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# Ectomycorrhizal community composition and structure of a mature red alder (*Alnus rubra*) stand

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ABSTRACT

Regional and global studies of ectomycorrhizal fungal (EMF) communities associated with *Alnus* have made progress in determining the key factors that influence EMF diversity and biogeography. Smaller scale studies provide a valuable complement by relating EMF to environmental gradients and describing how community composition is influenced by competition and niche partitioning. We examined controls on EMF community composition in an 80 y-old *Alnus rubra* stand. EMF species were identified using root tip morphology and DNA sequencing, and related to soil variables (soil moisture, total C, total N, C:N ratio, $PO_4$-P, soil pH) and to *Frankia* nodulation using Threshold Indicator Taxa ANalysis (TITAN) and multivariate techniques. Twenty-two EMF species were identified, including 14 that are new associates of *A. rubra*. EMF community composition varied temporally and was influenced by *Frankia* nodulation and soil chemistry. Species co-occurrence patterns suggest niche partitioning and competitive exclusion interact with subtle differences in the soil environment to influence EMF community composition.

## 1. Introduction

Environmental gradients (e.g., moisture, nutrients, temperature) affect ectomycorrhizal fungus (EMF) diversity, abundance, and community composition across plant communities (Worley and Hacskaylo, 1959; Harvey et al., 1978; Baar et al., 2002; Becerra et al., 2005a; Smith and Read, 2008; Ostonen et al., 2009; Akata et al., 2012; Põlme et al., 2013; Roy et al., 2013). Nitrogen availability (e.g., C:N ratio) is the primary determinant of 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) and influences EMF diversity in stands regardless of phosphorous levels applied (Alvarez et al., 2012). Changes in EMF community composition can occur based on subtle temporal differences in soil moisture content, 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 described (Fogel and Cromack, 1977), but how temporal fluctuations of the soil environment mediate EMF community composition has only recently begun to be examined (Becerra et al., 2005a,b; Koide et al., 2007).

The analysis of patterns in ectomycorrhizal fungal (EMF) community structure over spatial and temporal scales can provide information on key interactions within the EMF community (Peter et al., 2001b; Lilleskov et al., 2004; Izzo et al., 2005; Koide et al., 2005, 2007; Kennedy and Hill, 2010; Johnson et al., 2012; Kennedy et al., 2014; Barnes et al., 2016; Santalahti et al., 2016). EMF communities tend to have a clumped spatial distribution at small scales (<3 m on roots, < 8 m as sporocarps), with random spatial patterns at larger scales (Peter et al., 2001b; Lilleskov et al., 2004; Izzo et al., 2005). In some cases, these spatial relationships remain constant over time, even with fluctuations in EMF diversity (Izzo et al., 2005), however EMF temporal and spatial shifts in community structure have also been observed (Barnes et al., 2016; Santalahti et al., 2016). Spatial structure of EMF communities is thought to be determined by negative interspecific interactions within the community such as niche partitioning and competitive

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exclusion (Taylor and Bruns, 1999; Kennedy and Bruns, 2005; Koide et al., 2005; Kennedy et al., 2007, 2011), although positive interactions have also been reported (Kennedy et al., 2014). 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, 2011). Inclusion of environmental gradients also reveals EMF niche partitioning through differences in species resource and habitat preferences, or root colonization strategies (Taylor and Bruns, 1999; Geml et al., 2010). The outcome of interspecific interactions in terms of the overall structure of EMF communities may depend in part on local environmental variables (Horton and Bruns, 2001; Kennedy et al., 2007), and has yet to be intensively examined.

Ecological field studies examining these principles have been difficult because of the large sample sizes required to account for high EMF species diversity (Smith et al., 2002; Smith and Read, 2008). We studied the ectomycorrhizal community of *Alnus rubra* (red alder) a species characterized by high host specificity and a relatively low diversity of ectomycorrhizal associates (11–18 species associated with *A. rubra*) (Molina, 1979, 1981; Miller et al., 1992; Kennedy and Hill, 2010; Põlme et al., 2013; Kennedy et al., 2014; Walker et al., 2014). Our objective was to characterize this community and the environmental factors that structure it.

*A. rubra* forests are a model system to describe the effects of soil fertility and moisture on EMF communities, as *A. rubra* occupies a variety of soil moisture conditions (Baar et al., 2002; Deal and Harrington, 2006) and can colonize soils with a wide range of nitrogen levels (Harrington, 1984). Alders form root nodules containing nitrogen-fixing *Frankia* bacteria on almost all sites, with greater prevalence on lower nitrogen sites (Molina et al., 1994). The activity of *Frankia*, a genus of actinomycetes filamentous soil bacteria that converts atmospheric nitrogen to ammonium, increases foliar nitrogen levels and thus the soil total nitrogen content (2–23 g N $kg^{-1}$) due to the turnover and decomposition of *A. rubra* leaves (Martin et al., 2003). Higher soil nitrogen levels stimulate nitrification, leading over time to low pH values (4.5–4.9) and low phosphorous levels ($<7$ mg $kg^{-1}$) (Cole et al., 1990). The changes in total nitrogen due to *Frankia* presence may influence EMF community structure (Toljander et al., 2006; Kennedy and Hill, 2010; Kennedy et al., 2011; Põlme et al., 2013; Walker et al., 2014), making alder forests an ideal system for exploring the interaction between *Frankia* presence and EMF habitat requirements and species interactions.

Recent global (Põlme et al., 2013) and regional (Roy et al., 2013) studies of EMF communities associated with *Alnus* have made great strides in defining key factors that influence their biogeography and diversity. At the global scale, 146 EMF taxa have been identified in association with 22 *Alnus* species, with host and geography explaining much of the variation in community composition (Põlme et al., 2013). At the regional scale, 86 EMF taxa were associated with 5 *Alnus* species in France, with host, soil, altitude and longitude all significant factors in explaining the community composition (Roy et al., 2013).

Studies of large-scale patterns complement focused examinations at local scales that can include greater sampling depths and can tease out interspecific interactions such as competition and niche differentiation. Local analyses can be based on species co-occurrence patterns. For example, Kennedy et al. (2014) found that species co-occurrence patterns of EMF in *A. rubra* forests in Oregon at smaller scales ($<12$ m) suggested either neutral or positive interactions, though negative, competitive patterns have been reported in studies of EMF in other systems (Koide et al., 2005; Kennedy and Hill, 2010; Pickles et al., 2012). Kennedy et al. (2014) suggested that harsher conditions associated with elevated nitrate and soil acidity due to the presence of *Frankia* nodulation in alder forests could cause the EMF community composition to be influenced less by competition and more by facilitative interactions, however they could not directly test the role of the environment because soil environmental variables were not measured. When environmental variables are measured simultaneously with compositional data, statistical tools such as Indicator Species Analysis (ISA) (Dufrêne and Legendre, 1997) and Threshold Indicator Taxa ANalysis (TITAN) (Baker and King, 2010) can examine changes in community structure in response to environmental variation.

In this study, we described ectomycorrhizal species in a mature *A. rubra* stand and assessed the temporal and spatial influences of soil moisture, C, N, C:N ratio, *Frankia* presence, available phosphorous ($PO_4$-P), and soil pH on EMF community composition. This fine-scaled approach allowed us to closely examine the influence of soil environmental variables and species interactions on EMF community composition.

## 2. Materials and methods

### 2.1. Study site

A 0.45 ha plot was established at the Charles Lathrop Pack Experimental Forest in western Washington at an elevation of 427–457 m above sea level and on a 10–20% slope. The stand originated after a stand-replacing fire in 1926 (Swanson, 2006) and was quickly colonized by *A. rubra*; the stand was dominated by ~80-y-old *A. rubra* trees at the time of data collection. The stand had a total basal area (BA) of 28 $m^2$ $ha^{-1}$, more than half of which (15 $m^2$ $ha^{-1}$) was from *A. rubra*, and contained a few large *Populus balsamifera* ssp. *trichocarpa* (BA = 11 $m^2$ $ha^{-1}$) with *Tsuga heterophylla* interspersed in the understory and *Pseudotsuga menziesii* surrounding the stand. The dominant understory vegetation included *Rubus spectabilis*, *Polystichum munitum*, and *Ribes lacustre*, with dense herbaceous ground cover of various species including *Carex obnupta*, *Galium*, and *Ranunculus*. The climate is characterized by cool, wet winters, and summers with an extended drought (Franklin and Dyrness, 1988). Average January and July temperatures are 3.9 and 18.3 °C, respectively. Annual precipitation averages 98 cm, with 88% falling between September and May (Swanson, 2006).

The stand contains various microsites due to topographic variation and seasonal water availability (i.e., summer drought). The soils are heterogeneous, with a fine-loam texture (Wilkeson series, Soil Survey Staff, 2013). The microtopography of the site is complex and includes many small hummocks and valleys. Soil has variable carbon and moisture inputs due to the presence of decaying large woody debris and ephemeral springs.

### 2.2. Field sampling

Thirty randomly selected *A. rubra* trees of similar diameter and height were tagged within the plot. The ectomycorrhizal community of each tree was assessed by extracting root cores (10 $cm^3$) within 2 m of its stem. Each tree was sampled monthly from May to September 2011 for a total of 5 cores per tree (30 cores per month; 150 cores total). The location of root cores was systematically assigned in a clockwise manner around each tree prior to sampling to reduce spatial autocorrelation (Lilleskov et al., 2004) (Fig. S1). Cores were transported back to the laboratory on ice and kept cool until processing.

Soil moisture (volumetric water content) was measured in the center of each root core prior to coring ($n = 150$) using a portable time domain reflectometer fitted with a 12 cm probe (HydroSense, Campbell Scientific, Australia) and relativized to the maximum