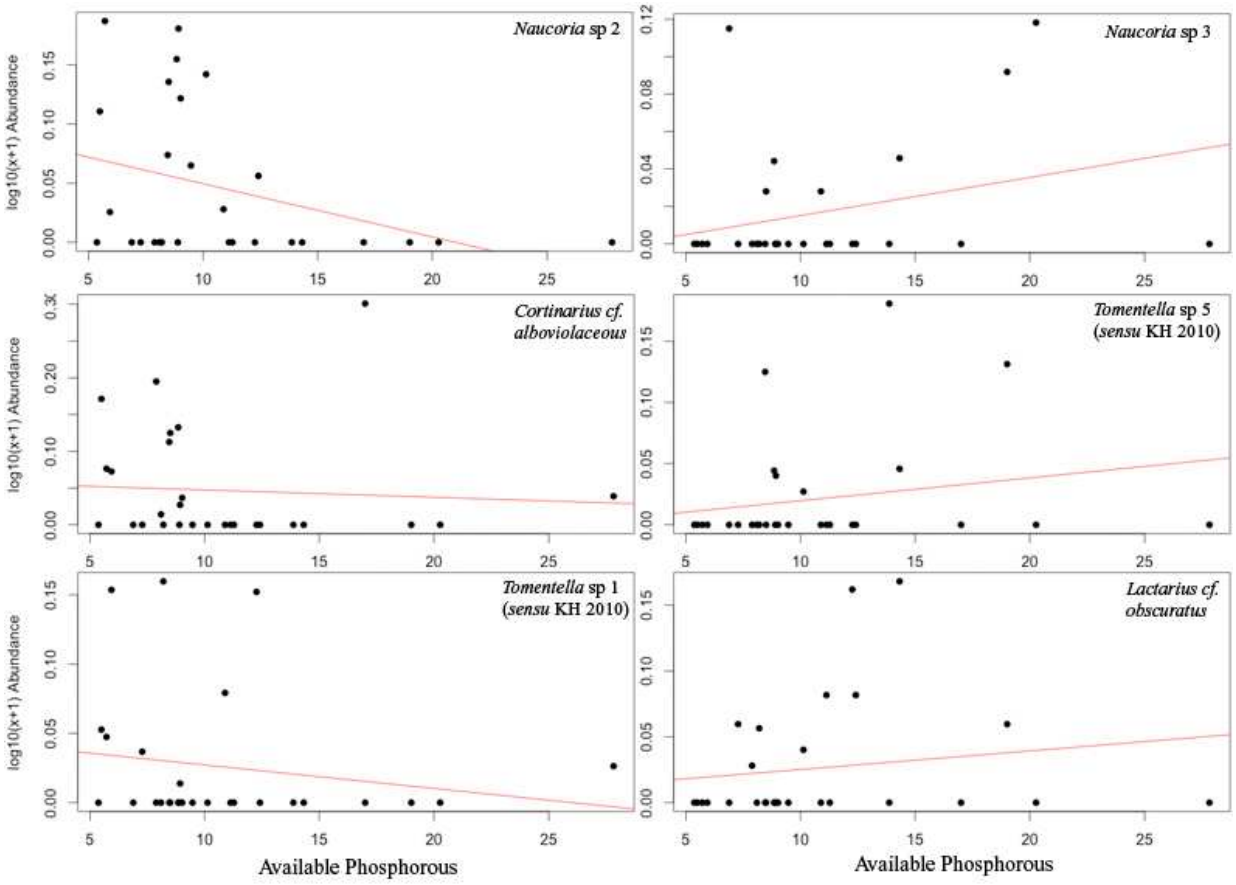


Figure 13: Plot of ectomycorrhizal fungal (EMF) species abundance (log10(x+1) transformed) (n = 30) relative to the available phosphorous. Species depicted are from TITAN (Figure 10a) and trend lines (abline in R.14.1) were applied to further visualize species abundance across the phosphorous gradient.

TITAN analyses and plots of abundance of species pairs inferred competition for root space in relation to the environmental gradients (Figure 10 – 14, Table 8). *Lactarius* cf. *obscuratus* and *Tomentella* sp 5 (*sensu* KH 2010) are both indicator species of low soil moisture and high available phosphorous levels (Figure 10a, c). The abundances of each species relative to moisture and phosphorous are similar (Figure 11, 13) but separation of species is apparent when occurrence is overlaid on the ordination space (Figure 15c). Despite similarity in species ranges relative to the soil environment, *L. obscuratus* and *Tomentella* sp 5 (*sensu* KH 2010) only co-occur in 6 cores (Figure 15c). Similar patterns can be seen between *Lactarius* cf. *brunneohepaticus* and *Tomentella* sp 1 (*sensu* KH 2010), which have significant Indicator Values for cores with higher soil moisture content (Figure 10a). Ranges of abundance relative to

soil moisture levels are similar, though *Tomentella* sp 1 has a broader range than *L. brunneohepaticus* (Figure 11). Both species occupy the outer cores on the ordination space, with only 4 cores occupied by both species (Figure 15d). There is thus evidence for potential competitive exclusion as well as niche partitioning for the EMF community structure; however, experimental manipulation of soil chemistry gradients to determine relative changes in abundance of each species would be useful in determining the cause the of patterns that were observed.

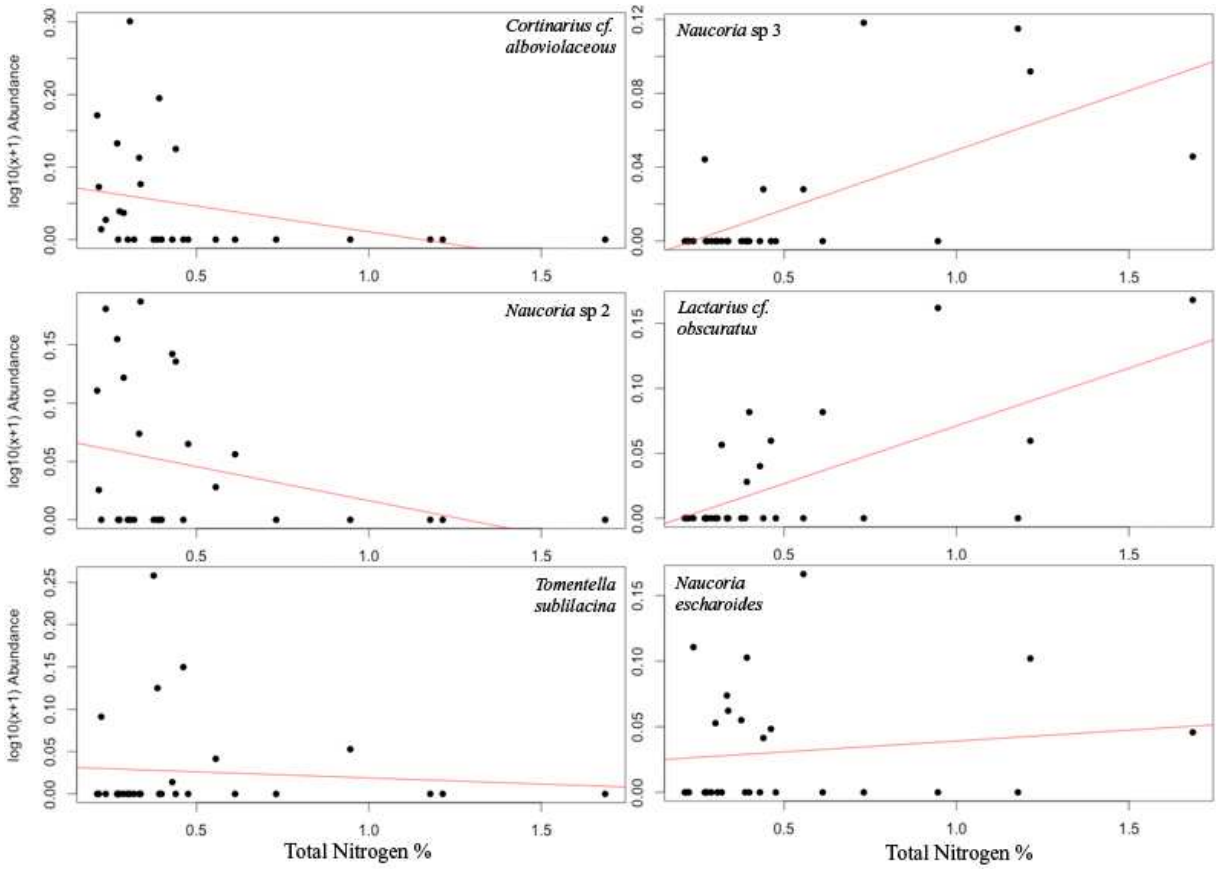


Figure 14: Plot of ectomycorrhizal fungal (EMF) species abundance (log10(x+1) transformed) (n = 30) relative to total nitrogen (%). Species depicted are from TITAN (Figure 10a) and trend lines (abline in R.14.1) were applied to further visualize species abundance across the nitrogen gradient.

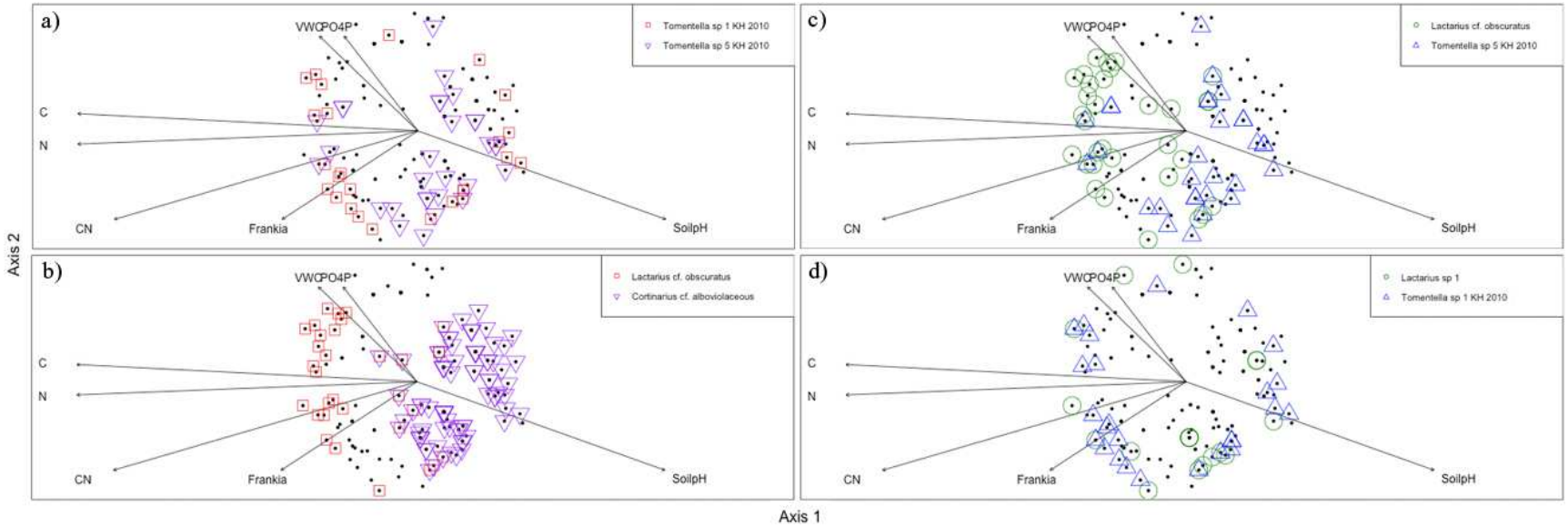


Figure 15: Non-metric MultiDimensional Scaling (NMDS) for visualizing ectomycorrhizal fungal (EMF) groupings of root cores (N=150) in two dimensions. Species exhibiting potential niche partitioning (a, b) and competitive exclusion (c, d) are shown. Niche partitioning: a) *Tomentella* sp 1 (*sensu* KH 2010) (□) and *Tomentella* sp 5 (*sensu* KH 2010) (∇), b) *Lactarius* cf. *obscuratus* (□) and *Cortinarius* cf. *alboviolaceous* (∇). Competitive exclusion: c) *Lactarius* cf. *obscuratus* (○) and *Tomentella* sp 5 (*sensu* KH 2010) (Δ), d) *Lactarius* cf. *brunneohepaticus* (○) and *Tomentella* sp 1 (*sensu* KH 2010) (Δ).

This study demonstrates the value of intensive sampling to investigate larger ecological questions over spatial and temporal scales. The EMF community composition associated with an older *Alnus rubra* stand is influenced by the presence of dominant EMF species, temporal variation of *Frankia* nodulation, and the changes in soil chemistry variables over space and time. Many EMF species in this study were found to have specific tolerances for each of the soil chemistry variables tested, resulting in variable responses to the environment within each taxon. Niche partitioning and competitive exclusion was found to occur between species in relation to the soil environment. The results parallel the findings of plant community structure and imply similar responses in belowground communities. The use of multivariate techniques in addition to intensive sampling effort provided the tools necessary to form a more complete picture of *Alnus rubra* ectomycorrhizal community composition than previously described. Future research on *Alnus* EMF community composition should focus on how microsite soil gradients may be influencing communities in other age stands with different disturbance histories. The potential niche partitioning and competitive exclusion relationships found in this study should be experimentally manipulated to verify interactions between EMF species in relation to soil chemistry gradients. Studies like this are a basis for further research in EMF interspecific interactions and provide the framework to eventually investigate how belowground communities influence aboveground community composition.

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## Appendix I: Ectomycorrhizal Morphotype Database

| Morphotype | Color | Luster | Texture | Branching | Rhizo-morphs | Comments |
|---|---|---|---|---|---|---|
| *Clavulina* sp 1 | Light yellow/orange-brown | Shiny | Smooth | Simple | None | Thick mantle short thin hyphae |
| *Clavulina* sp 2 | Tan with cream-yellow | Matte | Smooth/ velvety | Simple/whorled | None | straight tips |
| *Cortinarius* cf. *alboviolaceous* | variable: coral, orange, yellow, olive-tan, white, orange-brown | Matte/shiny | Smooth/sandy | Simple/one-sided | None | No hyphae/short thin hyphae, often holding sediment; straight to bent/tortuous tips |
| *Cortinarius* sp 2 (*sensu* KH 2010) | coral with pink overtones; lilac spots; white | Reflective | Felty/cottony | Simple/ monopodial-pinnate | White non-branching | Hyphae holding larger soil particles |
| *Entoloma* cf. *alpicola* | 5YR 8/2, 5/6, with tan/brown freckles | Shiny | Felty/cottony | Simple | None | Non-branching hyphae |
| *Inocybe rimosa* var. *rimosa* | Brown fading to cream/white | Matte/shiny | Velvety/ smooth | Simple/ monopodial-pinnate | None | No hyphae |
| *Inocybe* sp 1 | Brown with cream/yellow tips | Matte | Warty/sandy | Monopodial-pinnate | None | No hyphae |
| *Lactarius* cf. *obscuratus* | Cream/yellow to orange/dark brown | Shiny | Smooth | Simple/one-sided | None | No hyphae |
| *Lactarius* cf. *brunneohepaticus* | Brown/tan with orange/ cream/yellow areas | Matte | Smooth/sandy | One-sided/ monopodial-pinnate | None | No hyphae; bent to straight tips |
| *Naucoria escharoides* | Yellow/orange/cream/light brown Often dark pigment spots | Shiny | Smooth/ velvety | Simple/one-sided | None | Hyphae holding sand and covering tips |
| *Naucoria* sp 2 | Coral tan with areas of yellow/cream | Shiny | Felty/sandy | Simple/ monopodial-pinnate | None | Short fluffy hyphae holding soil particles; tips w/o hyphae |
| *Naucoria* sp 3 | Yellow/orange/brown | Matte | Sandy | Simple | None | No hyphae; thick mantle |
| *Tarzetta* sp | Black | Matte | Felty/warty | Simple | None | Short/straight tip; black hyphae |
| *Tomentella* sp 1 (*sensu* KH 2010) | Dark brown with red or purple tones | Matte | Warty | Simple/one-sided | None | No to sparse hyphae |
| *Tomentella* sp 2 | Coral/tan with yellow-cream | Matte | Felty/sandy | Monopodial-pinnate | None | Thin sparse hyphae |

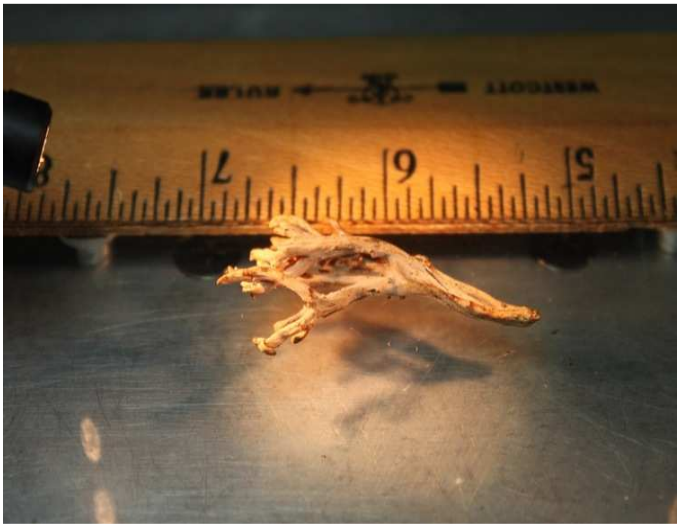
| | | | | | | |
|---|---|---|---|---|---|---|
| *Tomentella sublilacina* | Brown/tan fading into cream/yellow tips | Shiny | Smooth/felty | Simple/ monopodial-pinnate | None | Slightly bent tips |
| *Tomentella* sp 3 (*sensu* KH 2010) | Brown with orange/olive/tan/red hues | Matte/shiny | Sandy | Simple/ one-sided | None | Tips bend slightly, short to no hyphae |
| *Tomentella* sp 4 (*sensu* KH 2010) | Coral-tan/brown-orange with lighter cream areas | Matte | Sandy/felty | Simple/ monopodial-pinnate | None | Either no hyphae or hyphae thick holding sand |
| *Tomentella* sp 5 (*sensu* KH 2010) | Dark brown with red/orange tones | Reflective | Warty | Simple | None | Orange hyphae; thick mantle |
| *Tomentella* sp 6 | Cream/light yellow | Matte | Smooth | Simple | None | Straight tips |
| *Tomentella* sp 7 | Dark brown with light brown splotches; Newer growth: yellow brown | Matte | Warty/felty | Simple/ monopodial-pinnnate | None | Bent to straight tip |
| *Tuber* sp | Tan fading into yellow | Matte | Sandy | Monopodial-pinnate | None | No hyphae |

**"*sensu* KH 2010": Ectomycorrhizal species that matched closely too the species listed in Kennedy and Hill, 2010.

## Appendix I: Ectomycorrhizal Morphotype Database

*Clavulina* sp 1

KG0434_ALRU11_Lactarius cf. brunneohepaticus_35x_2011_Uncultured Clavulina_99_Clavulina sp 1.jpg



Clavulina sp 1/MF20 IMG_3274.JPG

*Clavulina* sp 2

KG0088_ALRU24_Tom2_2011_25x_Uncultured Clavulina_99_Clavulina sp 2.jpg

*Cortinarius* cf. *alboviolaceous*

KG0362_ALRU05_Alnsp_40x_2011_Cortinarius alboviolaceus_100_Cortinarius cf. alboviolaceous.jpg

KG0330_ALRU15_Tomellisii_50x_2011_Cortinarius alboviolaceus_99_Cortinarius cf. alboviolaceous.jpg

MF23 IMG_3277.JPG

*Cortinarius* sp 2 (*sensu* Kennedy and Hill, 2010)

KG0489_ALRU02_Cortinarius cf. alboviolaceous_40x_2011_Cortinarius alboviolaceus_98_Cortinarius sp 2 sensu KH2010.jpg

*Entoloma* cf. *alpicola*

KG0087_ALRU24_Aln2_2011_25x_Entoloma cf. alpicola_100_Entoloma cf. alpicola.jpg

*Inocybe rimosa* var. *rimosa*

KG0180_ALRU25_Lacsp_2011_50x_Inocybe rimosa var rimosa_99_ *Inocybe rimosa var rimosa*.jpg

*Inocybe* sp 1

KG0383_ALRU10_Tomsp_40x_2011_Inocybe fuscidula_100_Inocybe sp 1.jpg

*Lactarius* cf. *obscuratus*

KG0544_ALRU27_Lactarius brunneohepaticus_35x_2011_Lactarius cyathuliformis_98_ *Lactarius* cf. *obscuratus*.jpg


MF04 IMG_3305.JPG

*Lactarius* cf. *brunneohepaticus*

KG0420_1_ALRU29_Tomentella sp 1_40x_2011_Lactarius cyathuliformis_97_ *Lactarius* cf. *brunneohepaticus*.jpg

*Naucoria escharoides*

KG0062_ALRU20_Aln3_2011_30x_Naucoria escharoides_98_Naucoria escharoides.jpg

PA271646_JA06_Naucoria escharoides.JPG (photo by Joseph Ammirati)

*Naucoria* sp 2

KG0535_ALRU26_Tomentella sp_40x_2011_Alnicola umbrina_99_99_Naucoria sp 2.jpg

PA271649_JA15_Naucoria sp 2.JPG (photo by Joseph Ammirati)

*Naucoria* sp 3

KG0014_ALRU14_Tom1_2011_30x_Alnicola inculta_98_Naucoria sp 3.jpg

PA271648_JA09_Naucoria sp 3.JPG (photo by Joseph Ammirati)

*Tarzetta* sp

KG0381_ALRU24_Cenosp_60x_2011_Tarzetta sp_100_Tarzetta sp.jpg

*Tomentella* sp 1 (*sensu* Kennedy and Hill, 2010)

KG0078_ALRU28_Tom9_2011_30x_Uncultured Tomentella_97_Tomentella sp 1 sensu KH 2010.jpg

*Tomentella* sp 2

KG0519_ALRU25_Tomentella sublilacina_40x_2011_Uncultured EM fungi_99_Tomentella sp 2.jpg

*Tomentella sublilacina*

KG0114_ALRU12_Tom5_2011_35x_Uncultured Tomentella_99_Tomentella sublilacina.jpg

*Tomentella* sp 3 (*sensu* Kennedy and Hill, 2010)

KG0511_ALRU16_Tomentella stuposa_40x_2011_Uncultured Tomentella_100_Tomentella sp 3 sensu KH 2010.jpg

*Tomentella* sp 4 (*sensu* Kennedy and Hill, 2010)

KG0509_ALRU16_Tomentellasp_35x_2011_Uncultured Tomentella_99_Tomentella sp 4 sensu KH 2010.jpg

*Tomentella* sp 5 (*sensu* Kennedy and Hill, 2010)

KG0207_ALRU23_Tomstup_50x_2011_Uncultured Tomentella_97_Tomentella sp 5 sensu KH 2010.jpg

*Tomentella* sp 6

KG0177_ALRU15_Lac3_2011_40x_Uncultured Tomentella_99_Tomentella sp 6.jpg

*Tomentella* sp 7

KG0064_ALRU20_Tomentella sp_2011_30x_Uncultured EM fungus_98_Tomentella sp 7.jpg

*Tuber* sp

KG0553_ALRU11_Tuber sp_50x_2011_Uncultured EM Tuber_96_Tuber sp.jpg

## Appendix II: Phylogenetic trees

Trees were generated in the *MEGA* version 5.0 (Tamura et al. 2011). ClustalW alignment for each group was performed prior to tree formation. Trees are nearest neighbor trees using the maximum composite likelihood method, gaps treated as pairwise deletions, and trees were generated 500 times to determine the most parsimonious tree.

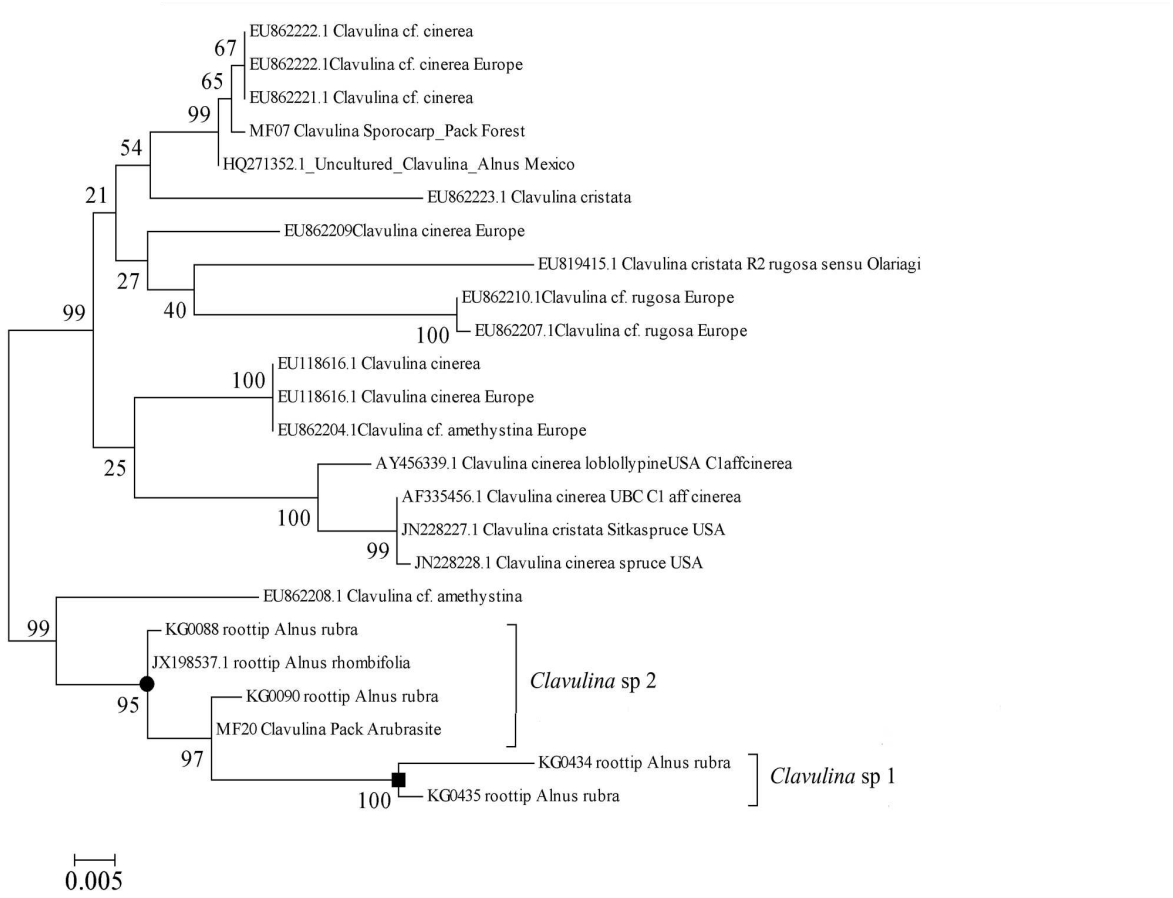



Figure 1: *Clavulina* phylogenetic tree. KGxxx are ectomycorrhizal root samples. MFxx is from a sporocarp collected at the site that could not be identified to the species. ITS sequences from Genbank and UNITE databases that matched >97% to the root tip and sporocarp samples are shown. Two *Clavulina* species were interpreted from the tree: *Clavulina* sp 1 (■) and *Clavulina* sp 2 (●). Each species is divergent over the 500 bootstrap permutations, despite the lack of resolution as a well-supported separate clade.

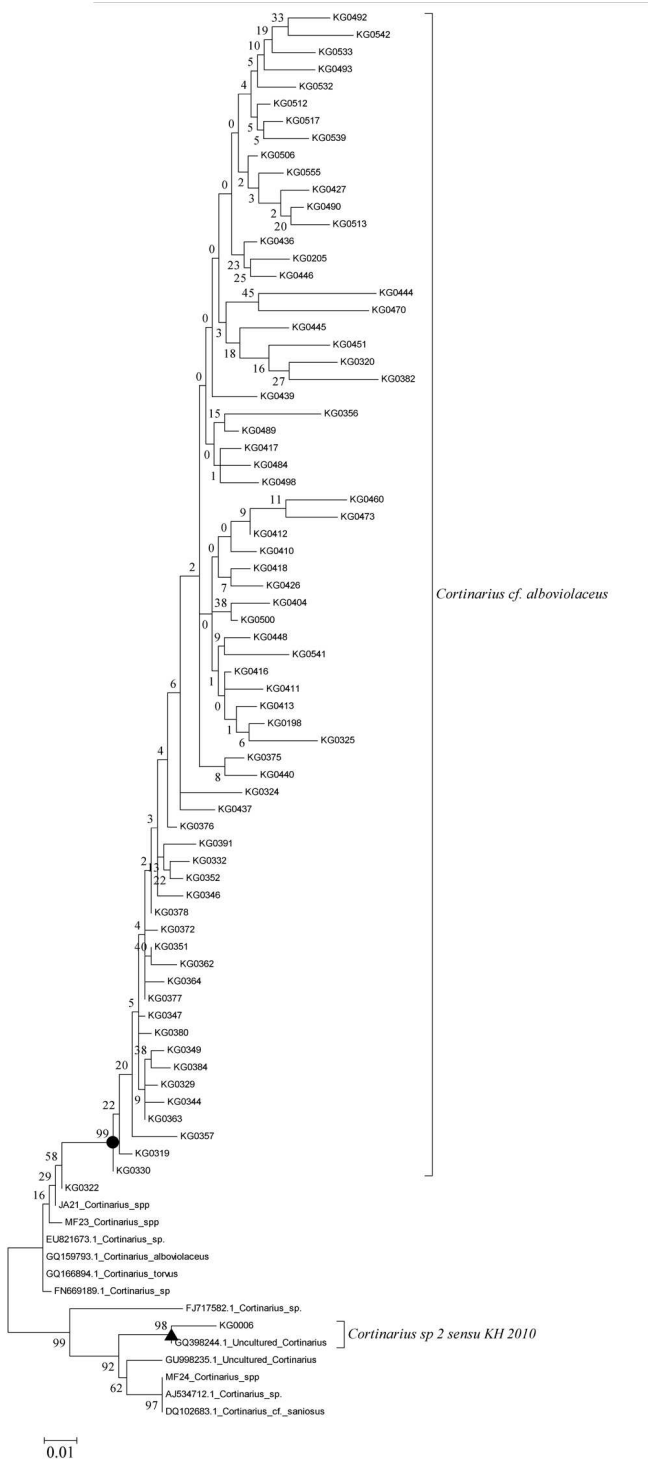



Figure 2: *Cortinarius* phylogenetic tree. KGxxx are ectomycorrhizal root samples. JAxx and MFxx are sporocarps collected at the site. ITS sequences from Genbank and UNITE databases that matched >97% to the root tip and sporocarp samples are shown. Two *Cortinarius* species were interpreted from the tree. *Cortinarius* cf. *alboviolaceous* consists of most of the samples (▼) and *Cortinarius* sp 2 (*sensu* KH 2010) (♦), matched to an *Alnus rubra Cortinarius* (GQ398244) from Kennedy and Hill (2010).

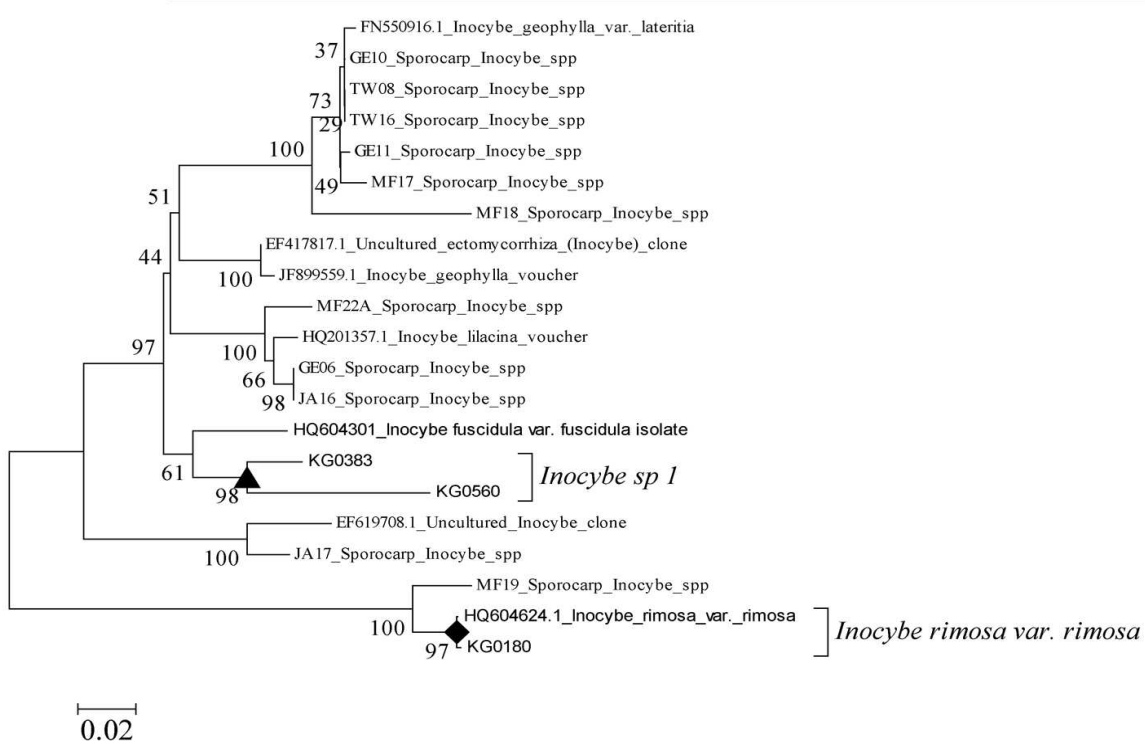



Figure 3: *Inocybe* phylogenetic tree. KGxxx are ectomycorrhizal root samples GE/JA/MF/TWxx are sporocarps collected at the site. ITS sequences from Genbank and UNITE databases that matched >97% to the root tip and sporocarp samples are shown. Most *Inocybe* sporocarps collected on the site fell within the *I. geophylla* and *I. lilacina* clades, but *Inocybe* found in association with *Alnus rubra* were instead: *Inocybe rimosa var rimosa* (♦) and *Inocybe* sp 1 (▲), in the *I. fuscidula* clade.

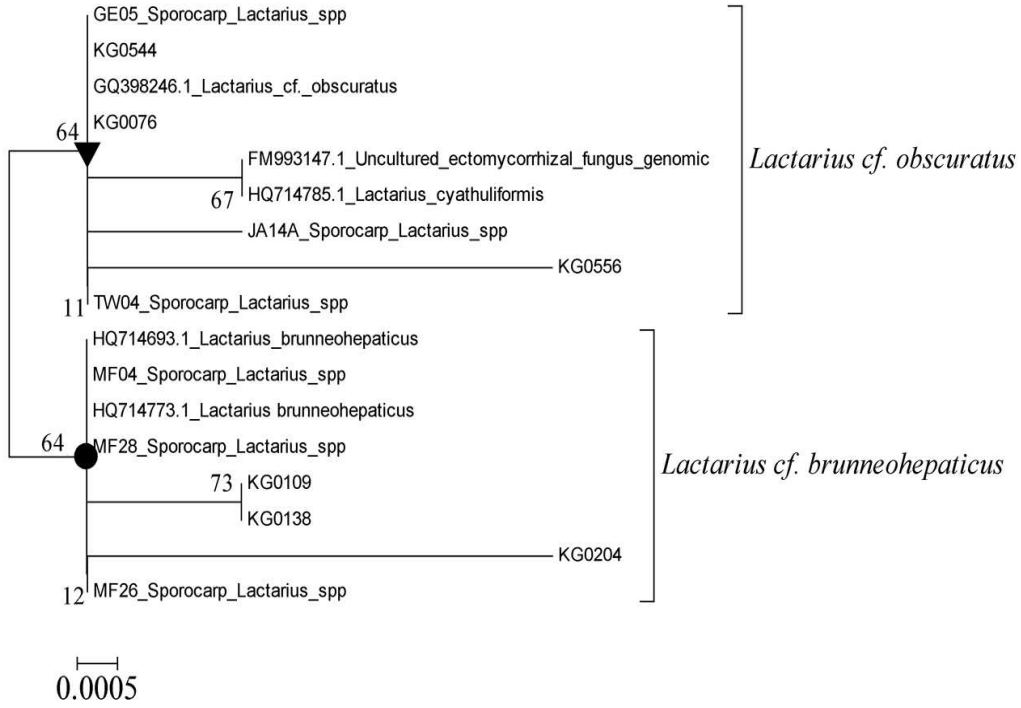



Figure 4: *Lactarius* phylogenetic tree. KGxxx are ectomycorrhizal root samples. GE/JA/MF/TWxx are sporocarps collected at the site. ITS sequences from Genbank and UNITE databases that matched >97% to the root tip and sporocarp samples are shown. Two *Lactarius* species were interpreted from the tree in relation to the root tips, despite the relatively weak resolution: *Lactarius* cf. *obscuratus* (▼) and *Lactarius* cf. *brunneohepaticus* (□).

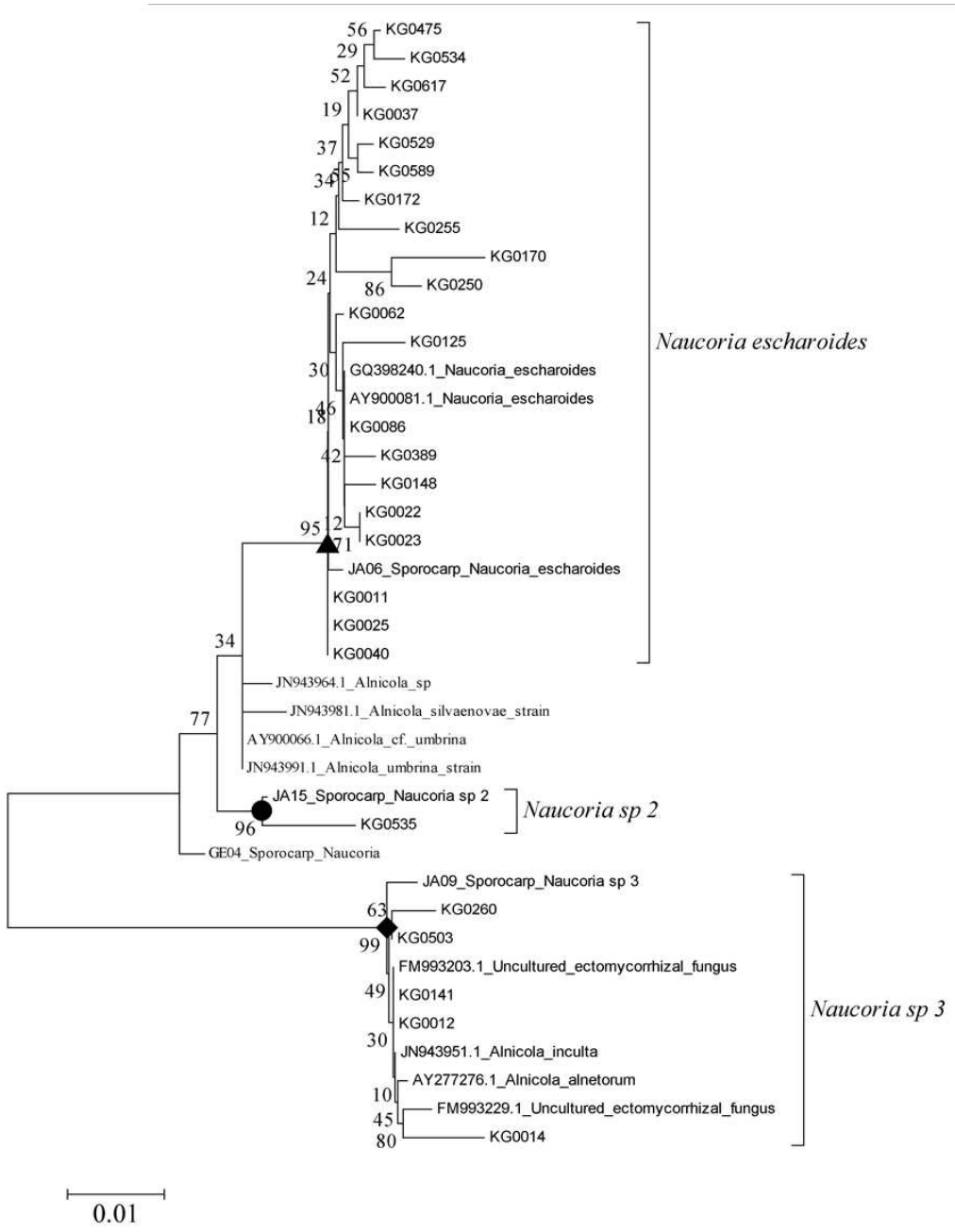



Figure 5: *Naucoria/Alnicola* phylogenetic tree. KGxxx are ectomycorrhizal root samples. GE/JAxx are sporocarps collected at the site. ITS sequences from Genbank and UNITE databases that matched >97% to the root tip and sporocarp samples are shown. Three *Naucoria* species were interpreted from the tree: *Naucoria escharoides* (▲), *Naucoria* sp 3 (●), *Naucoria* sp 3 (♦) which forms a distinct phylogenetic clade.

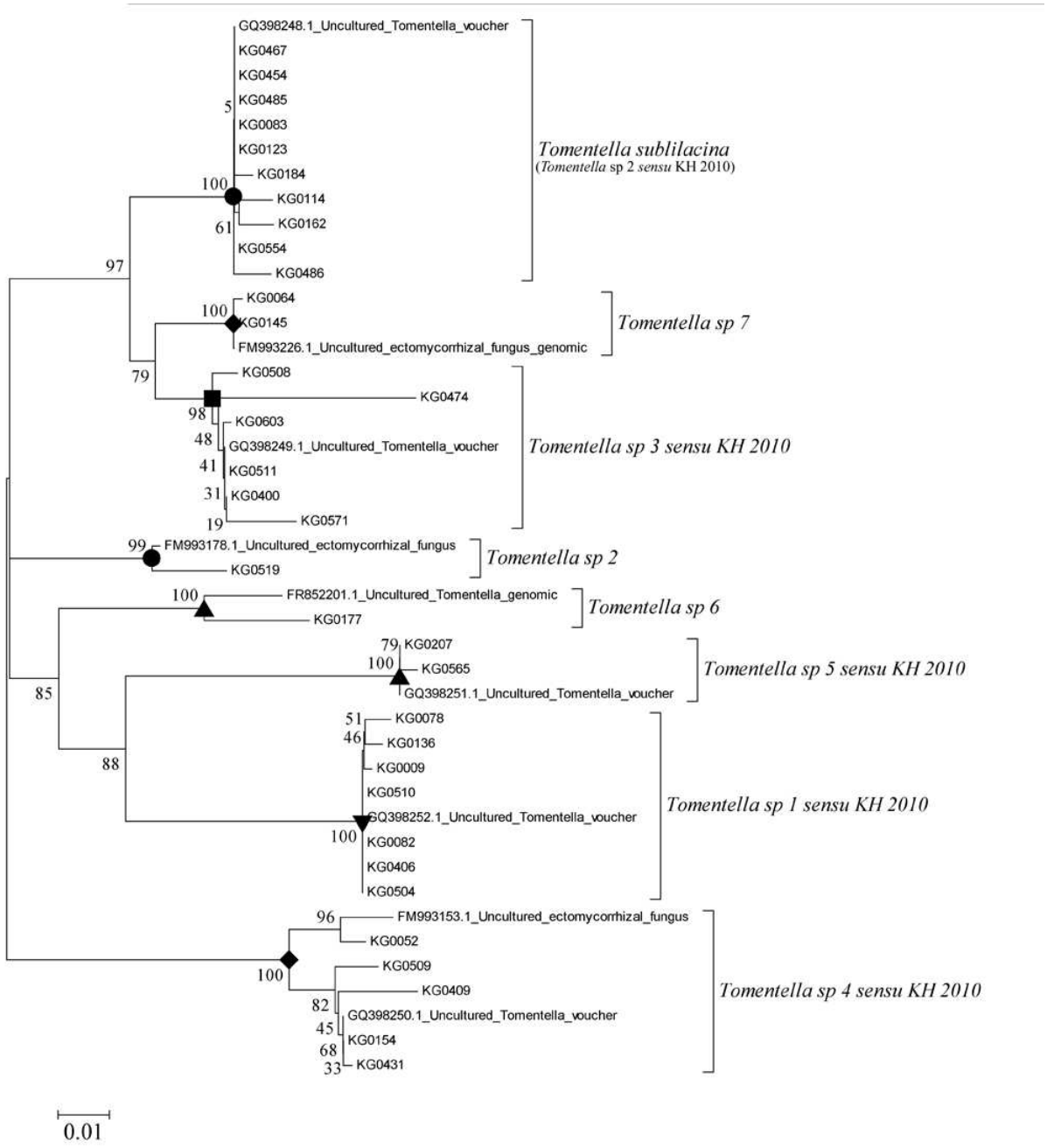



Figure 6: *Tomentella* phylogenetic tree. KGxxx are ectomycorrhizal root samples. ITS sequences from Genbank and UNITE databases that matched >97% to the root samples are shown. Eight species of *Tomentella* were found, three of which have not been identified on *Alnus rubra* before. New species to *A. rubra* include: *Tomentella* sp 2 (□), *Tomentella* sp 6 (▲), and *Tomentella* sp 7 (♦). The four other *Tomentella* species matched to *A. rubra* ectomycorrhizal species from Kennedy and Hill (2010): *Tomentella* sp 1 (*sensu* KH 2010) (▼), *Tomentella sublilacina* (□), *Tomentella* sp 3 (*sensu* KH 2010) (■), *Tomentella* sp 4 (*sensu* KH 2010) (♦), and *Tomentella* sp 5 (*sensu* KH 2010) (▲).

**Appendix III: Threshold Indicator Taxa ANalysis (TITAN) – Full Results**

Tables show the output of the TITAN analysis for each soil variable tested: soil moisture, soil pH, available phosphorous, and total nitrogen. EMF species from the entire sampling period were tested with soil moisture (N=150), and EMF species recorded in August, 2011 were compared to the other soil chemistry variables (N=30) as they only collected that month. Within the tables are individual z scores (+/-), indicator values, z scores, observed change point for each species (Obs), confidence intervals (5, 95%), *p*-values for CI coverage, the purity of the taxa for each z score classification, and how reliable each taxa was to have a *p*-value less than 5% or 1% over 500 bootstrap permutations. A TITAN sum (z-) and (z+) are the sum of Indval scores for all z- and z+ taxa, respectively, and nCPA thresholds which are the maximum deviance reduction among sample units using Bray-Curtis distance.

| | | | | Change Point | | | | | Reliability | |
|---|---|---|---|---|---|---|---|---|---|---|
| | -/+ | IndVal | z | Obs | 5% | 95% | *p* value | Purity | ≤0.05 | ≤0.01 |
| **Soil Moisture** | | | | | | | | | | |
| *Cortinarius* cf. *alboviolaceous* | + | 37.05 | 0.65 | 13.5 | 7.93 | 82.00 | 0.236 | 0.50 | 0.32 | 0.13 |
| *Lactarius* cf. *obscuratus* | - | 31.55 | 3.71 | 12 | 10.00 | 84.50 | 0.008 | 0.83 | 0.77 | 0.54 |
| *Lactarius* cf. *brunneohepaticus* | + | 41.76 | 5.46 | 82 | 23.00 | 84.50 | 0.004 | 1.00 | 1.00 | 0.98 |
| *Naucoria escharoides* | - | 38.39 | 1.40 | 73 | 8.00 | 79.50 | 0.108 | 0.72 | 0.46 | 0.11 |
| *Naucoria* sp 2 | - | 20.17 | 1.99 | 20 | 9.00 | 82.00 | 0.040 | 0.62 | 0.52 | 0.28 |
| *Naucoria* sp 3 | + | 40.86 | 1.86 | 84.5 | 18.95 | 84.50 | 0.092 | 0.85 | 0.68 | 0.35 |
| *Tomentella* sp 1 (*sensu* KH 2010) | + | 31.7 | 4.49 | 66.5 | 31.00 | 84.50 | 0.004 | 0.98 | 0.94 | 0.74 |
| *Tomentella sublilacina* | + | 26.87 | 3.27 | 50 | 15.00 | 64.55 | 0.012 | 0.96 | 0.88 | 0.59 |
| *Tomentella* sp 7 | + | 27.21 | 2.65 | 82 | 11.00 | 84.50 | 0.064 | 0.82 | 0.75 | 0.44 |
| *Tomentella* sp 3 (*sensu* KH 2010) | - | 20.67 | 4.20 | 36 | 5.00 | 39.50 | 0.004 | 0.96 | 0.95 | 0.83 |
| *Tomentella* sp 4 (*sensu* KH 2010) | + | 53.88 | 5.50 | 82 | 5.00 | 84.50 | 0.004 | 0.54 | 0.52 | 0.48 |
| *Tomentella* sp 5 (*sensu* KH 2010) | - | 20.01 | 2.72 | 45 | 5.00 | 52.03 | 0.016 | 0.94 | 0.86 | 0.60 |
| *Tuber* | + | 33.25 | 6.86 | 79.5 | 20.48 | 84.50 | 0.008 | 0.92 | 0.87 | 0.69 |
| | | | Sum z- | 5 | 15.00 | 39.65 | | | | |
| | | | Sum z+ | 82 | 80.00 | 84.50 | | | | |
| | | | nCPA | 20 | 25.25 | 84.50 | | | | |

| | | | | Change Point | | | | | Reliability | |
|---|---|---|---|---|---|---|---|---|---|---|
| | +/- | IndVal | z | Obs | 5% | 95% | *p* value | Purity | ≤0.05 | ≤0.01 |
| **Soil pH** | | | | | | | | | | |
| *Cortinarius* cf. *alboviolaceous* | - | 36.03 | 0.23 | 5.54 | 4.65 | 5.54 | 0.324 | 0.53 | 0.19 | 0.04 |
| *Lactarius* cf. *obscuratus* | - | 27.05 | 0.07 | 4.84 | 4.65 | 5.57 | 0.428 | 0.55 | 0.23 | 0.08 |
| *Naucoria escharoides* | - | 49.64 | 2.90 | 5.19 | 4.65 | 5.54 | 0.020 | 0.94 | 0.74 | 0.44 |
| *Naucoria* sp 2 | - | 32.76 | 0.37 | 5.45 | 4.65 | 5.54 | 0.312 | 0.55 | 0.21 | 0.06 |
| *Naucoria* sp 3 | - | 57.69 | 3.72 | 4.65 | 4.65 | 5.46 | 0.012 | 0.94 | 0.78 | 0.52 |
| *Tomentella* sp 1 (*sensu* KH 2010) | + | 45.00 | 2.13 | 4.98 | 4.84 | 5.57 | 0.032 | 0.90 | 0.63 | 0.18 |
| *Tomentella sublilacina* | + | 43.26 | 2.58 | 5.48 | 4.98 | 5.57 | 0.020 | 0.88 | 0.59 | 0.30 |
| *Tomentella* sp 3 (*sensu* KH 2010) | + | 38.41 | 1.68 | 5.30 | 4.98 | 5.57 | 0.088 | 0.86 | 0.57 | 0.29 |
| *Tomentella* sp 4 (*sensu* KH 2010) | - | 20.70 | 0.68 | 5.40 | 4.84 | 5.57 | 0.288 | 0.54 | 0.13 | 0.04 |
| *Tomentella* sp 5 (*sensu* KH 2010) | - | 83.69 | 8.37 | 4.84 | 4.65 | 5.25 | 0.004 | 1.00 | 1.00 | 0.97 |
| | | | Sum z- | 5.12 | 4.98 | 5.30 | | | | |
| | | | Sum z+ | 5.04 | 5.45 | 5.57 | | | | |
| | | | nCPA | 4.65 | 5.25 | 5.57 | | | | |

| | | | | Change Point | | | | | Reliability | |
|---|---|---|---|---|---|---|---|---|---|---|
| | +/- | IndVal | z | Obs | 5% | 95% | *p* value | Purity | ≤0.05 | ≤0.01 |
| **Available Phosphorous** | | | | | | | | | | |
| *Cortinarius* cf. *alboviolaceous* | - | 49.88 | 2.62 | 8.88 | 7.59 | 13.14 | 0.028 | 0.86 | 0.68 | 0.41 |
| *Lactarius* cf. *obscuratus* | + | 42.29 | 2.21 | 9.80 | 8.15 | 13.19 | 0.052 | 0.89 | 0.67 | 0.36 |
| *Naucoria escharoides* | - | 32.90 | 0.15 | 8.00 | 7.59 | 14.10 | 0.352 | 0.66 | 0.32 | 0.11 |
| *Naucoria* sp 2 | - | 53.06 | 3.05 | 10.51 | 8.00 | 13.19 | 0.016 | 0.91 | 0.81 | 0.49 |
| *Naucoria* sp 3 | + | 50.72 | 2.98 | 14.10 | 8.00 | 14.10 | 0.028 | 0.84 | 0.58 | 0.33 |
| *Tomentella* sp 1 (*sensu* KH 2010) | - | 47.46 | 2.05 | 7.59 | 7.59 | 12.34 | 0.036 | 0.86 | 0.68 | 0.35 |
| *Tomentella sublilacina* | - | 33.33 | 1.40 | 12.34 | 7.59 | 12.34 | 0.156 | 0.78 | 0.39 | 0.15 |
| *Tomentella* sp 3 (*sensu* KH 2010) | + | 36.27 | 1.63 | 8.88 | 8.15 | 13.14 | 0.088 | 0.65 | 0.40 | 0.15 |
| *Tomentella* sp 4 (*sensu* KH 2010) | - | 26.32 | 1.51 | 11.21 | 8.15 | 11.77 | 0.176 | 0.70 | 0.19 | 0.05 |
| *Tomentella* sp 5 (*sensu* KH 2010) | + | 42.37 | 1.96 | 13.14 | 8.15 | 14.10 | 0.028 | 0.92 | 0.63 | 0.29 |
| | | | Sum z- | 12.34 | 8.98 | 13.14 | | | | |
| | | | Sum z+ | 11.77 | 11.01 | 14.10 | | | | |
| | | | nCPA | 13.14 | 11.01 | 14.10 | | | | |

| | | | | Change Point | | | | | Reliability | |
|---|---|---|---|---|---|---|---|---|---|---|
| | +/- | IndVal | z | Obs | 5% | 95% | *p* value | Purity | ≤0.05 | ≤0.01 |
| **Total Nitrogen** | | | | | | | | | | |
| *Cortinarius* cf. *alboviolaceous* | - | 63.16 | 3.70 | 0.451 | 0.283 | 0.469 | 0.008 | 1.00 | 0.98 | 0.86 |
| *Lactarius* cf. *obscuratus* | + | 57.41 | 4.17 | 0.390 | 0.313 | 0.672 | 0.004 | 1.00 | 0.97 | 0.81 |
| *Naucoria escharoides* | + | 38.87 | 1.42 | 0.327 | 0.275 | 0.672 | 0.100 | 0.80 | 0.46 | 0.19 |
| *Naucoria* sp 2 | - | 42.71 | 1.56 | 0.295 | 0.275 | 0.672 | 0.080 | 0.89 | 0.61 | 0.28 |
| *Naucoria* sp 3 | + | 67.36 | 5.42 | 0.516 | 0.414 | 0.672 | 0.004 | 0.99 | 0.96 | 0.83 |
| *Tomentella* sp 1 (*sensu* KH 2010) | - | 34.79 | 0.78 | 0.283 | 0.275 | 0.672 | 0.184 | 0.76 | 0.42 | 0.17 |
| *Tomentella sublilacina* | + | 34.36 | 1.91 | 0.357 | 0.283 | 0.516 | 0.048 | 0.78 | 0.47 | 0.18 |
| *Tomentella* sp 3 (*sensu* KH 2010) | - | 39.13 | 1.15 | 0.672 | 0.275 | 0.672 | 0.120 | 0.71 | 0.34 | 0.09 |
| *Tomentella* sp 4 (*sensu* KH 2010) | - | 25.00 | 1.00 | 0.469 | 0.283 | 0.469 | 0.256 | 0.52 | 0.12 | 0.02 |
| *Tomentella* sp 5 (*sensu* KH 2010) | - | 23.93 | 0.46 | 0.336 | 0.283 | 0.672 | 0.292 | 0.55 | 0.22 | 0.06 |
| | | | Sum z- | 0.357 | 0.336 | 0.519 | | | | |
| | | | Sum z+ | 0.584 | 0.451 | 0.672 | | | | |
| | | | nCPA | 0.672 | 0.516 | 0.672 | | | | |

## Appendix IV: Metadata and R Code

### Metadata

emra.txt

File containing rows which represent the 150 sites, which are root cores taken May to September, 2011 around 30 *Alnus rubra* trees. The "site" is coded with the tree ID first (01 to 30) followed by month sampled (05 to 09). The columns consist of 22 ectomycorrhizal (EMF) species and a "dummy" variable, used to reduce the effect of zeros in the species dataset. The numbers in the data frame represent the abundance of each EM species colonizing root tips within each root core (# root tips of each species/150 root cores). Abbreviations for species names are listed below.

### List of Ectomycorrhizal Fungus Species Codes

Ectomycorrhizal fungal species identified by DNA sequencing matching to online BLAST (http://blast.ncbi.nlm.nih.gov) and UNITE (http://unite.ut.ee/) databases. Codes are listed below for species abbreviations. Species that matched to previously identified *Alnus rubra* EMF species (Kennedy and Hill, 2010) are labeled "*sensu* KH 2010". See Appendix I for further details about the species.

Clav1 – *Clavulina* species 1
Clav2 – *Clavulina* species 2
Cort1 – *Cortinarius* cf. *alboviolaceous*
Cort2 – *Cortinarius* species 2 (*sensu* KH 2010)
Ent – *Entoloma* cf. *aplicola*
Ino1 – *Inocybe rimosa* var. *rimosa*
Ino2 – *Inocybe* species 1
Lact1 – *Lactarius* cf. *obsuratus*
Lact2 – *Lactarius* cf. *brunneohepaticus*
Nauesc – *Naucoria escharoides*
Nau2 - *Naucoria* species 2
Nau3 - *Naucoria* species 3
Pstom - *Tomentella* species 1 (*sensu* KH 2010)
Tarz – *Tarzetta* species
Tom1 – *Tomentella* species 6
Tom2 – *Tomentella* species 2
Tom2.KH – *Tomentella sublilacina*
Tom3 – *Tomentella* species 7
Tom3.KH – *Tomentella* species 3 (*sensu* KH 2010)
Tom4.KH– *Tomentella* species 4 (*sensu* KH 2010)
Tom5.KH– *Tomentella* species 5 (*sensu* KH 2010)
Tuber – *Tuber* species
Dummy – "Dummy" variable

explan.txt

File containing the environmental variables collected May to September 2011 in root cores taken for EMF species analysis. Coding definitions are listed below.

**List of Environmental Variable Codes**

TreeID – The number of *Alnus rubra* tree sampled, numbers 01 to 30.

Month – The month sampled, May through September, 2011.

VWC – Soil moisture was estimated by measuring the volumetric water content (%) of the soil immediately prior to root coring. Data of VWC % for each root core/tree is listed in order for each *Alnus rubra* tree 01 to 30, from May to September, 2011.

Frankia – presence (1) and absence (0) of Frankia nodules on the *Alnus rubra* roots within each root core for each tree, listed in order for each *Alnus rubra* tree 01 to 30, from May to September, 2011.

EMCol – The percent of ectomycorrhizal roots out of non-mycorrhizal and necrotic roots in a subsample (60 tips/root core) of the roots sampled for each tree, listed in order from the top for each *Alnus rubra* tree 01 to 30 listed in order: May to September, 2011. Values are #EM tips/60 root tips.

SoilpH – Soil pH of soil samples taken adjacent to root cores from 8-30-2011 listed in order from top for each *Alnus rubra* tree 01 to 30. Values repeated for each month.

PO4P – Available phosphorous (mg/Kg) of soil samples taken adjacent to root cores listed in order from the top for each *Alnus rubra* tree 01 to 30. Values repeated for each month.

C – Carbon (%) of soil samples taken adjacent to root cores listed in order from the top for each *Alnus rubra* tree 01 to 30. Values repeated for each month.

N – Total inorganic nitrogen (%)of soil samples taken adjacent to root cores listed in sorder from the top for each *Alnus rubra* tree 01 to 30. Values repeated for each month.

CN – Carbon (%) to total nitrogen (%) ratio of soil samples taken adjacent to root cores listed in order from the top for each *Alnus rubra* tree 01 to 30. Values repeated for each month.

Core_x – x-coordinate of each root core location in relation to the 45m x 100m grid of the study site. Listed in order of the *Alnus rubra* trees 01 to 30, for each month sampled: May to September, 2011.

Core_y – y-coordinate of each root core location in relation to the 45m x 100m grid of the study site. Listed in order of the *Alnus rubra* trees 01 to 30, for each month sampled: May to September, 2011.

## R Code

Main R pacakges used for analyses: vegan (Oksanen et al. 2011), labdsv (Roberts 2012), mvpart (De ‘Ath 2002), and TITAN script used as part of the mvpart package (Baker and King, 2010).

**Data frames/matricies**

```
emra <- read.table(file.choose(),T) # Relative abundance EM data frame
explan <- read.table(file.choose(),T) #Explanatory variables
```

**Standardizatons/Transformations**

```
## Transform EM relative abundance dataset into a presence/absence matrix
emfreq <- ifelse(emra>0, 1, 0)
## Remove rare species: in less than 5% of the root cores over the sampling peroid
emra.1 <- vegtab(emra, min=(.05*150)) #EM RA dataset w/o rare species
dim(emra.1)
[1] 150  14
emfreq.1 <- vegtab(emfreq, min=(.05*150)) #EM P/A dataset w/o rare species
dim(emfreq.1)
[1] 150  14
## Standardize continuous explanatory variables
e.stand <- explan[, c(3, 5:8)] # Standardizing VWC, EMCol, SoilpH, PO4P, CN, C, N.
e.stand1 <- decostand(e.stand, method="range")
explan.stand <- cbind(explan[,c(1:2,4,9:10)], e.stand1) #Combine standardized continuous variables with categorical variables and core x-y coordinates.
```

**Distance Matricies**

```
emra.1.dist <- vegdist(emra.1, method="bray")
emfreq.1.dist <- vegdist(emfreq.1, method="bray")
```

**PERMANOVA Frequency**

```
adonis(emfreq.1.dist ~ CN, data=explan.stand, permutations=9999)

Call:
adonis(formula = emfreq.1.dist ~ CN, data = explan.stand, permutations = 9999)

          Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
CN         1    0.4617 0.46174  3.1107 0.02059 0.0085 **
Residuals 148   21.9690 0.14844         0.97941
Total     149   22.4307                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1 Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
adonis(emfreq.1.dist ~ PO4P, data=explan.stand, permutations=9999)

Call:
adonis(formula = emfreq.1.dist ~ PO4P, data = explan.stand, permutations = 9999)

          Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
PO4P       1    0.3967 0.39669  2.6645 0.01769 0.0272 *
Residuals 148   22.0340 0.14888         0.98231
Total     149   22.4307                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
adonis(emfreq.1.dist ~ VWC*PO4P, data=explan.stand, permutations=9999)

Call:
  adonis(formula = emfreq.1.dist ~ VWC * PO4P, data = explan.stand,      permutations = 9999)
```

```
Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
VWC         1    0.4030 0.40297 2.73397 0.01796 0.0245 *
  PO4P      1    0.4357 0.43569 2.95601 0.01942 0.0133 *
  VWC:PO4P  1    0.0728 0.07284 0.49416 0.00325 0.7596
Residuals 146   21.5192 0.14739         0.95936
Total     149   22.4307                 1.00000
---
  Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
adonis(emfreq.1.dist ~ VWC, data=explan.stand, permutations=9999)

Call:
adonis(formula = emfreq.1.dist ~ VWC, data = explan.stand, permutations = 9999)

          Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
VWC         1     0.403 0.40297  2.7074 0.01796 0.0271 *
Residuals 148    22.028 0.14884         0.98204
Total     149    22.431                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
 adonis(emfreq.1.dist ~ VWC*as.factor(Month), data=explan.stand, permutations=9999)

Call:
  adonis(formula = emfreq.1.dist ~ VWC * as.factor(Month), data = explan.stand,      permutations = 9999)

Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
VWC                   1    0.4030 0.40297  2.9360 0.01796 0.0150 *
  as.factor(Month)    4    2.1911 0.54778  3.9911 0.09768 0.0001 ***
  VWC:as.factor(Month)  4    0.6217 0.15543  1.1325 0.02772 0.3260
Residuals           140   19.2149 0.13725         0.85663
Total               149   22.4307                 1.00000
---
  Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
 adonis(emfreq.1.dist ~ SoilpH, data=explan.stand, permutations=9999)

Call:
adonis(formula = emfreq.1.dist ~ SoilpH, data = explan.stand,      permutations = 9999)

          Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
SoilpH      1    0.5539 0.55391  3.7473 0.02469 0.0026 **
Residuals 148   21.8768 0.14782         0.97531
Total     149   22.4307                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
adonis(emfreq.1.dist ~ Frankia, data=explan.stand, permutations=9999)

Call:
adonis(formula = emfreq.1.dist ~ Frankia, data = explan.stand,      permutations = 9999)

          Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
Frankia     1    1.0861 1.08611  7.5309 0.04842  1e-04 ***
Residuals 148   21.3446 0.14422         0.95158
Total     149   22.4307                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
adonis(emfreq.1.dist ~ EMCol, data=explan.stand, permutations=9999)
```

```
Call:
adonis(formula = emfreq.1.dist ~ EMCol, data = explan.stand,      permutations = 9999)

          Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
EMCol      1    1.3135 1.31345  9.2053 0.05856  1e-04 ***
Residuals 148   21.1173 0.14268         0.94144
Total     149   22.4307                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
adonis(emfreq.1.dist ~ as.factor(Month), data=explan,stand, permutations=9999)

Call:
adonis(formula = emfreq.1.dist ~ as.factor(Month), data = explan.stand,      permutations = 9999)

                Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
as.factor(Month)   4    2.2574 0.56436  4.0565 0.10064  1e-04 ***
Residuals        145   20.1733 0.13913         0.89936
Total            149   22.4307                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
adonis(emfreq.1.dist ~ as.factor(TreeID), data=explan.stand, permutations=9999)

Call:
adonis(formula = emfreq.1.dist ~ as.factor(TreeID), data = explan.stand,      permutations = 9999)

                 Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
as.factor(TreeID)  29    4.6307 0.15968  1.0765 0.20645 0.3053
Residuals         120   17.8000 0.14833         0.79355
Total             149   22.4307                 1.00000

adonis(emfreq.1.dist ~ C, data=explan.stand, permutations=9999)

Call:
adonis(formula = emfreq.1.dist ~ C, data = explan.stand, permutations = 9999)

Terms added sequentially (first to last)

          Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
C          1    0.3961 0.39607  2.6603 0.01766 0.0245 *
Residuals 148   22.0347 0.14888         0.98234
Total     149   22.4307                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

adonis(emfreq.1.dist ~ N, data=explan.stand, permutations=9999)

Call:
adonis(formula = emfreq.1.dist ~ N, data = explan.stand, permutations = 9999)

Terms added sequentially (first to last)

          Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
N          1    0.3796 0.37964   2.548 0.01692 0.0308 *
Residuals 148   22.0511 0.14899         0.98308
Total     149   22.4307                 1.00000
```

---
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

**CLUSTER**

**##Cluster analysis with EM frequency data**

```
emfreq.1.clust <- hclust(emfreq.1.dist, method="ward") # presence and absence data
plot(emfreq.1.clust)
heights <- cbind(emfreq.1.clust$height, emfreq.1.clust$merge)
colnames(heights) <- c("height", "joinsthis", "withthis")
heights
```

| | height | joinsthis | withthis |
|---|---|---|---|
| [1,] | 0.00000000 | -1 | -23 |
| [2,] | 0.00000000 | -26 | 1 |
| [3,] | 0.00000000 | -44 | 2 |
| [4,] | 0.00000000 | -106 | 3 |
| [5,] | 0.00000000 | -2 | -62 |
| [6,] | 0.00000000 | -5 | -54 |
| [7,] | 0.00000000 | -84 | 6 |
| [8,] | 0.00000000 | -92 | 7 |
| [9,] | 0.00000000 | -131 | 8 |
| [10,] | 0.00000000 | -144 | 9 |
| [11,] | 0.00000000 | -6 | -102 |
| [12,] | 0.00000000 | -9 | -14 |
| [13,] | 0.00000000 | -30 | 12 |
| [14,] | 0.00000000 | -96 | 13 |
| [15,] | 0.00000000 | -114 | 14 |
| [16,] | 0.00000000 | -15 | -49 |
| [17,] | 0.00000000 | -66 | 16 |
| [18,] | 0.00000000 | -16 | -21 |
| [19,] | 0.00000000 | -24 | -35 |
| [20,] | 0.00000000 | -27 | -83 |
| [21,] | 0.00000000 | -34 | -39 |
| [22,] | 0.00000000 | -87 | 21 |
| [23,] | 0.00000000 | -91 | 22 |
| [24,] | 0.00000000 | -36 | -86 |
| [25,] | 0.00000000 | -41 | -64 |
| [26,] | 0.00000000 | -75 | 25 |
| [27,] | 0.00000000 | -88 | 26 |
| [28,] | 0.00000000 | -42 | -127 |
| [29,] | 0.00000000 | -48 | -121 |
| [30,] | 0.00000000 | -57 | -89 |
| [31,] | 0.00000000 | -58 | -76 |
| [32,] | 0.00000000 | -85 | 31 |
| [33,] | 0.00000000 | -130 | 32 |
| [34,] | 0.00000000 | -146 | 33 |
| [35,] | 0.00000000 | -69 | -82 |
| [36,] | 0.00000000 | -94 | -124 |
| [37,] | 0.00000000 | -111 | -134 |
| [38,] | 0.00000000 | -122 | -139 |
| [39,] | 0.00000000 | -137 | -138 |
| [40,] | 0.00000000 | -140 | -147 |
| [41,] | 0.00000000 | -141 | -142 |
| [42,] | 0.09090909 | -71 | -133 |
| [43,] | 0.09090909 | -74 | -143 |
| [44,] | 0.09090909 | -105 | -115 |

```
 [45,] 0.09090909 -117 -120
 [46,] 0.09090909 -132 -148
 [47,] 0.11111111   -4 -150
 [48,] 0.11111111  -10 -126
 [49,] 0.11111111  -47  -60
 [50,] 0.11111111  -56  -77
 [51,] 0.11111111  -79  -95
 [52,] 0.11111111  -99 -125
 [53,] 0.11111111 -104 -108
 [54,] 0.14141414 -149   46
 [55,] 0.14285714   -3  -93
 [56,] 0.14285714   -7   -8
 [57,] 0.14285714  -11  -65
 [58,] 0.14285714  -17  -78
 [59,] 0.14285714  -37  -80
 [60,] 0.14285714  -38  -61
 [61,] 0.14285714  -40  -50
 [62,] 0.14285714  -53 -128
 [63,] 0.14285714  -68 -107
 [64,] 0.14285714  -97 -103
 [65,] 0.14814815 -112   28
 [66,] 0.14814815 -116   37
 [67,] 0.14814815 -145   40
 [68,] 0.16666667  -19   17
 [69,] 0.16666667  -13  -51
 [70,] 0.16666667  -22 -109
 [71,] 0.17037037  -81   50
 [72,] 0.17710438  -67   43
 [73,] 0.17710438  -70   42
 [74,] 0.19047619 -100   36
 [75,] 0.19747475 -118   54
 [76,] 0.20000000  -18  -46
 [77,] 0.20000000  -20  -63
 [78,] 0.20000000  -28 -110
 [79,] 0.20000000  -31  -55
 [80,] 0.20000000  -45  -52
 [81,] 0.21428571  -73   60
 [82,] 0.21428571 -129   61
 [83,] 0.22486772  -90   49
 [84,] 0.23082011 -123   65
 [85,] 0.23214286 -113   81
 [86,] 0.25000000   48   63
 [87,] 0.25396825  -72   59
 [88,] 0.25589226 -119   47
 [89,] 0.25885522   41   72
 [90,] 0.26565657   30   44
 [91,] 0.28571429   29   38
 [92,] 0.30000000 -136   69
 [93,] 0.30793651  -32   55
 [94,] 0.30793651  -98   57
 [95,] 0.33333333  -12 -101
 [96,] 0.33333333 -135   19
 [97,] 0.33730159   24   53
 [98,] 0.38461538  -29  -33
 [99,] 0.39020979  -25   78
[100,] 0.40000000   35   76
```

```
[101,] 0.40761905  51  96
[102,] 0.40816327  34  39
[103,] 0.42857143 -43 -59
[104,] 0.42974877  77  88
[105,] 0.46190476   5  56
[106,] 0.46857809  70  92
[107,] 0.47157287  45  86
[108,] 0.47619048  64  80
[109,] 0.48275132  52  83
[110,] 0.49760015  66  84
[111,] 0.53333333  11  23
[112,] 0.54289494  71  97
[113,] 0.54349206  79  93
[114,] 0.57601090  90 101
[115,] 0.60714286  18  62
[116,] 0.61335331  99 103
[117,] 0.62207792  58 107
[118,] 0.63041403  82  98
[119,] 0.64463856  73  85
[120,] 0.67261905  68 100
[121,] 0.70483726  75  89
[122,] 0.78039121  67 114
[123,] 0.85646259 110 112
[124,] 0.86349206  95 105
[125,] 0.86717071  87 117
[126,] 0.88253968  20  94
[127,] 0.92923410 104 106
[128,] 0.96000000  10  27
[129,] 1.00343915 115 126
[130,] 1.02645503  15  91
[131,] 1.03512876  74 118
[132,] 1.06311780 109 121
[133,] 1.19684284 102 124
[134,] 1.24373898 108 123
[135,] 1.29963777 113 127
[136,] 1.45901030 116 129
[137,] 1.65112031 111 119
[138,] 1.91602564 120 133
[139,] 1.95119159 128 131
[140,] 2.08815066 135 136
[141,] 2.15401371 122 132
[142,] 2.29327286   4 130
[143,] 2.47551373 137 139
[144,] 2.57860678 125 140
[145,] 3.43223538 138 141
[146,] 3.80346535 142 143
[147,] 4.12895183 134 145
[148,] 4.86457308 146 147
[149,] 6.37280236 144 148
scree <- plot(x=12:1, y=heights[(nrow(heights)-11):nrow(heights),1], xlab="Number of groups",
ylab="Height")
emfreq.1.clust.groups <- cutree(emfreq.1.clust, k=2)
plot(emfreq.1.clust)
rect.hclust(emfreq.1.clust, k=2, border="red")
```

**NMDS ORDINATION**

```
##Ordination using EM frequency data excluding rares
emfreq.1.nmds <- metaMDS(comm= emfreq.1, autotransform=FALSE, distance="bray", k=2,
engine="monoMDS", model="global", trymax=40, wascores=FALSE)
Run 0 stress 0.2717981
Run 1 stress 0.2725235
Run 2 stress 0.2769505
Run 3 stress 0.2712291
... New best solution
... procrustes: rmse 0.02113137  max resid 0.1583418
Run 4 stress 0.2844398
Run 5 stress 0.2896056
Run 6 stress 0.2956482
Run 7 stress 0.2799363
Run 8 stress 0.2975454
Run 9 stress 0.2727134
Run 10 stress 0.2746116
Run 11 stress 0.2913287
Run 12 stress 0.2723191
Run 13 stress 0.2843834
Run 14 stress 0.2735115
Run 15 stress 0.2831195
Run 16 stress 0.2771372
Run 17 stress 0.2779555
Run 18 stress 0.2727268
Run 19 stress 0.2739546
Run 20 stress 0.2731729
Run 21 stress 0.2929157
Run 22 stress 0.2721504
Run 23 stress 0.2830987
Run 24 stress 0.2728466
Run 25 stress 0.2728026
Run 26 stress 0.2794243
Run 27 stress 0.2770402
Run 28 stress 0.2728083
Run 29 stress 0.2897951
Run 30 stress 0.2752108
Run 31 stress 0.2853868
Run 32 stress 0.2871771
Run 33 stress 0.2720011
Run 34 stress 0.2798539
Run 35 stress 0.2962502
Run 36 stress 0.271428
... procrustes: rmse 0.01957089  max resid 0.1566983
Run 37 stress 0.2846353
Run 38 stress 0.2756913
Run 39 stress 0.2935781
Run 40 stress 0.2721261
emfreq.sp <- wascores(x=emfreq.1.nmds$points, w=emfreq.1, expand=TRUE)
emfreq.sp
              MDS1        MDS2
Dummy    0.01471600  0.13787046
Cort1    0.61286303 -0.03012039
Nauesc  -0.23472954 -0.76630647
Tom2.KH -0.47196319 -0.18283434
Nau2     0.41023178 -0.37889181
```

```
Nau3    -0.46957474  0.46207356
Lact1   -0.82914468  0.09444094
Tom5.KH  0.20086816 -0.20528728
Pstom   -0.29084822 -0.31745401
Tom3.KH  0.23057523 -0.38290068
Tom4.KH -0.09482048  0.56156577
Lact2   -0.08526496 -0.35163639
Tom3    -0.09208475  0.11577648
Tuber    0.39211771  0.13662342
attr(,"shrinkage")
    MDS1      MDS2
0.1911138 0.1363097
attr(,"centre")
      MDS1        MDS2
-0.01143024 -0.08069449
plot(emfreq.1.nmds, type = "n", display = "sites")
## Root cores are labeled on whether they were grouped into cluster 1 or 2 from cluster analysis and hulls
applied around each clustered group.
points(emfreq.1.nmds$points[emfreq.1.clust.groups == "2",], pch=3, col="black")
points(emfreq.1.nmds$points[emfreq.1.clust.groups == "1",], pch=1, col="black")
legend("topright", legend = paste("Cluster", 1:2),  bty = "n", pch = c(1,3))
ordihull(emfreq.1.nmds, groups = emfreq.1.clust.groups, display = "sites")

## NMDS plot with soil vectors
plot(emfreq.1.nmds, display="sites",type="p", main="NMDS of EM Frequency",xlab="Axis 1", xaxt="n",
ylab="Axis 2", yaxt="n")
frankia <- envfit(emfreq.1.nmds ~ Frankia, data=explan.stand)
vwc <- envfit(emfreq.1.nmds ~ VWC, data=explan.stand)
cn <- envfit(emfreq.1.nmds ~ CN, data=explan.stand)
phos <- envfit(emfreq.1.nmds ~ PO4P, data=explan.stand)
ph <- envfit(emfreq.1.nmds ~ SoilpH, data=explan.stand)
N <- envfit(emfreq.1.nmds ~ N, data=explan.stand)
C <- envfit(emfreq.1.nmds ~ C, data=explan.stand)
plot(ph, col="black", cex=.75)
plot(frankia, col="black", cex=.75)
plot(vwc, col="black", cex=.75)
plot(phos, col=”black”, cex=.75)
plot(cn, col="black", cex=.75)
plot(N, col="black", cex=.75)
 plot(C, col="black", cex=.75)

## Visualizing common species that explained clustered groups
plot(emfreq.1.nmds, "sites", type = "n", main = "", xlab="Axis 1", ylab="Axis 2", xaxt="n", yaxt="n")
plot(ph, col="black", cex=.75)
plot(frankia, col="black",  cex=.75)
plot(vwc, col="black", cex=.75)
plot(phos, col= "black", cex=.75)
plot(cn, col="black", cex=.75)
plot(N, col="black", cex=.75)
plot(C, col="black", cex=.75)
points(emfreq.1.nmds, "sites", cex =.5, pch=16)
points(emfreq.1.nmds, "sites", cex = emfreq.1[,"Cort1"] * 2.5, col="green", pch=1)
 points(emfreq.1.nmds, "sites", cex = emfreq.1[,"Nauesc"] * 2, col="blue", pch=2)
 legend(x="topright", pch=c(1,2), col=c("green","blue"), legend=c("Cortinarius cf.
alboviolaceous","Naucoria escharoides"), cex=.6)
```

```
## same EM frequency data NMDS ordination highlighting Frankia presence/absence
plot(emfreq.1.nmds, display="sites",type="n", main="NMDS of EM Frequency ",xlab="Axis 1", xaxt="n",
ylab="Axis 2", yaxt="n")
 points(emfreq.1.nmds$points[explan$Frankia == "0",], pch=1)
 points(emfreq.1.nmds$points[explan$Frankia == "1",], pch=15)
plot(ph, col="black", cex=.75)
plot(frankia, col="black", cex=.75)
plot(vwc, col="black", cex=.75)
plot(phos, col=”black”, cex=.75)
plot(cn, col="black", cex=.75)
plot(N, col="black", cex=.75)
plot(C, col="black", cex=.75)
legend(x="bottomright", pch=c(1,15), col=”black”, legend=c("Frankia Absent", "Frankia Present"),
cex=0.75)

## EM frequency data NMDS ordination but highlighting May and September
plot(emfreq.1.nmds, display="sites",type="n", main="",xlab="Axis 1", xaxt="n", ylab="Axis 2", yaxt="n")
points(emfreq.1.nmds$points[explan$Month == "1",], pch=1)
points(emfreq.1.nmds$points[explan$Month == "5",], pch=5)
plot(ph, col="black", cex=.75)
plot(frankia, col="black", cex=.75)
plot(vwc, col="black", cex=.75)
plot(phos, col=”black”, cex=.75)
plot(cn, col="black", cex=.75)
plot(N, col="black", cex=.75)
plot(C, col="black", cex=.75)

## same EM frequency data NMDS ordination but highlighting June and July
plot(emfreq.1.nmds, display="sites",type="n", main="",xlab="Axis 1", xaxt="n", ylab="Axis 2", yaxt="n")
points(emfreq.1.nmds$points[explan$Month == "2",], pch=2)
points(emfreq.1.nmds$points[explan$Month == "3",], pch=3)
plot(ph, col="black", cex=.75)
plot(frankia, col="black", cex=.75)
plot(vwc, col="black", cex=.75)
plot(phos, col=”black”, cex=.75)
plot(cn, col="black", cex=.75)
plot(N, col="black", cex=.75)
plot(C, col="black", cex=.75)
```

**FREQUENCY OF OCCURRENCE**

```
specnumber(emra, MARGIN = 2) # Frequency of occurrence
  Clav1   Clav2   Cort1   Cort2     Ent    Ino1    Ino2   Lact1   Lact2  Nauesc
     4       2      84       7       1       6       4      32      18      65
   Nau2    Nau3   Pstom    Tarz    Tom1    Tom2 Tom2.KH    Tom3 Tom3.KH Tom4.KH
     33      33      26       3       3       5      41      12      25      25
Tom5.KH   Tuber   Dummy
     30       9     150
```

**SPECIES RICHNESS**

```
##Shannon Diversity Indicies
diversity(emfreq[1:30,1:22], index="shannon")#May Shannon Diversity
01_05     02_05     03_05     04_05     05_05     06_05
0.0000000 0.0000000 0.6931472 1.0986123 0.0000000 0.0000000
   07_05     08_05     09_05     10_05     11_05     12_05
1.0986123 1.0986123 0.0000000 1.3862944 1.0986123 1.0986123
```

```
   13_05     14_05     15_05     16_05     17_05     18_05
1.3862944 0.0000000 1.3862944 1.0986123 0.6931472 1.3862944
   19_05     20_05     21_05     22_05     23_05     24_05
1.0986123 1.0986123 1.0986123 1.6094379 0.0000000 1.3862944
   25_05     26_05     27_05     28_05     29_05     30_05
1.7917595 0.0000000 1.0986123 1.6094379 1.9459101 0.0000000
 summary(diversity(emfreq[1:30,1:22], index="shannon"))
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
 0.0000  0.0000  1.0990  0.8753  1.3860  1.9460

 diversity(emfreq[31:60,1:22], index="shannon")#June Shannon Diversity
   01_06     02_06     03_06     04_06     05_06     06_06
0.6931472 0.6931472 1.6094379 0.6931472 0.6931472 0.6931472
   07_06     08_06     09_06     10_06     11_06     12_06
1.9459101 0.6931472 0.6931472 1.0986123 0.6931472 1.3862944
   13_06     14_06     15_06     16_06     17_06     18_06
0.6931472 0.0000000 0.6931472 1.3862944 1.0986123 1.0986123
   19_06     20_06     21_06     22_06     23_06     24_06
1.3862944 1.0986123 1.7917595 0.6931472 0.6931472 0.6931472
   25_06     26_06     27_06     28_06     29_06     30_06
1.0986123 1.3862944 1.0986123 0.6931472 1.0986123 1.3862944
 summary(diversity(emfreq[31:60,1:22], index="shannon"))
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
 0.0000  0.6931  0.8959  0.9891  1.3140  1.9460

 diversity(emfreq[61:90,1:22], index="shannon")#July Shannon Diversity
   01_07     02_07     03_07     04_07     05_07     06_07
1.0986123 0.0000000 1.6094379 0.6931472 0.6931472 1.3862944
   07_07     08_07     09_07     10_07     11_07     12_07
1.0986123 0.6931472 0.6931472 1.3862944 1.6094379 1.6094379
   13_07     14_07     15_07     16_07     17_07     18_07
1.0986123 1.3862944 0.6931472 0.6931472 1.3862944 1.0986123
   19_07     20_07     21_07     22_07     23_07     24_07
1.0986123 1.6094379 1.3862944 0.6931472 1.0986123 0.0000000
   25_07     26_07     27_07     28_07     29_07     30_07
0.6931472 0.6931472 0.6931472 0.6931472 1.0986123 0.6931472
 summary(diversity(emfreq[61:90,1:22], index="shannon"))
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
 0.0000  0.6931  1.0990  0.9792  1.3860  1.6090

 diversity(emfreq[91:120,1:22], index="shannon")#August Shannon Diversity
   01_08     02_08     03_08     04_08     05_08     06_08
1.3862944 0.0000000 1.0986123 0.6931472 1.3862944 0.0000000
   07_08     08_08     09_08     10_08     11_08     12_08
1.0986123 1.6094379 1.0986123 1.3862944 0.0000000 1.0986123
   13_08     14_08     15_08     16_08     17_08     18_08
1.3862944 1.3862944 1.6094379 0.0000000 1.3862944 1.3862944
   19_08     20_08     21_08     22_08     23_08     24_08
1.6094379 1.0986123 1.3862944 1.3862944 1.3862944 0.0000000
   25_08     26_08     27_08     28_08     29_08     30_08
1.3862944 1.0986123 1.6094379 1.0986123 1.7917595 1.3862944
 summary(diversity(emfreq[91:120,1:22], index="shannon"))
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
  0.000   1.099   1.386   1.108   1.386   1.792

 diversity(emfreq[121:150,1:22], index="shannon")#September Shannon Diversity
```

```
   01_09     02_09     03_09     04_09     05_09     06_09
1.0986123 1.3862944 0.6931472 1.0986123 1.6094379 1.3862944
   07_09     08_09     09_09     10_09     11_09     12_09
1.0986123 1.0986123 1.0986123 0.6931472 1.0986123 1.6094379
   13_09     14_09     15_09     16_09     17_09     18_09
1.6094379 1.3862944 1.3862944 1.3862944 1.0986123 1.0986123
   19_09     20_09     21_09     22_09     23_09     24_09
0.6931472 1.0986123 1.6094379 1.6094379 1.6094379 1.0986123
   25_09     26_09     27_09     28_09     29_09     30_09
1.6094379 0.6931472 1.0986123 1.6094379 1.6094379 1.3862944
 summary(diversity(emfreq[121:150,1:22], index="shannon"))
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
 0.6931  1.0990  1.2420  1.2550  1.6090  1.6090

 ##Simpson Diversity Indicies
 diversity(emfreq[1:30,1:22], index="simpson") #May Simpson Diversity
   01_05     02_05     03_05     04_05     05_05     06_05
1.0000000 0.0000000 0.5000000 0.6666667 0.0000000 0.0000000
   07_05     08_05     09_05     10_05     11_05     12_05
0.6666667 0.6666667 0.0000000 0.7500000 0.6666667 0.6666667
   13_05     14_05     15_05     16_05     17_05     18_05
0.7500000 0.0000000 0.7500000 0.6666667 0.5000000 0.7500000
   19_05     20_05     21_05     22_05     23_05     24_05
0.6666667 0.6666667 0.6666667 0.8000000 1.0000000 0.7500000
   25_05     26_05     27_05     28_05     29_05     30_05
0.8333333 1.0000000 0.6666667 0.8000000 0.8571429 0.0000000
 summary(diversity(emfreq[1:30,1:22], index="simpson"))
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
 0.0000  0.5417  0.6667  0.5902  0.7500  1.0000

diversity(emfreq[31:60,1:22], index="simpson")#June Simpson Diversity
   01_06     02_06     03_06     04_06     05_06     06_06
0.5000000 0.5000000 0.8000000 0.5000000 0.5000000 0.5000000
   07_06     08_06     09_06     10_06     11_06     12_06
0.8571429 0.5000000 0.5000000 0.6666667 0.5000000 0.7500000
   13_06     14_06     15_06     16_06     17_06     18_06
0.5000000 1.0000000 0.5000000 0.7500000 0.6666667 0.6666667
   19_06     20_06     21_06     22_06     23_06     24_06
0.7500000 0.6666667 0.8333333 0.5000000 0.5000000 0.5000000
   25_06     26_06     27_06     28_06     29_06     30_06
0.6666667 0.7500000 0.6666667 0.5000000 0.6666667 0.7500000
 summary(diversity(emfreq[31:60,1:22], index="simpson"))
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
 0.5000  0.5000  0.6667  0.6302  0.7500  1.0000

 diversity(emfreq[61:90,1:22], index="simpson")#July Simpson Diversity
   01_07     02_07     03_07     04_07     05_07     06_07
0.6666667 0.0000000 0.8000000 0.5000000 0.5000000 0.7500000
   07_07     08_07     09_07     10_07     11_07     12_07
0.6666667 0.5000000 0.5000000 0.7500000 0.8000000 0.8000000
   13_07     14_07     15_07     16_07     17_07     18_07
0.6666667 0.7500000 0.5000000 0.5000000 0.7500000 0.6666667
   19_07     20_07     21_07     22_07     23_07     24_07
0.6666667 0.8000000 0.7500000 0.5000000 0.6666667 0.0000000
   25_07     26_07     27_07     28_07     29_07     30_07
0.5000000 0.5000000 0.5000000 0.5000000 0.6666667 0.5000000
```

```
 summary(diversity(emfreq[61:90,1:22], index="simpson"))
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
 0.0000  0.5000  0.6667  0.5872  0.7500  0.8000

 diversity(emfreq[91:120,1:22], index="simpson")#August Simpson Diversity
    01_08     02_08     03_08     04_08     05_08     06_08
0.7500000 0.0000000 0.6666667 0.5000000 0.7500000 0.0000000
    07_08     08_08     09_08     10_08     11_08     12_08
0.6666667 0.8000000 0.6666667 0.7500000 0.0000000 0.6666667
    13_08     14_08     15_08     16_08     17_08     18_08
0.7500000 0.7500000 0.8000000 1.0000000 0.7500000 0.7500000
    19_08     20_08     21_08     22_08     23_08     24_08
0.8000000 0.6666667 0.7500000 0.7500000 0.7500000 0.0000000
    25_08     26_08     27_08     28_08     29_08     30_08
0.7500000 0.6666667 0.8000000 0.6666667 0.8333333 0.7500000
 summary(diversity(emfreq[91:120,1:22], index="simpson"))
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
 0.0000  0.6667  0.7500  0.6400  0.7500  1.0000

diversity(emfreq[121:150,1:22], index="simpson")#September Simpson Diversity
    01_09     02_09     03_09     04_09     05_09     06_09
0.6666667 0.7500000 0.5000000 0.6666667 0.8000000 0.7500000
    07_09     08_09     09_09     10_09     11_09     12_09
0.6666667 0.6666667 0.6666667 0.5000000 0.6666667 0.8000000
    13_09     14_09     15_09     16_09     17_09     18_09
0.8000000 0.7500000 0.7500000 0.7500000 0.6666667 0.6666667
    19_09     20_09     21_09     22_09     23_09     24_09
0.5000000 0.6666667 0.8000000 0.8000000 0.8000000 0.6666667
    25_09     26_09     27_09     28_09     29_09     30_09
0.8000000 0.5000000 0.6666667 0.8000000 0.8000000 0.7500000
summary(diversity(emfreq[121:150,1:22], index="simpson"))
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
 0.5000  0.6667  0.7083  0.7011  0.8000  0.8000
```

**SPECIES ACCUMULATION CURVES**

```
plot(specaccum(mayem, method="random", permutations=100, gama="chao"), main="", xlab="Root
Cores", ylab="EMF Species", xlim=c(0,30), ylim=c(0,25), col="red")
plot(specaccum(juneem, method="random", permutations=100, gama="chao"), add=TRUE, col="orange")
plot(specaccum(julyem, method="random", permutations=100, gama="chao"), add=TRUE, col="green")
plot(specaccum(augustem, method="random", permutations=100, gama="chao"), add=TRUE, col="blue")
plot(specaccum(septemberem, method="random", permutations=100, gama="chao"), add=TRUE,
col="purple")
legend(x="bottomright", pch=19, col=c("red", "orange", "green", "blue", "purple"), legend=c("May",
"June", "July", "August", "September"), cex=0.75)
```

**MANTEL**

```
##EMF frequency dataset vs all EMF core locations using Pearson method
mantel(emfreq.1.dist, coord.dist, method="pearson", permutations = 9999)
Mantel statistic based on Pearson's product-moment correlation
Call:
mantel(xdis = emfreq.1.dist, ydis = coord.dist, method = "pearson",      permutations = 9999)
Mantel statistic r: 0.01484
      Significance: 0.2153
Empirical upper confidence limits of r:
   90%    95%  97.5%    99%
0.0246 0.0318 0.0390 0.0471
```

```
Based on 9999 permutations

##May EM vs May core locations
mayfreq <- emfreq.1[c(1:30),]
maycore <- coord[c(1:30),]
mayfreq.dist <- vegdist(mayfreq, method="bray")
maycore.dist <- vegdist(maycore, method="euc")
mantel(mayfreq.dist, maycore.dist, method="pearson", permutations = 9999)
Mantel statistic based on Pearson's product-moment correlation
Call:
mantel(xdis = mayfreq.dist, ydis = maycore.dist, method = "pearson",      permutations = 9999)
Mantel statistic r: 0.07771
      Significance: 0.0788
Empirical upper confidence limits of r:
  90%    95%  97.5%    99%
0.0692 0.0931 0.1137 0.1416
Based on 9999 permutations

##June EM vs June core locations
junefreq <- emfreq.1[c(31:60),]
junecore <- coord[c(31:60),]
junefreq.dist <- vegdist(junefreq, method="bray")
junecore.dist <- vegdist(junecore, method="euc")
mantel(junefreq.dist, junecore.dist, method="pearson", permutations = 9999)
Mantel statistic based on Pearson's product-moment correlation
Call:
mantel(xdis = junefreq.dist, ydis = junecore.dist, method = "pearson",      permutations = 9999)
Mantel statistic r: 0.002689
      Significance: 0.4688
Empirical upper confidence limits of r:
  90%    95%  97.5%    99%
0.0871 0.1138 0.1380 0.1639
Based on 9999 permutations

##July EM vs July core locations
julyfreq <- emfreq.1[c(61:90),]
junecore <- coord[c(61:90),]
julycore <- coord[c(61:90),]
julycore.dist <- vegdist(julycore, method="euc")
julyfreq.dist <- vegdist(julyfreq, method="bray")
mantel(julyfreq.dist, julycore.dist, method="pearson", permutations = 9999)
Mantel statistic based on Pearson's product-moment correlation
Call:
mantel(xdis = julyfreq.dist, ydis = julycore.dist, method = "pearson",      permutations = 9999)
Mantel statistic r: 0.0645
      Significance: 0.1543
Empirical upper confidence limits of r:
  90%    95%  97.5%    99%
0.0839 0.1093 0.1339 0.1617
Based on 9999 permutations

##August EM vs August core locations
augfreq <- emfreq.1[c(91:120),]
augcore <- coord[c(91:120),]
augfreq.dist <- vegdist(augfreq, method="bray")
augcore.dist <- vegdist(augcore, method="euc")
```

```
mantel(augfreq.dist, augcore.dist, method="pearson", permutations = 9999)
Mantel statistic based on Pearson's product-moment correlation
Call:
mantel(xdis = augfreq.dist, ydis = augcore.dist, method = "pearson",      permutations = 9999)
Mantel statistic r: 0.05422
     Significance: 0.1427
Empirical upper confidence limits of r:
  90%    95%  97.5%    99%
0.0672 0.0907 0.1096 0.1361
Based on 9999 permutations

##September vs September core locations
septfreq <- emfreq.1[c(121:150),]
septcore <- coord[c(121:150),]
septfreq.dist <- vegdist(septfreq, method="bray")
septcore.dist <- vegdist(septcore, method="euc")
mantel(septfreq.dist, septcore.dist, method="pearson", permutations = 9999)
Mantel statistic based on Pearson's product-moment correlation
Call:
mantel(xdis = septfreq.dist, ydis = septcore.dist, method = "pearson",      permutations = 9999)
Mantel statistic r: 0.03926
     Significance: 0.2676
Empirical upper confidence limits of r:
  90%    95%  97.5%    99%
0.0843 0.1090 0.1311 0.1625
Based on 9999 permutations
```

**INDICATOR SPECIES**

```
## With the EM frequency dataset
indval(x=emfreq.1, clustering=emfreq.1.clust.groups, numirt=1000)
$relfrq
             1     2
Dummy   1.00000000 1.000
Cort1   0.71818182 0.125
Nauesc  0.38181818 0.575
Tom2.KH 0.22727273 0.400
Nau2    0.23636364 0.175
Nau3    0.17272727 0.350
Lact1   0.07272727 0.600
Tom5.KH 0.20000000 0.200
Pstom   0.10000000 0.375
Tom3.KH 0.19090909 0.100
Tom4.KH 0.11818182 0.300
Lact2   0.08181818 0.225
Tom3    0.06363636 0.125
Tuber   0.07272727 0.025

$relabu
             1         2
Dummy   0.5000000 0.5000000
Cort1   0.8517520 0.1482480
Nauesc  0.3990499 0.6009501
Tom2.KH 0.3623188 0.6376812
Nau2    0.5745856 0.4254144
Nau3    0.3304348 0.6695652
Lact1   0.1081081 0.8918919
```

```
Tom5.KH 0.5000000 0.5000000
Pstom   0.2105263 0.7894737
Tom3.KH 0.6562500 0.3437500
Tom4.KH 0.2826087 0.7173913
Lact2   0.2666667 0.7333333
Tom3    0.3373494 0.6626506
Tuber   0.7441860 0.2558140

$indval
                1           2
Dummy   0.500000000 0.500000000
Cort1   0.611712815 0.018530997
Nauesc  0.152364500 0.345546318
Tom2.KH 0.082345191 0.255072464
Nau2    0.135811150 0.074447514
Nau3    0.057075099 0.234347826
Lact1   0.007862408 0.535135135
Tom5.KH 0.100000000 0.100000000
Pstom   0.021052632 0.296052632
Tom3.KH 0.125284091 0.034375000
Tom4.KH 0.033399209 0.215217391
Lact2   0.021818182 0.165000000
Tom3    0.021467689 0.082831325
Tuber   0.054122622 0.006395349

$maxcls
  Dummy   Cort1  Nauesc Tom2.KH    Nau2    Nau3   Lact1 Tom5.KH   Pstom Tom3.KH
      1       1       2       2       1       2       2       1       2       1
Tom4.KH   Lact2    Tom3   Tuber
      2       2       2       1

$indcls
     Dummy      Cort1     Nauesc    Tom2.KH       Nau2       Nau3      Lact1
0.50000000 0.61171282 0.34554632 0.25507246 0.13581115 0.23434783 0.53513514
   Tom5.KH      Pstom    Tom3.KH    Tom4.KH      Lact2       Tom3      Tuber
0.10000000 0.29605263 0.12528409 0.21521739 0.16500000 0.08283133 0.05412262

$pval
  Dummy   Cort1  Nauesc Tom2.KH    Nau2    Nau3   Lact1 Tom5.KH   Pstom Tom3.KH
  1.000   0.001   0.032   0.035   0.659   0.033   0.001   1.000   0.001   0.325
Tom4.KH   Lact2    Tom3   Tuber
  0.011   0.026   0.227   0.460

attr(,"class")
[1] "indval"
> summary(indval(x=emfreq.1, clustering=emfreq.1.clust.groups, numirt=1000))
        cluster indicator_value probability
Cort1         1          0.6117       0.001
Lact1         2          0.5351       0.001
Nauesc        2          0.3455       0.034
Pstom         2          0.2961       0.001
Tom2.KH       2          0.2551       0.026
Nau3          2          0.2343       0.024
Tom4.KH       2          0.2152       0.011
Lact2         2          0.1650       0.023
```

Sum of probabilities = 3.748

Sum of Indicator Values = 3.66

Sum of Significant Indicator Values = 2.66

Number of Significant Indicators = 8

Significant Indicator Distribution

1 2
1 7

**TITAN**

### The relative abundance dataset (emra) was log transformed and had rare species in less than 5% of cores taken out. Uploaded separate files for each soil variable, but could have sampled the explanatory dataset (choosing certain columns) instead. Both would be identical. See Appendix of Baker and King (2010) for analysis codes.

```
emvwc <- em.vwc[c(1:21, 23:24, 26:42, 44:104, 106:145), ]
## Transforming relative abundance dataset
emra.nodummy <- emra[,1:22] #removing dummy variable used in other analyses
emtaxarare <- emra.nodummy[which(rowSums(emra.nodummy) > 0),] #removing rows with 0 species in
them
emtaxa.rare <- vegtab(emtaxarare, min=(0.05*145)) #removing species in <5% cores
emtaxa.log <- log10(emtaxa.rare + 1)# log10(x+1) transformation of matrix

##Log transformed full data set compared to soil moisture data
 emvwctitan <- titan(em.vwc, emtaxa.log, minsplt=5, numprm=250, nboot=500, boot=TRUE,
deviance=TRUE)
[1] "Taxa frequency screen complete"
[1] "Function definition complete"
[1] "IndVal z score calculation complete"
[1] "Deviance reduction calculation complete"
[1] "Begin bootstrap resampling..."
[1] "Bootstrap resampling complete"
[1] "TITAN complete"
       env.cp freq maxgrp IndVal  pval    z     5%   10%   50%   90%    95% purity
Cort1    13.5   84      2  37.05 0.236 0.65  7.925  9.90 27.00 72.55 82.000  0.496
Nauesc   73.0   65      1  38.39 0.108 1.40  8.000  9.90 46.00 74.50 79.500  0.716
Tom2.KH  50.0   41      2  26.87 0.012 3.27 15.000 18.00 46.00 58.00 64.550  0.962
Nau2     20.0   33      1  20.17 0.040 1.99  9.000 10.00 21.00 79.50 82.000  0.622
Nau3     84.5   33      2  40.86 0.092 1.86 18.950 21.00 73.00 84.50 84.500  0.852
Lact1    12.0   32      1  31.55 0.008 3.71 10.000 11.00 14.00 69.30 84.500  0.834
Tom5.KH  45.0   30      1  20.01 0.016 2.72  5.000  6.50 21.00 46.00 52.025  0.938
Pstom    66.5   26      2  31.70 0.004 4.49 31.000 46.00 63.00 79.50 84.500  0.984
Tom3.KH  36.0   25      1  20.67 0.004 4.20  5.000  6.35 25.00 36.00 39.500  0.962
Tom4.KH  82.0   25      2  53.88 0.004 5.50  5.000  5.00 74.00 84.50 84.500  0.536
Lact2    82.0   18      2  41.76 0.004 5.46 23.000 26.45 53.75 84.50 84.500  1.000
Tom3     82.0   12      2  27.21 0.064 2.65 11.000 11.50 54.50 84.50 84.500  0.818
Tuber    79.5    9      2  33.25 0.008 6.86 20.475 52.45 73.00 82.00 84.500  0.920
       rel05 rel01
Cort1   0.320 0.130
Nauesc  0.458 0.108
Tom2.KH 0.878 0.594
Nau2    0.516 0.284
```

```
Nau3    0.680 0.354
Lact1   0.766 0.542
Tom5.KH 0.862 0.604
Pstom   0.944 0.740
Tom3.KH 0.950 0.834
Tom4.KH 0.518 0.476
Lact2   1.000 0.980
Tom3    0.746 0.440
Tuber   0.872 0.688
        cp   0.05 0.10  0.50 0.90  0.95
sumz-     5  5.000  5.0 15.00 36.0 39.65
sumz+    82 45.975 49.0 80.00 84.5 84.50
ncpa.bc  20  8.000  9.9 25.25 82.0 84.50
ncpa.euc 20  8.000  9.9 21.00 82.0 84.50

##Plot with 80% purity and p value at 0.05
plot.taxaz(emvwctitan, boot=TRUE, xlab=expression("Soil Moisture (%)"), cex.taxa=.9, cex.axis=1.35,
cex=1.35, xmax=100, fil2="red", col2="red", pur.cut=0.80, pval.cut=0.05, rel05.cut=0.5, rel01.cut=.1)
$sppsub1
        env.cp freq maxgrp IndVal  pval    z     5% 10%50%  90%  95%      purity rel05
Lact1       12   32      1       31.55 0.008 3.71 10 11.00   14 69.3 84.500  0.834 0.766
Tom5.KH     45   30      1  20.01 0.016 2.72  5  6.50     21 46.0 52.025  0.938 0.862
Tom3.KH     36   25      1  20.67 0.004 4.20  5  6.35     25 36.0 39.500  0.962 0.950
        rel01
Lact1   0.542
Tom5.KH 0.604
Tom3.KH 0.834

$sppsub2
        env.cp freq maxgrp IndVal  pval    z         5%   10%   50%  90%   95% purity
Tom2.KH   50.0   41      2  26.87 0.012 3.27 15.000 18.00 46.00 58.0 64.55  0.962
Pstom     66.5   26      2      31.70 0.004 4.49 31.000 46.00 63.00 79.5 84.50  0.984
Lact2     82.0   18      2      41.76 0.004 5.46 23.000 26.45 53.75 84.5 84.50  1.000
Tuber     79.5    9      2       33.25 0.008 6.86 20.475 52.45 73.00 82.0 84.50  0.920
        rel05 rel01
Tom2.KH 0.878 0.594
Pstom   0.944 0.740
Lact2   1.000 0.980
Tuber   0.872 0.688

##TITAN of pH with August data
augtaxa <- read.table(file.choose(),T)
aug.ph <- em.ph[c(87:96, 98:115),]
> augphtitan.log <- titan(aug.ph, augemlog.norare, minsplt=5, numprm=250, nboot=500, boot=TRUE,
deviance=TRUE)
[1] "Taxa frequency screen complete"
[1] "Function definition complete"
[1] "IndVal z score calculation complete"
[1] "Deviance reduction calculation complete"
[1] "Begin bootstrap resampling..."
[1] "Bootstrap resampling complete"
[1] "TITAN complete"
        env.cp freq maxgrp IndVal  pval    z    5%   10%   50%   90%   95% purity
Cort1    5.535   12      1  36.03 0.324 0.23 4.645 4.645 5.190 5.495 5.535  0.528
Nauesc   5.190   11      1  49.64 0.020 2.90 4.645 4.645 5.190 5.495 5.535  0.942
Tom2.KH  5.480    7      2  43.26 0.020 2.58 4.975 5.115 5.475 5.565 5.565  0.884
```

```
Nau2     5.450   12      1  32.76 0.312 0.37 4.645 4.645 5.245 5.495 5.535  0.552
Nau3     4.645    7      1  57.69 0.012 3.72 4.645 4.645 4.835 5.440 5.460  0.936
Lact1    4.835    9      1  27.05 0.428 0.07 4.645 4.645 5.350 5.535 5.565  0.552
Tom5.KH  4.835    7      1  83.69 0.004 8.37 4.645 4.645 4.975 5.190 5.245  1.000
Pstom    4.975    9      2  45.00 0.032 2.13 4.835 4.835 5.190 5.565 5.565  0.896
Tom3.KH  5.295    9      2  38.41 0.088 1.68 4.975 5.115 5.395 5.565 5.565  0.862
Tom4.KH  5.395    5      1  20.70 0.288 0.68 4.835 5.035 5.350 5.535 5.565  0.538
       rel05 rel01
Cort1   0.192 0.036
Nauesc  0.740 0.436
Tom2.KH 0.588 0.300
Nau2    0.210 0.056
Nau3    0.776 0.520
Lact1   0.226 0.080
Tom5.KH 0.998 0.974
Pstom   0.628 0.180
Tom3.KH 0.574 0.286
Tom4.KH 0.134 0.044
          cp  0.05  0.10  0.50  0.90  0.95
sumz-    5.115 4.645 4.645 4.975 5.245 5.295
sumz+    5.035 4.645 4.835 5.450 5.565 5.565
ncpa.bc  4.645 4.645 4.645 5.245 5.535 5.565
ncpa.euc 4.645 4.645 4.645 5.295 5.535 5.565

plot.taxaz(augphtitan.log, boot=TRUE, xlab=expression("Soil pH"), cex.taxa=.9, cex.axis=1.35, cex=1.35,
xmax=6, fil2="red", col2="red", pur.cut=0.70, pval.cut=0.1, rel05.cut=0.3, rel01.cut=.1)
$sppsub1
       env.cp freq maxgrp IndVal  pval    z      5%   10%   50%   90%   95%      purity
Nauesc   5.190   11      1  49.64 0.020 2.90 4.645 4.645 5.190 5.495 5.535  0.942
Nau3       4.645    7      1  57.69 0.012 3.72 4.645 4.645 4.835 5.440 5.460  0.936
Tom5.KH 4.835    7      1  83.69 0.004 8.37 4.645 4.645 4.975 5.190 5.245  1.000
       rel05 rel01
Nauesc   0.740 0.436
Nau3       0.776 0.520
Tom5.KH 0.998 0.974

$sppsub2
       env.cp freq maxgrp IndVal  pval    z       5%   10%   50%   90%   95%      purity
Tom2.KH  5.480    7      2  43.26 0.020 2.58 4.975 5.115 5.475 5.565 5.565  0.884
Pstom       4.975    9      2  45.00 0.032 2.13 4.835 4.835 5.190 5.565 5.565  0.896
Tom3.KH  5.295    9      2  38.41 0.088 1.68 4.975 5.115 5.395 5.565 5.565  0.862
                rel05 rel01
Tom2.KH 0.588 0.300
Pstom       0.628 0.180
Tom3.KH 0.574 0.286

##August data with phosphorous
augem.log <- emtaxa.log[87:115,] ## Rows that are the species abundance for August
augemlog <- augem.log[,1:10] ## Pruning dataset so all species occur in >5 cores
augphos <- em.phos[c(87:96, 98:115),] #August phosphorous values

augphostitan.log <- titan(augphos, augemlog.norare, minsplt=5, numprm=250, nboot=500, boot=TRUE,
deviance=TRUE)
[1] "Taxa frequency screen complete"
[1] "Function definition complete"
[1] "IndVal z score calculation complete"
```

```
[1] "Deviance reduction calculation complete"
[1] "Begin bootstrap resampling..."
[1] "Bootstrap resampling complete"
[1] "TITAN complete"
          env.cp freq maxgrp IndVal  pval    z    5%   10%    50%     90%      95%
Cort1    8.875   12      1  49.88 0.028 2.62 7.585 8.150  8.975 12.3350 13.14000
Nauesc   7.995   11      1  32.90 0.352 0.15 7.585 7.995  9.245 13.1400 14.09500
Tom2.KH 12.335    7      1  33.33 0.156 1.40 7.585 7.585  8.675 11.7700 12.33500
Nau2    10.510   12      1  53.06 0.016 3.05 7.995 8.330 10.760 12.4155 13.18775
Nau3    14.095    7      2  50.72 0.028 2.98 7.995 8.312 12.335 14.0950 14.09500
Lact1    9.800    9      2  42.29 0.052 2.21 8.150 8.675 10.760 13.1400 13.18775
Tom5.KH 13.140    7      2  42.37 0.028 1.96 8.150 8.150  9.245 14.0950 14.09500
Pstom    7.585    9      1  47.46 0.036 2.05 7.585 7.585  8.330 11.2050 12.33500
Tom3.KH  8.875    9      2  36.27 0.088 1.63 8.150 8.480  9.245 12.3350 13.14000
Tom4.KH 11.205    5      1  26.32 0.176 1.51 8.150 8.330  9.245 11.2050 11.77000
         purity rel05 rel01
Cort1    0.862 0.680 0.414
Nauesc   0.660 0.324 0.114
Tom2.KH  0.778 0.394 0.146
Nau2     0.908 0.806 0.492
Nau3     0.844 0.584 0.332
Lact1    0.886 0.672 0.360
Tom5.KH  0.920 0.634 0.294
Pstom    0.862 0.680 0.354
Tom3.KH  0.648 0.396 0.146
Tom4.KH  0.696 0.188 0.050
            cp  0.05  0.10   0.50   0.90   0.95
sumz-    12.335 7.585 7.585  8.975 12.335 13.140
sumz+    11.770 8.150 8.330 11.010 14.095 14.095
ncpa.bc  13.140 7.995 8.330 11.010 14.095 14.095
ncpa.euc  8.875 7.585 7.995  9.800 13.140 14.095

## August Phosphorous graphed with purity = 70%, p value = 0.10
plot.taxaz(augphostitan.log, boot=TRUE, xlab=expression("Available Phosphorous"), cex.taxa=.9,
cex.axis=1.35, cex=1.35, xmax=15, fil2="red", col2="red", pur.cut=0.70, pval.cut=0.1, rel05.cut=0.3,
rel01.cut=.1)
$sppsub1
     env.cp freq maxgrp IndVal  pval    z    5%   10%    50%     90%      95%
Cort1    8.875   12      1  49.88 0.028 2.62 7.585 8.150  8.975 12.3350 13.14000
Nau2    10.510   12      1  53.06 0.016 3.05 7.995 8.330 10.760 12.4155 13.18775
Pstom    7.585    9      1  47.46 0.036 2.05 7.585 7.585  8.330 11.2050 12.33500
            purity rel05 rel01
Cort1    0.862 0.680 0.414
Nau2     0.908 0.806 0.492
Pstom    0.862 0.680 0.354

$sppsub2
       env.cp freq maxgrp     IndVal  pval    z       5%   10%    50%    90%      95%
Nau3        14.095    7      2  50.72 0.028 2.98 7.995 8.312 12.335 14.095 14.09500
Lact1        9.800    9      2  42.29 0.052 2.21 8.150 8.675 10.760 13.140 13.18775
Tom5.KH 13.140    7      2  42.37 0.028 1.96 8.150 8.150  9.245 14.095 14.09500
       purity rel05 rel01
Nau3     0.844 0.584 0.332
Lact1    0.886 0.672 0.360
Tom5.KH  0.920 0.634 0.294
```

```
##August species with total N
augN <- read.table(file.choose(),T) # %N values collected in August minus a few rows that have no EMF species in them (to make the matrices match)

augNtitan.log <- titan(augN, augemlog.norare, minsplt=5, numprm=250, nboot=500, boot=TRUE, deviance=TRUE)
[1] "Taxa frequency screen complete"
[1] "Function definition complete"
[1] "IndVal z score calculation complete"
[1] "Deviance reduction calculation complete"
[1] "Begin bootstrap resampling..."
[1] "Bootstrap resampling complete"
[1] "TITAN complete"
      env.cp freq maxgrp IndVal  pval    z     5%    10%    50%    90%    95%
Cort1   0.4510   12      1  63.16 0.008 3.70 0.28260 0.2950 0.3895 0.4510 0.4690
Nauesc  0.3265   11      2  38.87 0.100 1.42 0.27500 0.2830 0.3570 0.5840 0.6715
Tom2.KH 0.3570    7      2  34.36 0.048 1.91 0.28300 0.3040 0.3815 0.4510 0.5160
Nau2    0.2950   12      1  42.71 0.080 1.56 0.27500 0.2750 0.3360 0.5840 0.6715
Nau3    0.5160    7      2  67.36 0.004 5.42 0.41355 0.4350 0.5160 0.6715 0.6715
Lact1   0.3895    9      2  57.41 0.004 4.17 0.31300 0.3265 0.3955 0.5840 0.6715
Tom5.KH 0.3360    7      1  23.93 0.292 0.46 0.28300 0.2950 0.3895 0.6715 0.6715
Pstom   0.2830    9      1  34.79 0.184 0.78 0.27500 0.2750 0.3570 0.5840 0.6715
Tom3.KH 0.6715    9      1  39.13 0.120 1.15 0.27500 0.2830 0.3815 0.5840 0.6715
Tom4.KH 0.4690    5      1  25.00 0.256 1.00 0.28260 0.2950 0.3815 0.4690 0.4690
       purity rel05 rel01
Cort1    0.996 0.982 0.862
Nauesc   0.796 0.464 0.194
Tom2.KH  0.782 0.472 0.176
Nau2     0.892 0.608 0.284
Nau3     0.986 0.958 0.828
Lact1    1.000 0.970 0.806
Tom5.KH  0.550 0.220 0.064
Pstom    0.758 0.424 0.170
Tom3.KH  0.708 0.338 0.094
Tom4.KH  0.522 0.124 0.016
           cp   0.05   0.10  0.50   0.90   0.95
sumz-    0.3570 0.2750 0.2750 0.336 0.4690 0.5194
sumz+    0.5840 0.3265 0.3570 0.451 0.6715 0.6715
ncpa.bc  0.6715 0.3040 0.3265 0.516 0.6715 0.6715
ncpa.euc 0.6715 0.2830 0.3040 0.460 0.6715 0.6715

#Graph of N with purity =70%, p value = 0.10
plot.taxaz(augNtitan.log, boot=TRUE, xlab=expression("Total Nitrogen (%)"), cex.taxa=.9, cex.axis=1.35, cex=1.35, xmax=0.7, fil2="red", col2="red", pur.cut=0.70, pval.cut=0.1, rel05.cut=0.3, rel01.cut=.1)
$sppsub1
     env.cp freq maxgrp IndVal  pval    z     5%     10%     50%    90%    95%    purity
Cort1   0.451   12      1  63.16 0.008 3.70 0.2826 0.295 0.3895 0.451 0.4690  0.996
Nau2    0.295   12      1  42.71 0.080 1.56 0.2750 0.275 0.3360 0.584 0.6715  0.892
         rel05 rel01
Cort1 0.982 0.862
Nau2  0.608 0.284

$sppsub2
       env.cp freq maxgrp     IndVal  pval    z      5%        10%       50%    90%       95%
Nauesc    0.3265   11      2  38.87 0.100 1.42 0.27500 0.2830 0.3570 0.5840 0.6715
Tom2.KH 0.3570    7       2  34.36 0.048 1.91 0.28300 0.3040 0.3815 0.4510 0.5160
```

```
Nau3        0.5160    7       2  67.36 0.004 5.42 0.41355 0.4350 0.5160 0.6715 0.6715
Lact1       0.3895    9       2  57.41 0.004 4.17 0.31300 0.3265 0.3955 0.5840 0.6715
              purity rel05 rel01
Nauesc     0.796 0.464 0.194
Tom2.KH  0.782 0.472 0.176
Nau3        0.986 0.958 0.828
Lact1       1.000 0.970 0.806
```

**SCATTER PLOTS**

```
##Moisture
Lactobsvwcres <- lm(emtaxa.log$Lact1 ~ as.matrix(em.vwc))
plot(as.matrix(em.vwc), emtaxa.log$Lact1, main="Lactarius obscuratus Soil Moisture", xlab= "Soil
Moisture (%)", ylab="log10(x+1) Abundance", pch=16, col="black")
 abline(Lactobsvwcres, col="red")

 Tom3KHvwcres <- lm(emtaxa.log$Tom3.KH ~ as.matrix(em.vwc))
 plot(as.matrix(em.vwc), emtaxa.log$Tom3.KH, main="Tomentella sp 3 KH2010 Soil Moisture", xlab=
"Soil Moisture (%)", ylab="log10(x+1) Abundance", pch=16, col="black")
 abline(Tom3KHvwcres, col="red")

 Tom5KHvwcres <- lm(emtaxa.log$Tom5.KH ~ as.matrix(em.vwc))
 plot(as.matrix(em.vwc), emtaxa.log$Tom5.KH, main="Tomentella sp 5 KH2010 Soil Moisture", xlab=
"Soil Moisture (%)", ylab="log10(x+1) Abundance", pch=16, col="black")
 abline(Tom5KHvwcres, col="red")

 Tom2KHvwcres <- lm(emtaxa.log$Tom2.KH ~ as.matrix(em.vwc))
 plot(as.matrix(em.vwc), emtaxa.log$Tom2.KH, main="Tomentella sp 2 KH2010 Soil Moisture", xlab=
"Soil Moisture (%)", ylab="log10(x+1) Abundance", pch=16, col="black")
 abline(Tom2KHvwcres, col="red")

 Lact1vwcres <- lm(emtaxa.log$Lact2 ~ as.matrix(em.vwc))
 plot(as.matrix(em.vwc), emtaxa.log$Lact2, main="Lactarius cf. brunneohepaticus Soil Moisture", xlab=
"Soil Moisture (%)", ylab="log10(x+1) Abundance", pch=16, col="black")
 abline(Lact1vwcres, col="red")

 Tubervwcres <- lm(emtaxa.log$Tuber ~ as.matrix(em.vwc))
 plot(as.matrix(em.vwc), emtaxa.log$Tuber, main="Tuber sp Soil Moisture", xlab= "Soil Moisture (%)",
ylab="log10(x+1) Abundance", pch=16, col="black")
 abline(Tubervwcres, col="red")

 Tom1KHvwcres <- lm(emtaxa.log$Pstom ~ as.matrix(em.vwc))
 plot(as.matrix(em.vwc), emtaxa.log$Pstom, main="Tomentella sp 1 KH2010 Soil Moisture", xlab= "Soil
Moisture (%)", ylab="log10(x+1) Abundance", pch=16, col="black")
 abline(Tom1KHvwcres, col="red")

#pH
Tom1phres <- lm(augemlog.norare$Pstom ~ aug.ph)
plot(aug.ph, augemlog.norare$Pstom, main="Tomentella 1 sensu KH 2010 Soil pH distribution", xlab=
"Soil pH", ylab="log10(x+1) Tom 1 KH 2010 Abundance")
 abline(Tom1phres, col="red")

Tom5phres <- lm(augemlog.norare$Tom5.KH ~ aug.ph)
plot(aug.ph, augemlog.norare$Tom5.KH, main="Tomentella 5 sensu KH 2010 Soil pH distribution", xlab=
"Soil pH", ylab="log10(x+1) Tom 5 KH 2010 Abundance")
abline(Tom5phres)
```

```
Nauescphres <- lm(augemlog.norare$Nauesc ~ aug.ph)
plot(aug.ph, augemlog.norare$Nauesc, main="Naucoria escharoides Soil pH distribution", xlab= "Soil pH",
ylab="log10(x+1) Naucoria escharoides Abundance")
abline(Nauescphres, col="red")

Nau3phres <- lm(augemlog.norare$Nau3 ~ aug.ph)
plot(aug.ph, augemlog.norare$Nau3, main="Naucoria sp 3 Soil pH distribution", xlab= "Soil pH",
ylab="log10(x+1) Naucoria sp 3 Abundance", pch=16, col="black")
 abline(Nau3phres, col="red")

 Tom2KHphres <- lm(augemlog.norare$Tom2.KH ~ aug.ph)
 plot(aug.ph, augemlog.norare$Tom2.KH, main="Tomentella sp 2 KH2010 Soil pH", xlab= "Soil pH",
ylab="log10(x+1) Tomentella sp 2 KH2010 Abundance", pch=16, col="black")
 abline(Tom2KHphres, col="red")

 Tom3KHphres <- lm(augemlog.norare$Tom3.KH ~ aug.ph)
 plot(aug.ph, augemlog.norare$Tom3.KH, main="Tomentella sp 3 KH2010 Soil pH", xlab= "Soil pH",
ylab="log10(x+1) Tomentella sp 3 KH2010 Abundance", pch=16, col="black")
 abline(Tom3KHphres, col="red")

#Available phosphorous
Tom5phosres <- lm(augemlog.norare$Tom5.KH ~ augphos)
plot(augphos, augemlog.norare$Tom5.KH, main="Tomentella 5 KH 2010 Available Phosphorous", xlab=
"Available Phosphorous", ylab="log10(x+1) Tom 5 KH2010 Abundance", pch=16, col="black")
abline(Tom5phosres, col="red")

 Tom1phosres <- lm(augemlog.norare$Pstom ~ augphos)
 plot(augphos, augemlog.norare$Pstom, main="Tomentella 1 KH 2010 Available Phosphorous", xlab=
"Available Phosphorous", ylab="log10(x+1) Tom 1 KH2010 Abundance", pch=16, col="black")
 abline(Tom1phosres, col="red")

Nau3phosres <- lm(augemlog.norare$Nau3 ~ augphos)
 plot(augphos, augemlog.norare$Nau3, main="Naucoria sp 3 Available Phosphorous", xlab= "Available
Phosphorous", ylab="log10(x+1) Naucoria sp 3 Abundance", pch=16, col="black")
abline(Nau3phosres, col="red")

 Lactobsphosres <- lm(augemlog.norare$Lact1 ~ augphos)
plot(augphos, augemlog.norare$Lact1, main="Lactarius cf. obscuratus Available Phosphorous", xlab=
"Available Phosphorous", ylab="log10(x+1) Lactarius cf. obscuratus Abundance", pch=16, col="black")
 abline(Lactobsphosres, col="red")

 cortalbphosres <- lm(augemlog.norare$Cort1 ~ augphos)
 plot(augphos, augemlog.norare$Cort1, main="Cortinarius alboviolaceous Available Phosphorous", xlab=
"Available Phosphorous", ylab="log10(x+1) Cortinarius alboviolaceous Abundance", pch=16, col="black")
 abline(cortalbphosres, col="red")

 nau2phosres <- lm(augemlog.norare$Nau2 ~ augphos)
plot(augphos, augemlog.norare$Nau2, main="Naucoria sp 2 Available Phosphorous", xlab= "Available
Phosphorous", ylab="log10(x+1) Naucoria sp 2 Abundance", pch=16, col="black")
abline(nau2phosres, col="red")

# Total N
TotalN <- augN
Nau2nres <- lm(augemlog.norare$Nau2 ~ as.matrix(TotalN))
plot(as.matrix(TotalN), augemlog.norare$Nau2, main="Naucoria sp 2 Total N", xlab= "Total Nitrogen
(%)", ylab="log10(x+1) Naucoria sp 2 Abundance", pch=16, col="black")
```

```
abline(Nau2nres, col="red")

Cort1nres <- lm(augemlog.norare$Cort1 ~ as.matrix(TotalN))
plot(as.matrix(TotalN), augemlog.norare$Cort1, main="Cortinarius alboviolaceus Total N", xlab= "Total Nitrogen (%)", ylab="log10(x+1) Abundance", pch=16, col="black")
abline(Cort1nres, col="red")

Nau3nres <- lm(augemlog.norare$Nau3 ~ as.matrix(TotalN))
plot(as.matrix(TotalN), augemlog.norare$Nau3, main="Naucoria sp 3 Total N", xlab= "Total Nitrogen (%)", ylab="log10(x+1) Abundance", pch=16, col="black")
abline(Nau3nres, col="red")

Lactobsnres <- lm(augemlog.norare$Lact1 ~ as.matrix(TotalN))
plot(as.matrix(TotalN), augemlog.norare$Lact1, main="Lactarius cf obscuratus Total N", xlab= "Total Nitrogen (%)", ylab="log10(x+1) Abundance", pch=16, col="black")
abline(Lactobsnres, col="red")

Tomsubnres <- lm(augemlog.norare$Tom2.KH ~ as.matrix(TotalN))
plot(as.matrix(TotalN), augemlog.norare$Tom2.KH, main="Tomentella sublilacina Total N", xlab= "Total Nitrogen (%)", ylab="log10(x+1) Abundance", pch=16, col="black")
abline(Tomsubnres, col="red")

Nauescnres <- lm(augemlog.norare$Nauesc ~ as.matrix(TotalN))
plot(as.matrix(TotalN), augemlog.norare$Nauesc, main="Naucoria escharoides Total N", xlab= "Total Nitrogen (%)", ylab="log10(x+1) Abundance", pch=16, col="black")
abline(Nauescnres, col="red")
```

**NICHE PARTITIONING/COMPETTIVE EXCLUSION**

```
##NMDS ordination with Tomentella sp 1 KH and Tomentella sp 5 KH which displayed niche partitioning in TITAN analyses highlighted on the ordination space
plot(emfreq.1.nmds, "sites", type = "n", main = "Niche Partitioning", xlab="Axis 1", ylab="Axis 2", xaxt="n", yaxt="n")
plot(ph, col="black", cex=.75)
plot(frankia, col="black",  cex=.75)
plot(vwc, col="black", cex=.75)
plot(phos, col= "black", cex=.75)
plot(cn, col="black", cex=.75)
plot(N, col="black", cex=.75)
plot(C, col="black", cex=.75)
points(emfreq.1.nmds, "sites", cex =.5, pch=16)
points(emfreq.1.nmds, "sites", cex = emfreq.1[,"Tom5.KH"] * 2.5, col="blue")
points(emfreq.1.nmds, "sites", cex = emfreq.1[,"Pstom"] * 2, col="red")
legend(x="topright", pch=1, col=c("red", "blue"), legend=c("Tomentella sp 1 KH 2010", "Tomentella sp 5 KH 2010"), cex=.6)

##NMDS ordination with Lactarius cf. obscuratus and Cortinarius cf. alboviolaceous which displayed niche partitioning in TITAN analyses highlighted on the ordination space
plot(emfreq.1.nmds, "sites", type = "n", main = "", xlab="Axis 1", ylab="Axis 2", xaxt="n", yaxt="n")
plot(ph, col="black", cex=.75)
plot(frankia, col="black",  cex=.75)
plot(vwc, col="black", cex=.75)
plot(phos, col= "black", cex=.75)
plot(cn, col="black", cex=.75)
plot(N, col="black", cex=.75)
plot(C, col="black", cex=.75)
points(emfreq.1.nmds, "sites", cex =.5, pch=16)
```

```
  points(emfreq.1.nmds, "sites", cex = emfreq.1[,"Lact1"] * 1.5, col="red2", pch=0)
  points(emfreq.1.nmds, "sites", cex = emfreq.1[,"Cort1"] * 2, col="purple2", pch=6)
 legend(x="topright", pch=c(0,6), col=c("red2", "purple2"), legend=c("Lactarius cf.
obscuratus","Cortinarius cf. alboviolaceous"), cex=.6)

##NMDS ordination with Lactarius cf. obscuratus and Tomentella sp 5 KH which displayed niche
partitioning in TITAN analyses highlighted on the ordination space
plot(emfreq.1.nmds, "sites", type = "n", main = "", xlab="Axis 1", ylab="Axis 2", xaxt="n", yaxt="n")
 plot(ph, col="black", cex=.75)
 plot(frankia, col="black",  cex=.75)
  plot(vwc, col="black", cex=.75)
  plot(phos, col= "black", cex=.75)
  plot(cn, col="black", cex=.75)
  plot(N, col="black", cex=.75)
  plot(C, col="black", cex=.75)
  points(emfreq.1.nmds, "sites", cex =.5, pch=16)
  points(emfreq.1.nmds, "sites", cex = emfreq.1[,"Lact1"] * 2.5, col="green4", pch=1)
points(emfreq.1.nmds, "sites", cex = emfreq.1[,"Tom5.KH"] * 2, col="blue", pch=2)
legend(x="topright", pch=c(1,2), col=c("green4", "blue"), legend=c("Lactarius cf. obscuratus","Tomentella
sp 5 KH 2010"), cex=.6)

##NMDS ordination with Lactarius cf. brunneohepaticus and Tomentella sp 1 KH which displayed niche
partitioning in TITAN analyses highlighted on the ordination space
plot(emfreq.1.nmds, "sites", type = "n", main = "", xlab="Axis 1", ylab="Axis 2", xaxt="n", yaxt="n")
 plot(ph, col="black", cex=.75)
 plot(frankia, col="black",  cex=.75)
  plot(vwc, col="black", cex=.75)
  plot(phos, col= "black", cex=.75)
  plot(cn, col="black", cex=.75)
  plot(N, col="black", cex=.75)
  plot(C, col="black", cex=.75)
  points(emfreq.1.nmds, "sites", cex =.5, pch=16)
  points(emfreq.1.nmds, "sites", cex = emfreq.1[,"Lact2"] * 2.5, col="green4", pch=1)
  points(emfreq.1.nmds, "sites", cex = emfreq.1[,"Pstom"] * 2, col="blue", pch=2)
legend(x="topright", pch=c(1,2), col=c("green4", "blue"), legend=c("Lactarius cf.
brunneohepaticus","Tomentella sp 1 KH 2010"), cex=.6)
```