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Red alder (*Alnus rubra*) alters community-level soil microbial function in conifer forests of the Pacific Northwest, USA

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Abstract

Nitrogen-fixing tree species have been shown to improve site fertility and increase N transformation rates, but the influence of N-fixing plants on the soil microbial community as a whole is largely unknown. We used patterns of individual carbon-source utilization and enzyme activities to assess the relative effects of N-fixing red alder on the soil microbial community in three adjacent stands (pure conifer, mixed alder-conifer, and pure alder) of a highly productive coastal Oregon forest where the density of red alder has been experimentally manipulated for over 65 years. Two major patterns were revealed: (1) bacterial and fungal carbon-source utilization patterns in soil from pure conifer stands were significantly different from both pure alder soils and mixed conifer-alder soils, while there was no difference in substrate utilization patterns between soils from the mixed alder-conifer and pure alder stands; and (2) the activities of nine extracellular enzymes involved in ligno-cellulose degradation and the mineralization of organic nitrogen, phosphate, and sulfate compounds were all significantly greater in pure alder soils compared to either pure conifer or mixed conifer-alder soils. Our results show that, in addition to an overall increase in soil fertility, microbial biomass, and microbial activity, the presence of N-fixing red alder significantly alters the physiological profile of the microbial community-even in an ecosystem already of high N status.

Keywords: *Alnus rubra*; BiOLOG; Community-level physiological profile (CLPP); Extra-cellular enzyme activity; N-fixing tree species; Pacific Northwest

1. Introduction

Many studies have demonstrated that plant species may differentially affect soil chemical and physical properties (Klemmedson, 1987; Hobbie, 1992; Dahlgren et al., 1997; Berendse, 1998; Binkley and Giardina, 1998), and a number of authors have suggested that nitrogen (N)-fixing species, as a functional group, may exert a substantially different influence on soil properties than non-N-fixing species (Van Breeman and Finzi, 1998; Spears et al., 2001). However, we know of no previous research that has examined how N-fixing plant species might affect soil microbial function.

The presence of red alder (*Alnus rubra* Bong.) in conifer forests of the Pacific Northwest (PNW) has been shown to alter ecosystem N dynamics, primarily because of the association with the N-fixing actinomycete *Frankia*. On N-limited sites, red alder increases aboveground net primary productivity (ANPP) by increasing soil N capital, N availability, and levels of exchangeable base cations. On more fertile sites, soil N capital and N availability also increase with the presence of red alder trees, but with no concomitant change in ANPP and often an increase in nitrate leaching and a decrease in soil pH (Binkley and Sollins, 1990; Binkley et al., 1992; Hart et al., 1997).

Despite long-standing knowledge of its effects on ecosystem N dynamics, the influence of red alder on the soil microbial community as a whole is largely unknown. This is important because soil microorganisms regulate many aspects of ecosystem function besides N transformations, including litter decomposition, soil organic matter formation, and the availability of other essential elements, most notably phosphorus (P) and sulfur (S). Previous research has shown that artificial N amendments increase the activity of extracellular enzymes involved in the degradation of cellulose (Johnson et al., 1998; Ajwa et al., 1999; Carreiro et al., 2000; Saiya-Cork et al., 2002) and

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the hydrolysis of organic phosphate esters (Olander and Vitousek, 2000). The inclusion of N-fixing tree species has also been shown to increase the activity of phosphatase enzymes in soil (Giardina et al., 1995; Zou et al., 1995). However, N addition or increased N availability may decrease the activity of lignin and chitin degrading enzymes (Fog, 1988; Carreiro et al., 2000; Olander and Vitousek, 2000; Saiya-Cork et al., 2002). Changes in soil N status have also been shown to dramatically alter the community-level physiological profile (CLPP) of soil microorganisms based on utilization patterns of individual carbon (C) substrates (Grayston et al., 1998; Waldrop et al., 2000; Collins and Cavigelli, 2003).

Because the leaves and roots of red alder have higher N and lower lignin concentrations than those of conifers (Edmonds, 1980; Harmon et al., 1990), it is reasonable to assume that this improved substrate quality may influence the activity and functional diversity of the soil microbial community (Wardle, 2002; Schimel and Weintraub, 2003). We chose to investigate the relative influence of red alder on the soil microbial community in a highly productive coastal Oregon forest where the density of red alder has been experimentally manipulated for over 65 years, and the presence of red alder has been shown to increase soil N status and gross rates of N transformations, but not ANPP (Binkley et al., 1992; Hart et al., 1997). We compared the CLPP of soil bacteria and fungi as well as soil extracellular enzyme activities in three adjacent forest stands: pure conifer, mixed alder-conifer, and pure alder. We predicted that: (1) there would be a shift in CLPPs for both bacteria and fungi as alder density increased; (2) the activities of extracellular enzymes involved in cellulose degradation and the hydrolysis of phosphate and sulfate esters would increase with increasing alder density; and (3) the activities of enzymes involved in lignin and chitin degradation would decrease with increasing alder density.

2. Materials and methods

2.1. Site description and soil sampling

The study site is the Cascade Head Experimental Forest near the Oregon coast (45°03′N) at approximately 180 m elevation. Mean annual precipitation is 2400 mm with little in the form of snow. Air temperature averages 20 °C in July and 10 °C in January (Berntsen, 1961). The soil is a well-drained, Typic Dystrandept developed from tuffaceous siltstone overlying basalt (Binkley et al., 1992). The three plots we sampled are within an 8 ha stand on a moderate slope (~15%, SW aspect) that was cleared for agriculture and then abandoned in 1925. By 1935, a naturally regenerated mixed stand had developed, containing Douglas-fir (*Pseudotsuga menziesii* Mirb., Franco), western hemlock (*Tsuga heterophylla* Raf., Sarg.), Sitka spruce (*Picea sitchensis* Bong., Carr.), and red alder. Conifer density at this stage was approximately 4500 trees ha^{-1}, while alder density was about 3000 trees ha^{-1}. Three plots were established by the U.S. Forest Service within this 8 ha stand between 1935 and 1936 in an effort to compare growth rates of alder and conifers in pure vs. mixed stands: a 0.4 ha unthinned alder-conifer plot; a 0.2 ha pure conifer plot where all the alders and many of the smaller conifers were removed reducing stand density to 2800 trees ha^{-1}; and a 0.2 ha pure alder plot where all the conifers were removed and alders were thinned to a density of about 1800 trees ha^{-1} (Berntsen, 1961). Selected soil characteristics of the three stands are listed in Table 1.

In September of 2001 we collected 10 soil samples from each of the three stands: pure conifer, mixed alder-conifer, and pure alder. Selected soil characteristics of each of these three stands are listed in Table 1. Within each stand, a 50 m transect was established along a random azimuth from the center of the stand. After removing the O horizon, soil samples were taken from the top 5 cm of mineral soil by hand trowel at 5 m intervals along the transect. Stand edges were avoided, as it has been shown that the influence of red alder roots and litter can extend up to 5 m into adjacent conifer stands (Rhoades and Binkley, 1992). Soils were sieved to <2 mm fraction and stored at 4 °C for less than 48 h until analyzed.

2.2. Community-level physiological profiles

Bacterial and fungal community-level physiological profiles (CLPPs) in each stand were characterized by assessing the utilization pattern of individual C substrates using 96-well BiOLOG microtiter plates (Hayward, CA, USA). We used BiOLOG ECO plates with 31 unique C substrates replicated three times to assess bacterial CLPPs and BiOLOG SFN2 plates with 95 unique C substrates to assess fungal CLPPs (Garland and Mills, 1991; Buyer et al., 2001; Classen et al., 2003). Measurement of substrate metabolism in ECO plates is based on color formation from

Table 1
Soil pH, total and microbial C and N, and C:N ratio in the upper 15 cm of mineral soil from adjacent alder, conifer, and mixed alder-conifer stands

Stand	pH	Total C ($g\ kg^{-1}$soil)	Total N ($g\ kg^{-1}$soil)	C:N	Microbial C ($mg\ kg^{-1}$soil)	Microbial N ($mg\ kg^{-1}$soil)
Alder	3.9 (0.1)	160 (13)	9.74 (0.87)	16.4 (2.0)	2172 (244)	419 (43)
Conifer	5.4 (0.4)	118 (9)	4.33 (0.28)	27.1 (2.7)	1681 (222)	332 (50)
Mixed	4.3 (0.4)	118 (8)	6.70 (0.88)	17.6 (2.6)	2264 (324)	444 (62)

Data are means +1 SE; $n=5$; from Hart et al. (1997).

tetrazolium dye, a redox indicator. Substrate utilization in fungal (SFN2) plates is assessed turbidimetrically.

Plates were prepared as described in Classen et al. (2003). Briefly, 4 g of soil was extracted with 36 ml of 50 mM K_2HPO_4 buffer adjusted to pH 6, and the resulting soil suspensions were shaken for 30 min on a reciprocal shaker. After settling for 30 min, an 8 ml aliquot of the supernatant was diluted in 792 ml of inoculating solution for a final 1:1000 dilution (A.C. Kennedy, USDA-ARS, personal communication). Fungal inoculations also contained 1 μg of streptomycin sulfate and 0.5 μg chlortetracycline per microtiter plate well to limit bacterial growth (Dobranic and Zak, 1999).

All plates were placed in polyethylene bags to reduce desiccation while incubating in dark growth chambers. Bacterial plates were incubated for 72 h at 25 °C and fungal plates were incubated for 168 h at 25 °C (Classen et al., 2003). Both bacterial and fungal plates were read on an Emax plate reader (Molecular Devices, Inc., Sunnyvale, CA, USA). For bacterial plates, optical density at both 590 nm (color development plus turbidity) and 750 nm (turbidity only) were read. The final values used to denote activity in each well were the 590 nm values minus the 750 nm values after correcting for readings in the A1 (control) well at these wavelengths (Classen et al., 2003). Final values for the fungal plates were the 750 nm optical density readings minus the A1 well values at that wavelength. Optical density values in wells that were negative or under 0.06 (the detection limit of the spectrophotometer) were set to zero (Classen et al., 2003). We calculated total activity per plate by summing corrected absorption values for each well, allowing us to examine differences in potential bacterial and fungal activity among the three stands. Relative use of each individual C substrate was calculated as corrected absorption values in each well divided by total absorption in the plate (Garland and Mills, 1991; Hungate et al., 2000). This normalization procedure effectively reduces the influence of differences in inoculum density on CLPPs (Garland and Mills, 1991, Classen et al. 2003).

2.3. Extracellular enzyme activity

Soil suspensions from each of the three stands were assayed for the activity of nine extracellular enzymes involved in ligno-cellulose degradation as well as the mineralization of nitrogen, phosphorus, and sulfur (Table 2). We assessed activity for all enzymes except phenol oxidase by measuring the fluorescence of methylumbelliferyl (MUB)-linked substrates (Table 2) in the presence of soil extracts on 96-well microtiter plates (Sinsabaugh et al., 2000; Waldrop et al., 2000; Waldrop and Firestone, 2004). Soil suspensions were prepared by combining 1 g of soil per 100 ml of 5 mM bicarbonate buffer adjusted to pH 8 (Waldrop and Firestone, 2004). Standards for quenching were included on each plate, and each enzyme assay was replicated six times per plate to account for variability (Sinsabaugh et al., 2000). Fluorescence was measured using a Fluoromax fluorometer (Jobin Yvon-Spex, Edison, NJ) attached to a MicroMax Microwell plate reader (Jobin Yvon-Spex, Edison, NJ) with 360 nm excitation and 450 nm emission filters. Activities of enzymes assessed fluorometrically are reported as μmoles product kg^{-1} oven-dried soil h^{-1}.

Table 2
Extracellular enzymes assayed in soil collected from adjacent alder, conifer, and mixed alder-conifer stands, their commission number (EC), corresponding substrate, and the abbreviation used in this study

Enzyme	Abbreviation	EC	Substrate
Ligno-cellulolytic			
β-1,4-Glucosidase	β-gluc	3.2.1.21	4-MUB-β-D-glucoside
α-1,4-Glucosidase	α-gluc	3.2.1.20	4-MUB-α-D-glucoside
β-Galactosidase	β-galac	3.2.1.22	4-MUB-β-D-galactoside
β-1,4-Xylosidase	β-xylo	3.2.1.37	4-MUB-β-D-xyloside
Cellobiohydrolase	CBH	3.2.1.91	4-MUB-β-D-cellobioside
Phenol oxidase	PhOx	1.10.3.2	L-DOPA
Nutrient mineralizing			
β-1,4-*N*-Acetyl-glucosaminidase	NAG	3.2.1.14	4-MUB-*N*-acetyl-β-D-glucosaminide
Phosphatase	Phos	3.1.3.1	4-MUB-phosphate
Sulfatase	Sulf	3.1.6.1	4-MUB-sulfate

L-DOPA = L-3,4-dihydroxyphenylalanine; 4-MUB = 4-methylumbelliferyl.

Phenol oxidase activity was assessed spectrophotometrically on 96-well microtiter plates with 5 mM L-3, 4-dihydroxyphenylalanine (L-DOPA) as the substrate. Soil suspensions were prepared as described above and plates were incubated at 27 °C for 1 h. Absorbance at 460 nm was read every 5 min with a plate-reading spectrophotometer. Phenol oxidase activity is expressed as absorbance units g^{-1} dry soil h^{-1}.

2.4. Statistical analyses

We used non-metric multidimensional scaling (NMS) to examine bacterial and fungal CLPPs in the pure alder, pure conifer, and mixed stands. We also used NMS to assess variation among the three stands in activity patterns of enzymes involved in ligno-cellulose degradation (β-gluc, α-gluc, β-galac, β-xylo, CBH, and PhOx; Table 2). NMS is an iterative best-fit ordination technique that arranges samples so that the distance between soil samples in ordination space is in rank order with either their similarities in C substrate utilization pattern or ligno-cellulolytic enzyme activity pattern (Clarke, 1993). The Sorensen (Bray-Curtis) distance metric was used as a measure of dissimilarity in the three NMS ordinations (Faith et al., 1987; McCune, 1997).

We used multi-response permutation procedures (MRPP) to test the null hypothesis of no difference in the CLPPs of bacterial and fungal communities, as well as ligno-cellulolytic enzyme activity patterns, among the three stand types. MRPP is a non-parametric, multivariate method

used to make statistical comparisons among two or more a priori groups (Zimmerman et al., 1985). The *P*-value associated with the MRPP test statistic describes how likely an observed difference between groups is due to chance, and the agreement statistic (*A*) describes within-group homogeneity compared to random expectation, i.e. *A* is a measure of the effect size independent of sample size (McCune and Grace, 2002). The Sorensen distance metric was used to quantify the dissimilarity in relative use of C substrates, as well as the dissimilarity in ligno-cellulolytic enzyme activity pattern, as it has been shown to be more robust than other distance measures (Faith et al., 1987; McCune and Mefford, 1999), and also because we used it as the distance metric in the NMS ordination. For pairwise comparisons of CLPPs and ligno-cellulolytic enzyme activity patterns using MRPP, the comparisonwise error rate was adjusted for the number of comparisons using the Bonferroni method (Sokal and Rohlf, 1995), such that the adjusted level of $\alpha = 0.017$.

Grouping of C substrates into guilds (Dobranic and Zak, 1999) allowed us to assess which types of substrates contributed the most to community separation as determined by the NMS ordination and MRPP analyses. Bacterial and fungal total C-use and C-use by substrate guilds, as well as individual extracellular enzyme activities, were analyzed among the three stands using one-way Analyses of Variance (ANOVAs). Tukey's multiple pairwise comparisons tests were used to compare means when ANOVAs were significant. Kruskal–Wallis ANOVA on ranks was used for data with unequal variances. Data from relative C-use by substrate guilds were arcsine square-root transformed before analyses to approximate normality (Sokal and Rohlf, 1995). We used an alpha level of 0.05 as a measure of statistical significance for all tests.

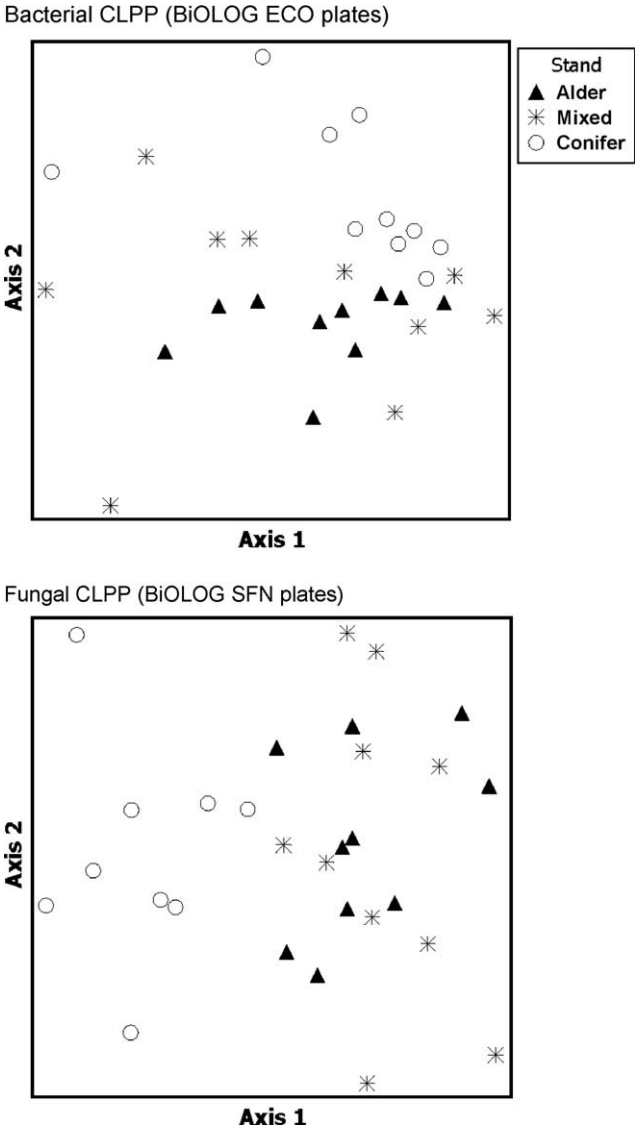

Fig. 1. Non-metric multi-dimensional scaling of bacterial and fungal community-level physiological profiles from pure conifer, mixed alder-conifer, and pure alder forest stands of coastal Oregon.

3. Results

Among the three stands, CLPPs were significantly different for both bacteria ($A = 0.070$, $P = 0.002$) and fungi ($A = 0.085$, $P < 0.001$; Fig. 1). Pairwise comparisons of bacterial CLPPs reveal a significant difference between the conifer stand and the alder stand ($A = 0.095$, $P < 0.001$), but mixed stand bacterial CLPPs overlap with both conifer ($A = 0.044$, $P = 0.036$) and alder stand CLPPs ($A = 0.027$, $P = 0.085$; Fig. 1). Pairwise comparisons of fungal CLPPs reveal a slightly different pattern, in that the conifer stand was significantly different from both the alder stand ($A = 0.085$, $P < 0.001$) and the mixed stand ($A = 0.099$, $P < 0.001$), but there was no difference in fungal CLPPs between the alder and mixed stands ($A = 0.015$, $P = 0.093$; Fig. 1). There was a significant difference in total bacterial metabolic activity as measured by CLPPs among the three stands ($F = 3.85$, $P = 0.03$), with the mixed stand significantly lower compared to both the alder and conifer stands (Fig. 2), but there was no difference in total fungal metabolic activity among the three stands ($F = 1.33$, $P = 0.28$; Fig. 2).

Substrate guild analysis of CLPPs showed that bacteria in the conifer stand utilized significantly fewer carbohydrates and more amino acids than bacteria in either the alder or the mixed stand, and bacteria in the mixed stand used significantly fewer carboxylic acids than bacteria in either the alder or the conifer stand (Fig. 3). Bacteria in the mixed stand utilized more compounds in the miscellaneous group than did bacteria in the conifer stand, while usage of miscellaneous compounds by bacteria in the alder stand was intermediate between that of bacteria in the mixed stand and the conifer stand. Fungi showed significantly higher use of carboxylic acids in the conifer stand compared to the alder stand (Fig. 3).

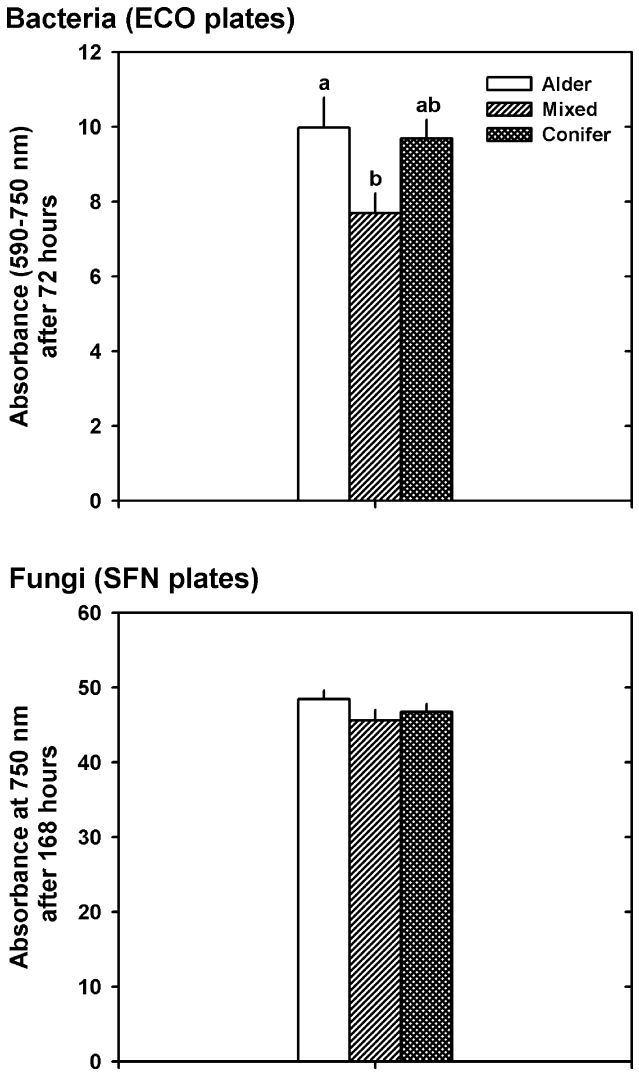

Fig. 2. Total metabolic activity of bacteria on BiOLOG ECO plates and fungi on BiOLOG SFN plates from pure conifer, mixed alder-conifer, and pure alder forest stands of coastal Oregon. Different letters indicate significant differences ($P<0.05$) based on ANOVA and Tukey's multiple pairwise comparisons test.

Activity patterns of enzymes involved in ligno-cellulose degradation were significantly different among the three stands ($A=0.099$, $P=0.005$; Fig. 4). The ligno-cellulolytic enzyme activity pattern in the alder stand was significantly different from that of the conifer stand ($A=0.144$, $P=0.003$), but enzyme activity pattern in the mixed stand was not significantly different from either the alder stand ($A=0.055$, $P=0.050$) or the conifer stand ($A=0.033$, $P=0.125$; Fig. 5). Individually, enzyme activities displayed two contrasting trends among the three stands. Four of the enzymes assayed (β-gluc, β-xylo, PhOx, and NAG) had significantly lower activities in the conifer stand than the alder stand, with intermediate activity levels in the mixed stand (Fig. 5). However, four other enzymes (α-gluc, β-galac, CBH, and sulf) had significantly lower activities in the mixed stand compared to the alder stand, with intermediate activity levels in the conifer stand (Fig. 5). Phosphatase had similar activity levels in both the alder and conifer stands but significantly lower activity in the mixed stand (Fig. 5).

4. Discussion

The presence of red alder in conifer forests of the PNW has been shown to alter ecosystem N dynamics, increase NPP (in N-limited systems), increase soil organic matter content, decrease soil bulk density, as well as alter soil pH and exchangeable base cation concentrations (Tarrant and Miller, 1963; Binkley and Sollins, 1990; Binkley et al., 1992; Cole et al., 1995; Hart et al., 1997). Our results suggest that, in addition to altering soil chemical and physical properties, N-fixing red alder can have a dramatic influence on community-level soil microbial function, even in an ecosystem already of high N status. Both the CLPP and enzyme activity assays showed strong differences between conifer soils and soils where alder is present, as well as strong overlap between pure alder and mixed alder-conifer soils, suggesting that the influence of N-fixing red alder on community-level soil microbial function is a robust phenomenon and not simply an artifact of a particular assay.

Consistent with our first hypothesis, both bacterial and fungal CLPPs shifted with increasing alder density, suggesting that the presence of red alder at intermediate densities is enough to alter the physiological profile of the microbial community. These results are also consistent with other research that has shown that bacterial communities from soil dominated by different plant species can produce distinct CLPPs (Grayston et al., 1998). Differences in bacterial and fungal CLPPs among the three stands can be partially explained when C substrates are grouped into substrate guilds. The shift in bacterial CLPPs with increasing alder density is due in part to increased use of carbohydrates and decreased use of N-rich amino acids among bacteria in the alder and mixed stands compared to the conifer stand. Differential use of C compounds classified as 'miscellaneous' by soil bacteria was also evident. Compounds grouped into the miscellaneous guild on the bacterial (ECO) BiOLOG plates are all organic phosphates (e.g. 2 glucose-1-phosphate, 2 D,L-a-glycerol phosphate), which suggests a greater P limitation to bacterial growth in the mixed stand, and to some extent the alder stand, compared to the conifer stand. Most N-fixing plants, including alder, have high P requirements (Giardina et al., 1995), so free-living soil bacteria may face more intense competition for P in the mixed and pure alder stands than in the conifer stand.

Differences in fungal CLPPs are more difficult to explain based on grouping C substrates into guilds. Only the use of carboxylic acids was significantly different among the three stands and no significant difference in total C-use by fungi was found among stands. Increased carboxylic acid usage

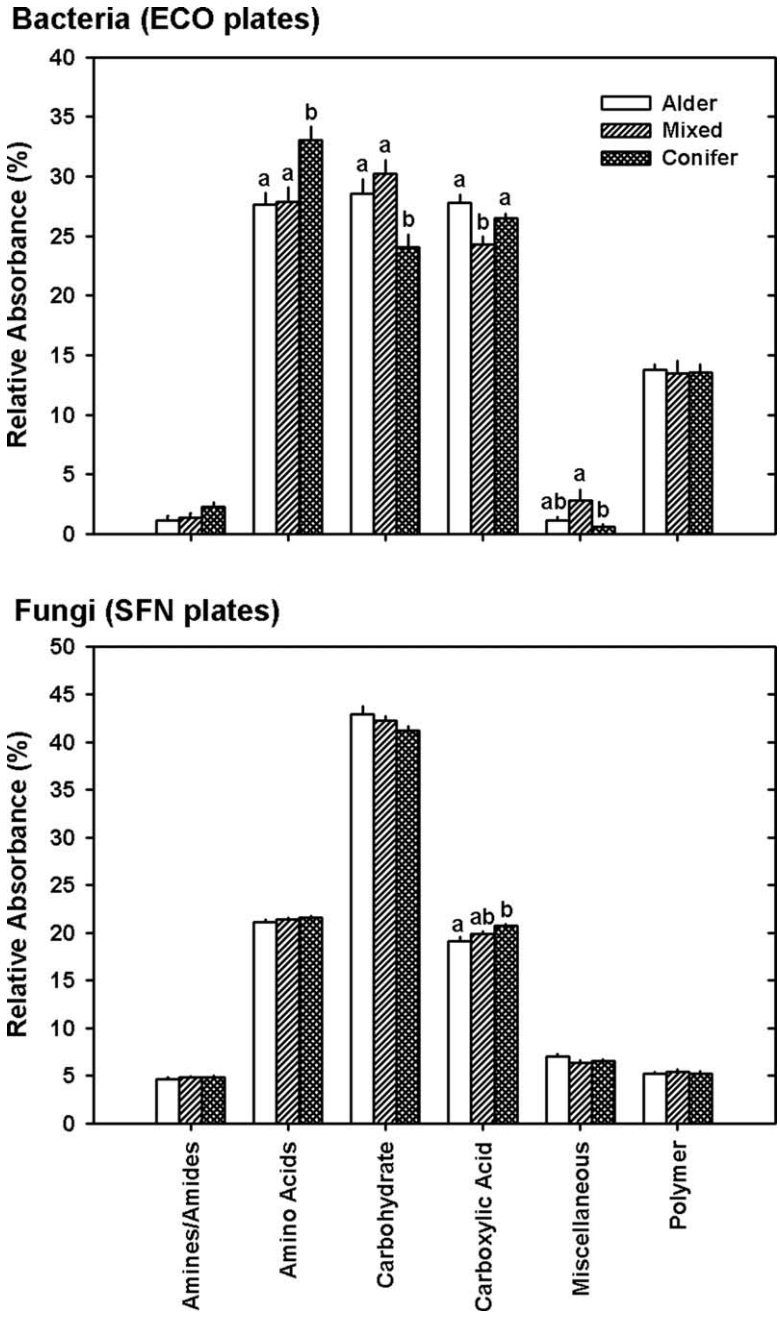

Fig. 3. Relative use of carbon-substrate guilds by bacteria on BiOLOG ECO plates and fungi on BiOLOG SFN plates from pure conifer, mixed alder-conifer, and pure alder forest stands of coastal Oregon. Different letters indicate significant differences ($P<0.05$) based on ANOVA and Tukey's multiple pairwise comparisons test.

by fungal communities with increasing conifer density suggests a shift toward a greater affinity for metabolizing the more humified organic matter common to coniferous forests (Schlesinger, 1997).

The activities of all nine enzymes assayed were greatly enhanced in the alder stand. However, enzyme activities in the mixed stand were not consistently intermediate between those of the alder and conifer stands, nor was there a distinct separation between conifer and mixed stand ligno-cellulolytic enzyme activity patterns as one would expect from the trend in bacterial and fungal CLPPs. This suggests that there may be a critical density of red alder trees that must be reached before litter inputs, root turnover, and root exudates from red alder can significantly enhance production and activity of some extra-cellular enzymes.

Our hypothesis that cellulolytic enzymes, as well as those involved in the hydrolysis of phosphate and sulfate esters, would increase with increasing red alder density was only partially supported. Two of the five cellulolytic enzymes, β-gluc, the activity of which is the rate-limiting step in

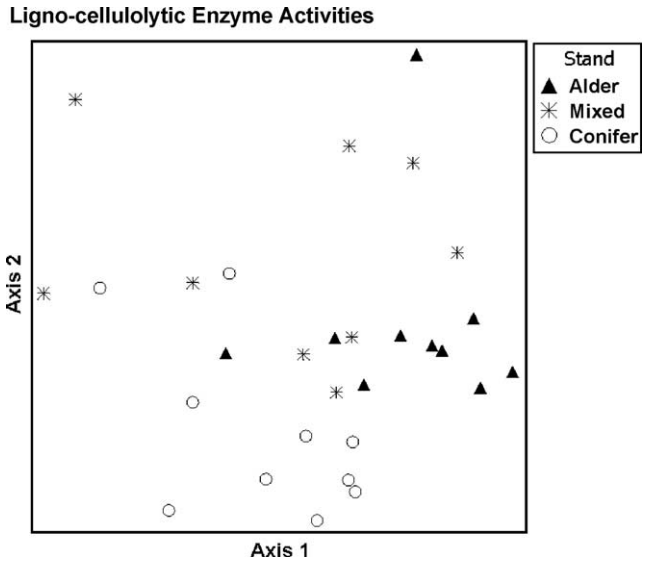

Fig. 4. Non-metric multi-dimensional scaling of extra-cellular ligno-cellulolytic enzymes from pure conifer, mixed alder-conifer, and pure alder forest stands of coastal Oregon.

the hydrolysis of cellulose (Alef and Nannapieri, 1995), and β-xylo, which is involved in the later stages of hemicellulose degradation (Dekker, 1985), followed the predicted pattern. Results from these two enzymes alone would lead us to the conclusion that the presence of red alder may greatly enhance C cycling in soils. However, three other cellulolytic enzymes, as well as phos and sulf, displayed an apparent depression in activity in the mixed alder-conifer stand.

We suggest three potential explanations for the apparent depression of α-gluc, β-galac, CBH, phos, and sulf activity in the mixed stand. First, lower activity of some enzymes in the mixed stand may be related to the lower overall metabolic activity of the bacterial community in this stand, as shown by the BiOLOG ECO plates. We consider this the least likely explanation because fungi are primarily responsible for the production of extra-cellular ligno-cellulolytic enzymes (Eriksson and Wood, 1985), and fungi make up the majority of microbial biomass in relatively low pH forest ecosystems (Chapin et al., 2002). Second, the depression in activity of these enzymes in the mixed alder-conifer stand may be the result of inhibition by unique humic-phenolic condensates generated during the decomposition of mixed alder and conifer detritus. Experiments with mixed-species litter decomposition have shown that basal respiration from mixed *Alnus* and *Picea* litter was significantly reduced compared with that of either litter type decomposing individually (Gartner and Cardon, 2004). Third, extra-cellular enzymes that displayed reduced activity in the mixed alder-conifer stand may have been inhibited by their reaction products. For example, cellobiose is a powerful inhibitor of CBH (Eriksson and Wood, 1985), the presence of orthophosphate inhibits phosphatase activity, and sulfatase activity is strongly inhibited by the presence of both inorganic sulfate and inorganic phosphate (Alef and Nannapieri, 1995). Previous work in the same three stands we studied revealed soil inorganic P concentrations in the mixed alder-conifer stand that were

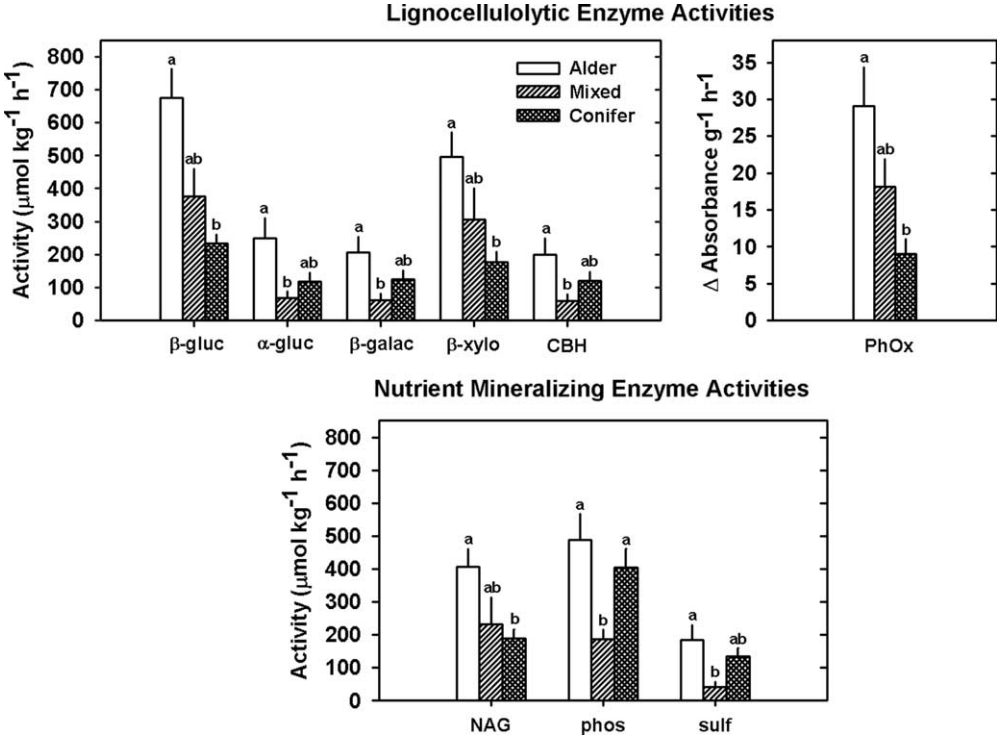

Fig. 5. Individual activity levels of ligno-cellulolytic and nutrient mineralizing extra-cellular enzymes from pure conifer, mixed alder-conifer, and pure alder forest stands of coastal Oregon. Different letters indicate significant differences ($P<0.05$) based on ANOVA and Tukey's multiple pairwise comparisons test.

triple the concentrations in either the pure alder or pure conifer stand (Zou et al., 1995), which could explain the suppression of both phosphatase and sulfatase activity in the mixed stand compared to the pure alder and pure conifer stands. In the case of CBH, it may be that β-gluc activity, although higher in the mixed stand than in the pure conifer stand, has not increased enough to catalyze the hydrolysis of excess cellobiose, the accumulation of which feeds back on and inhibits CBH activity. It may also be the case that some extra-cellular enzymes in the mixed stand are being inhibited by a combination of humic-phenolic condensates unique to this stand and by an excess of reaction product in the soil solution.

We also hypothesized that the activity of enzymes involved in lignin and chitin degradation, PhOx and NAG, would decrease with increasing alder density. In fact, we found that PhOx and NAG activities increase with increasing alder density, the exact opposite of our predicted pattern. This may be because all of the stands we studied are of high-N status, and that the inclusion of red alder, with its more labile root and leaf tissue, may induce a 'priming effect' (Fontaine et al., 2003; Waldrop and Firestone, 2004) increasing the activities of many of the extra-cellular enzymes involved in microbial heterotrophic metabolism. In the case of NAG activity, an essential enzyme in the mineralization of N from chitin (Olander and Vitousek, 2000), this is consistent with results from Hart et al. (1997) who found that all of the assessed N transformation rates catalyzed by soil microorganisms increased with increasing alder density.

In the case of PhOx, produced primarily by white-rot basidiomycetes as an initial step in lignin degradation, fungi typically require high amounts of readily available C sources to subsidize the production of this relatively expensive enzyme (Kirk and Farrell, 1987). Hence, we speculate that higher PhOx activity in soil from the alder stand is the result of higher C availability in this stand relative to the other stands; higher C availability in alder than conifer stands of the PNW has also been suggested in other studies (Edmonds, 1980; Harmon et al., 1990). Furthermore, we speculate that we did not observe an inhibitory effect of N on PhOx activity because of the already high N-status of all three stands.

In summary, we found that a N-fixing plant can significantly affect the extra-cellular enzyme activities and physiological profiles of the soil microbial community even in an ecosystem already of high N status. Because our study is unreplicated, we cannot generalize beyond the site we studied. However, our results strongly suggest that individual plant species can have profound impacts on soil microbial communities. One of the key questions that remains in soil biology is whether or not such plant-driven changes in soil microbial function feed back to affect plant performance, as well as the functioning of the ecosystem as a whole. Well-replicated field studies are needed to experimentally evaluate these potential feedbacks in order to elucidate the importance of microbial functional diversity in ecosystems (Binkley and Giardina, 1998; Waldrop and Firestone, 2004).

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