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Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition

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ABSTRACT

The relative roles of ectomycorrhizal (ECM) and saprotrophic communities in controlling the decomposition of soil organic matter remain unclear. We tested the hypothesis that ECM community structure and activity influences the breakdown of nutrient-rich biopolymers in soils, while saprotrophic communities primarily regulate the breakdown of carbon-rich biopolymers. To test this hypothesis, we used high-throughput techniques to measure ECM and saprotrophic community structure, soil resource availability, and extracellular enzyme activity in whole soils and on ECM root tips in a coastal pine forest. We found that ECM and saprotroph richness did not show spatial structure and did not co-vary with any soil resource. However, species richness of ECM fungi explained variation in the activity of enzymes targeting recalcitrant N sources (protease and peroxidase) in bulk soil. Activity of carbohydrate- and organic P- targeting enzymes (e.g. cellobiohydrolase, β-glucosidase, α-glucosidase, hemicellulases, *N*-acetyl-glucosaminidase, and acid phosphatase) was correlated with saprotroph community structure and soil resource abundance (total soil C, N, and moisture), both of which varied along the soil profile. These observations suggest independent roles of ECM fungi and saprotrophic fungi in the cycling of N-rich, C-rich, and P-rich molecules through soil organic matter. Enzymatic activity on ECM root tips taken from the same soil cores used for bulk enzyme analysis did not correlate with the activity of any enzyme measured in the bulk soil, suggesting that ECM contributions to larger-scale soil C and nutrient cycling may occur primarily via extramatrical hyphae outside the rhizosphere.

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1. Introduction

Ectomycorrhizal (ECM) fungi are a critical component of the diversity and function of terrestrial ecosystems. They receive a large, direct share of net primary productivity and play an important role in cycling key plant macronutrients through soils (Smith and Read, 2008). ECM communities on roots can vary spatially, temporally, and along environmental gradients from the local to regional scales (Courty et al., 2008; Cox et al., 2010; Peay et al., 2010; Peay et al., 2011; Taylor et al., 2010). However, the functional consequences of these different communities for ecosystem processes, like soil carbon (C) and nitrogen (N) cycling, are poorly understood. Traditionally, saprotrophic (i.e. free-living) microbes were thought to control rates of decomposition in soil. However, many ECM fungi have the physiological capability to break down nutrient- and C-rich molecules present in soil and plant litter (Talbot et al., 2008). Species of both ECM and saprotrophic fungi can vary in this decomposer ability (Buée et al., 2007; Courty et al., 2005; Gessner et al., 2010; Sinsabaugh, 2005), which may cause different assemblages of fungi to have unique effects on rates of soil C and N cycling. On the other hand, if different assemblages contain species that are functionally redundant with respect to these traits, rates of C and N cycling may be consistent across fungal communities regardless of differences in species composition. Identifying the structure–function relationships of ECM and saprotrophic communities is an important step toward resolving uncertainty about the roles of these two fungal groups in soil processes (Baldrian, 2009).

ECM and saprotrophic communities may be responsible for degrading different C and nutrient fractions in soil organic matter. For example, evidence has accumulated from laboratory and greenhouse studies that ECM fungi may target recalcitrant

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nutrients in soil (Read and Perez-Moreno, 2003; Talbot and Treseder, 2010). The genetic and/or physiological capability to produce enzymes that degrade N-rich or phenolic polymers appears to be distributed across a wide phylogenetic range of ECM taxa (Bödeker et al., 2009; Plett and Martin, 2011; Talbot and Treseder, 2010). By contrast, few ECM fungi tested can produce enzymes that break down large C-rich biopolymers like cellulose, pectin, or lipids in culture (Talbot and Treseder, 2010). ECM fungi are also consistently dominant in lower soil horizons, where nutrient availability is high relative to C availability (Allison et al., 2007; McGuire et al., 2011; O'Brien et al., 2005). Saprotrophs dominate the litter layer in soils, where the activity of labile C-targeting hydrolase enzymes is high (Sinsabaugh et al., 2008), suggesting that saprotrophs may play a more important role in production of these enzymes compared to ECM fungi. Nevertheless, the contribution of ECM fungi to enzyme production in soil remains unclear, and the importance of ECM fungi to soil C turnover in natural ecosystems, relative to saprotrophs, is a matter of debate (Baldrian, 2009; Cullings and Courty, 2009). Over 90% of N in soils is present in organic forms, which typically consist of over 50% C (Talbot and Treseder, 2010). If ECM fungi mobilize even a small fraction of this organic N pool in soils, it may have large consequences for both N and C cycling at the ecosystem-level (Orwin et al., 2011).

Patterns of extracellular enzyme production across ECM species suggest that ECM diversity may affect soil C and nutrient degradation rates. Root tips colonized by different species of ECM fungi can vary in their capacity to produce extracellular enzymes that target nutrient- and C-rich substrates (Buée et al., 2007). In addition, the enzymatic diversity of ECM root tips can increase linearly with taxonomic diversity when the initial diversity of the community is low (<50 species, Rineau and Courty, 2011), suggesting that species effects on extracellular enzyme production in the rhizosphere (i.e. in close proximity to the plant root tip) may be additive. The same taxonomic-functional diversity relationships may hold true for ECM hyphae that extend outside the rhizosphere, as all ECM fungi tested have the capacity to produce some extracellular enzymes in their vegetative states in culture (Talbot et al., 2008). The extent to which ECM processes in the rhizosphere contribute to rates of decomposition in bulk soil is unclear; if enzymatic activity on root tips is low relative to the activity of fungal hyphae in soils, functional diversity of ECM hyphae in soil may have greater consequences for total soil C and nutrient cycling than ECM communities on roots.

In addition to diversity effects, resource availability to soil fungi may act as a control over enzyme activity in soils. Enzyme production by ECM root tips can increase with decreasing access to plant photosynthate (Courty et al., 2007; Courty et al., 2010), suggesting that ECM fungi may mine soil for C when easily-accessible C supplies are low. If this mechanism occurs in ECM hyphae, plant photosynthate availability may control the activity of extracellular enzymes in bulk soils. Similarly, activity of C-targeting hydrolases in bulk soil is correlated with total soil organic matter content both within and across biomes (Sinsabaugh et al., 2008), suggesting that these enzymes are regulated by resource availability to saprotrophic soil microbes. Determining the strength of resource effects on extracellular enzyme production, relative to fungal community effects, has been challenging to assess directly in the field. This is due in part to the difficulty of simultaneously assessing community structure and enzyme production in large sample sizes. High-throughput sequencing technologies coupled with recent developments in high-throughput microplate procedures for assaying extracellular enzyme production in soils (Allison et al., 2008; German et al., 2011) and on root tips (Pritsch et al., 2011) now make it possible to address this issue by exploring relationships between resource availability, community structure, and enzyme activity directly in field soil.

The objective of this study was to determine the relative roles of ECM and saprotrophic community structure and resource availability on rates of extracellular enzyme activity in soils. To do this, we used a spatially explicit sampling design to capture turnover in fungal community composition and diversity, resource abundance, and extracellular enzyme activity in a model ECM system that allows for minimal variation in other factors (e.g. plant type and soil parent material). We expected that protease and oxidase activity would vary with ECM diversity in bulk soil, while hydrolases that target more labile C-rich substrates would be correlated with the diversity of saprotrophic fungi. We also expected that resource availability to both ECM and saprotrophic fungi would influence enzyme activity, such that activity of proteases/oxidases would decrease with increases in ECM host tree density and C-targeting hydrolases would increase with increasing resource availability to saprotrophs (e.g. soil C content). Finally, we expected that extracellular enzymes produced by root tips would make little contribution to bulk soil enzymatic activity, such that any species effects on ECM tip enzyme production at the rhizosphere scale may not be observed at the whole soil level.

2. Materials and methods

2.1. Study site

To identify patterns of resource availability, ectomycorrhizal community structure, and soil function across spatial scales, we sampled soils from three plots in a *Pinus muricata* D. Don (Bishop pine) forest at Point Reyes National Seashore, located in west Marin County, California (38°04′N by 122°50′W). The area has a Mediterranean climate, with cold wet winters and warm dry summers (Peay et al., 2007). Soils at each plot are either mollisols or alfisols formed from quartz-diorite (Kashiwagi, 1985, Table 1). Elevation of the study sites ranged from 152 to 244 m. We calculated tree basal area (m^2) at four points in each sampling plot by measuring DBH (diameter at breast height) of all trees with diameter >10 cm within in a 10 m-diameter circle around the sampling point (Supplementary Fig. 1). A single ectomycorrhizal host plant, *P. muricata*, dominated all plots while one plot had an understory of *Morella californica* (Cham.) Wilbur (Table 1).

2.2. Soil and ECM root tip sampling

In January of 2012, soils were collected from each of three plots that were 40 m × 40 m in size and spaced 1 km apart. To describe

Table 1
Plot characteristics at sampling site in Point Reyes National Seashore.

Plot	Stand age	Elevation (m)	Slope	Aspect	Soil type	Total basal area (m^2)	Total ECM basal area (m^2)	Percent *P. muricata*	Percent *M. californica*
Mount Vision old (MVO)	NA	240	31	S	Ultic Haplustalf	2.63	2.63	100	0
Pierce Point Road (PPR)	53	156	0	none	Pachic Haploxeroll	2.11	1.99	94	6
Mount Vision young (MVY)	17	350	<5	WNW	Ultic Haplustalf	2.25	2.25	100	0

local ECM diversity at each plot, we used a spatially explicit, nested sampling design (Supplementary Fig. 1). This design was chosen because it facilitates analysis of turnover at fine spatial scales by enabling multiple comparisons for each sampling distance. At each point in the plot, a 10 cm deep, 5 cm diameter soil core was taken and immediately separated into O horizon and A horizon. This resulted in a total of 26 soil samples collected per plot (13 sample points × 2 horizons). Soils were kept on ice and transported to University of California, Berkeley within 8 h of collection. Soils were sieved through a 2 mm mesh, all Pinaceae roots were removed, and fine roots were observed in water with a stereomicroscope (×40). Up to 8 ECM root tips were selected per soil core and preserved for extracellular enzyme analysis (4 °C in tap water). Enzyme analyses were conducted within 1 week of collection, after which the root tips were frozen at −20 °C for DNA extraction. Sieved soils were mixed in Ziploc bags, a 0.20 g subsample was placed directly into a bead tube from the Powersoil DNA Extraction Kit (MoBio, Carlsbad, CA USA), and the samples were stored at 4 °C until DNA extraction. Before extraction, samples were homogenized for 30 s at 75% power using a Mini-Beadbeater (BioSpec, Bartlesville, OK USA). A second subset was preserved for extracellular enzyme analysis and characterization of soil chemistry (frozen at −80 °C). Soils and ECM root tips were preserved within 48 h of field sampling.

2.3. EMF community composition

To determine the ectomycorrhizal and saprotrophic community composition in each soil sample, DNA was extracted from bulk soil and amplified using the fungal specific primers ITS1f and ITS4 (Gardes and Bruns, 1993; White et al., 1990). We then identified the fungal community in each soil sample using 454-pyrosequencing of the ITS region with primer barcoding methods (Amend et al., 2010). Each of the 78 soil samples was individually barcoded in the initial PCR as in Peay et al. (2012) with the exception that PCR was run for 25 cycles. Individual PCR reactions were cleaned using an Agencourt Ampure XP kit (Beckman Coulter, USA), quantified fluorescently with the Qubit dsDNA HS kit (Life Technologies, USA), and pooled at equimolar concentration prior to pyrosequencing. Pyrosequencing was performed using a 1/8th GS-FLX run at the Duke Institute for Genome Sciences & Policy. Sequence data was analyzed using the QIIME pipeline (Caporaso et al., 2010). Initial sequence processing and sample assignment was done using the split_libraries.py command with a min/max sequence length cutoff of 350/1200 bp, max homopolymer run length of 10 bp, and max barcode error number of 1.5. To account for sequencing error that can arise as part of the PCR and 454 sequencing process, we used flowgram clustering (Reeder and Knight, 2010) implemented in the denoise_wrapper.py command using a Titanium error profile. Denoised sequences were chimera checked and clustered into operational taxonomic units (OTUs) using the usearch option (Edgar, 2010) in the pick_otus.py command. Chimera checking was also implemented in usearch using both de novo and reference based methods (with the UNITE ITS dataset provided on QIIME). Only sequences identified as chimeric using both denovo and reference methods were considered chimeric and removed from the analysis. Sequences were clustered into OTUs in usearch using a 96% sequence similarity threshold and a minimal cluster size of one. Although 97% is the canonical species cutoff used in many fungal ecology studies (Peay et al., 2008), our previous work with NGS data from this system has suggested that a slightly more conservative approach to OTU delineation can help compensate for spurious OTUs generated via sequencing error (Peay et al., 2012). Such cutoffs are always biological approximations (Hughes et al., 2009) and the choice has little effect on our results (<1% change is total species richness). Taxonomy was assigned by searching representative sequences from each OTU against a previously published fungal ITS database (Tedersoo et al., 2010a), including well curated sequences from the National Center for Biotechnology's GenBank and UNITE, using the Basic Local Alignment Search Tool (BLAST) with a minimum expected value of 0.001 with the QIIME assign_taxonomy.py script. OTUs were categorized as ECM or saprotrophic based on current knowledge of metabolic lifestyle of the closest BLAST matches for each individual taxa (Tedersoo et al., 2010a).

We also determined the ECM species colonizing root tips that were assayed for extracellular enzyme activity. DNA was extracted from individual root tips with the Extract-n-Amp kit (Sigma–Aldrich) and amplified with the same ITS1f and ITS4 primers. Amplicons were sequenced directly using Sanger sequencing at Beckman Coulter Genomics (Danvers, MA USA).

2.4. Soil chemistry

Each soil horizon at each sampling point was analyzed for total C, total N, pH, and percent moisture content. Subsamples of fresh soil from each horizon at each sampling point in a plot were analyzed for soil water content by drying at 60 °C for 48 h. Frozen soils were thawed and analyzed for pH in a 1:1 water ratio using a glass electrode. To generate C/N ratios, total C and N was analyzed using dry combustion on a Vario MAX CNS Elemental Analyzer (Elementar Inc.) at University of Minnesota.

2.5. Soil and ECM root tip enzyme analyses

We assayed the potential activities of 10 extracellular enzymes involved in soil C and nutrient cycling: cellobiohydrolase (CBH, an exocellulase), β-glucosidase (BG, which hydrolyzes cellobiose into glucose), α-glucosidase (AG, which hydrolyzes starch and glycogen), β-xylosidase (BX, which degrades the xylose component of hemicellulose), β-glucuronidase (BGLU, which degrades the glucuronic acid component of hemicellulose), polyphenol oxidase (PPO, which oxidizes phenols) and peroxidase (PER, including oxidases that degrade lignin), acid phosphatase (AP, which releases inorganic phosphate from organic matter), *N*-acetyl-glucosaminidase (NAG, which breaks down chitin), and leucine-amino-peptidase (LAP, which breaks down polypeptides). Potential enzyme activities were measured sequentially on individual ECM root tips following the procedure of Pritsch et al. (2011). Only about half of the soil samples contained ECM tips that were suitable for enzyme analyses (46 out of 78 samples). Some samples did not have 8 ECM tips available for enzyme analysis (13 out of 46), resulting in a total sample size of 324 ECM tips (out of a potential 624 = 78 samples × 8 tips). Potential enzyme activities in bulk soil were measured in each soil horizon collected at a sampling point using fluorometric and colorimetric procedures on a microplate reader following Allison et al. (2008).

2.6. Data analysis and statistics

To determine patterns of resource availability in soil, we performed ANOVA with plot and horizon (nested within plot) as independent variables and soil chemistry parameters or basal area of ECM host tree species (in m^2) as the dependent variables. Percent soil moisture, total soil C, and total soil N were highly correlated, while pH was weakly correlated with soil C:N ratio and total soil C (Supplementary Table 1). Therefore, we collapsed the soil variables into vectors using principal components analysis (PCA). After examining scree plots we chose to retain the first two principal components, which explained 86% (PC1 62.4%, PC2 23.4%) of the

variation in soil chemistry variables. Mantel tests were used to identify spatial autocorrelation in soil chemistry variables and ECM host basal area across plots.

To determine factors controlling patterns of ECM and saprotrophic species richness in soils, we performed univariate regression analyses with plot, horizon (nested within plot), basal area of ECM plant species, or the first two soil chemistry principal components as independent variables and ECM or saprotrophic richness (as Chao1 estimates) as the dependent variable. Mantel tests were used to further explore spatial effects (distance between sample points in m^2) on ECM and saprotrophic richness across plots.

To determine the factors controlling ECM or saprotrophic community composition in soils, we first used one-way non-parametric PERMANOVA analyses with plot, horizon (nested within plot), soil chemistry PC1 or PC2, or ECM basal area as independent variables and Bray–Curtis community dissimilarity among samples as the dependent variable (Anderson, 2001). To determine the relative importance of spatial and environmental factors in structuring communities, we then conducted a multivariate PERMANOVA using only those variables that showed significant correlation with community composition in the univariate analyses. Because this method is sensitive to the order of analyses, we randomized the order of analysis for all variables and only retained those that were significant in all 999 runs. These differences were visualized with non-metric multi-dimensional scaling (NMS) (McCune et al., 2002). In addition, Mantel tests were used to analyze correlations between matrices for geographic distance and the Bray–Curtis community dissimilarity in community structure, with distances calculated between all possible pairs of samples.

Soil enzymes were highly correlated (Supplementary Table 2), so to reduce the measured enzymes to a reasonable number of factors, we used a PCA on the ranked variables. After examining scree plots, we chose to retain the first two principal components, which explained 78% (PC1 64.5%, PC2 13.5%) of the variation in soil enzymes. The first principal component was associated with variation in carbohydrate- and phosphorus-targeting enzymes, the second with protein (LAP) and polyphenolic (PPO, PER) targeting enzymes (Supplementary Table 2). ECM tip enzymes were correlated as well, and separated into two principal components that together explained 63% (PC1 47.7%, PC2 15.3%) of the variation in ECM tip enzyme activity. Similar to the soil enzymes, carbohydrate- and phosphorus-targeting enzymes were associated with principal component 1, while LAP, PPO, and PER were correlated with principal component 2 (Supplementary Table 3).

To determine the effect of ECM diversity, saprotrophic diversity, and resource availability on enzyme activity, we first conducted univariate analyses with plot, horizon (nested within plot), ECM species richness, saprotrophic species richness, soil chemistry PC1 or PC2, or ECM host tree basal area as independent variables and enzyme activity in bulk soil, or on ECM root tips, as independent variables. To determine the relative importance of these factors in structuring soil enzyme activity, we then conducted multiple regression analysis using only those variables that showed significant correlation with soil enzyme PC1 and PC2 in the univariate analyses. To explore the relationship between ECM community structure and function, we looked for univariate correlations between enzyme PCA scores the Bray–Curtis community similarity matrices for ECM or saprotrophic fungi using Mantel tests.

All statistical tests and graphics were done using the program R version 2.7.2 (R Core Development Team, 2008). Distance matrices and NMDS ordination were performed with the package Vegan, and Mantel tests with the package Ecodist. In cases where data did not conform to assumptions of normality and homogeneity of variance, values were log transformed prior to analysis. In cases where transformed data did not conform to assumptions of normality, analyses were performed on ranked data. All statistical tests were considered significant at $P < 0.05$.

3. Results

3.1. Community overview

Across sites and soil horizons, pyrosequencing yielded a total of 121,499 sequences averaging 447 bp in length from 76 samples. After denoising and quality control, 90,009 sequences remained (median of 1215 and mean of 1184 sequences per sample), which clustered into 1650 operational taxonomic units. Ten sequences could not be identified to domain. Prior to statistical analyses, unclassifiable sequences were removed, leaving 165 unique ECM taxa and 895 unique saprotrophic taxa. Altogether, 726 (46%) taxa were present in more than one soil core and 493 taxa (30%) were present in more than one plot. Observed ECM species richness ranged from 5 to 22 species for horizons in individual soil cores (median 13, SD 4.0). Chao 1 estimates of ECM species richness ranged from 5 to 44 for soil horizons within a core (median 17, SD 7.9). Observed saprotrophic richness ranged from 25 to 99 species per sample (median 60.2, SD 17.5), while Chao 1 estimates ranged from 43 to 213 per sample (median 125, SD 40.5). Species accumulation curves did not plateau for plots or individual soil samples (data not shown). For this reason, unless indicated all measures of species richness used in further analyses are rarefied Chao1 estimates. We obtained fungal DNA sequences from 74 ECM tips that were also analyzed for extracellular enzyme activity. Twenty-three unique ECM taxa were identified on root tips; 12 OTUs were based on single root tips, while the remaining 11 were found in multiple root tips and represented species from across the Basidiomycota (Supplementary Fig. 2). Only five species were present in more than one soil core, and only two species (*Tomentella* sp. 1 and *Russula aff. sanguinea*) were identified on root samples from more than one plot.

3.2. Patterns of resource availability

Soil resources varied significantly among soil horizons. Resources associated with the first principal component in soil chemistry (PC1: percent soil moisture, total soil C, and total soil N) were higher in the O horizon compared to the A horizon (Fig. 1A). Soil chemistry PC1 and PC2 both varied among plots (Fig. 1A, Supplementary Fig. 3F), but did not vary with distance among individual soil samples (PC1: $P = 0.325$, PC2: $P = 0.346$). Basal area of ECM plant species did not vary by plot or distance between samples, and was not correlated with soil chemistry variables (data not shown).

3.3. Patterns of ECM and saprotrophic species richness and community structure

ECM species richness (estimated by Chao 1) was similar across plots and soil horizons, despite significant differences in soil chemistry among sites and soil horizons (Fig. 1A, Supplementary Fig. 3F). Saprotrophic richness also did not vary among plots or horizons (Table 2). Neither ECM richness nor saprotrophic species richness was correlated with any environmental parameter, and did not show spatial structure across plots. However, ECM community composition varied significantly across plots and by the distance (in m) between individual soil samples (Table 2). ECM community composition was also correlated with environmental variables, including both soil chemistry PCA scores (PC1: $P = 0.001$, PC2: $P = 0.001$) and ECM host tree basal area ($P = 0.002$). In multiple

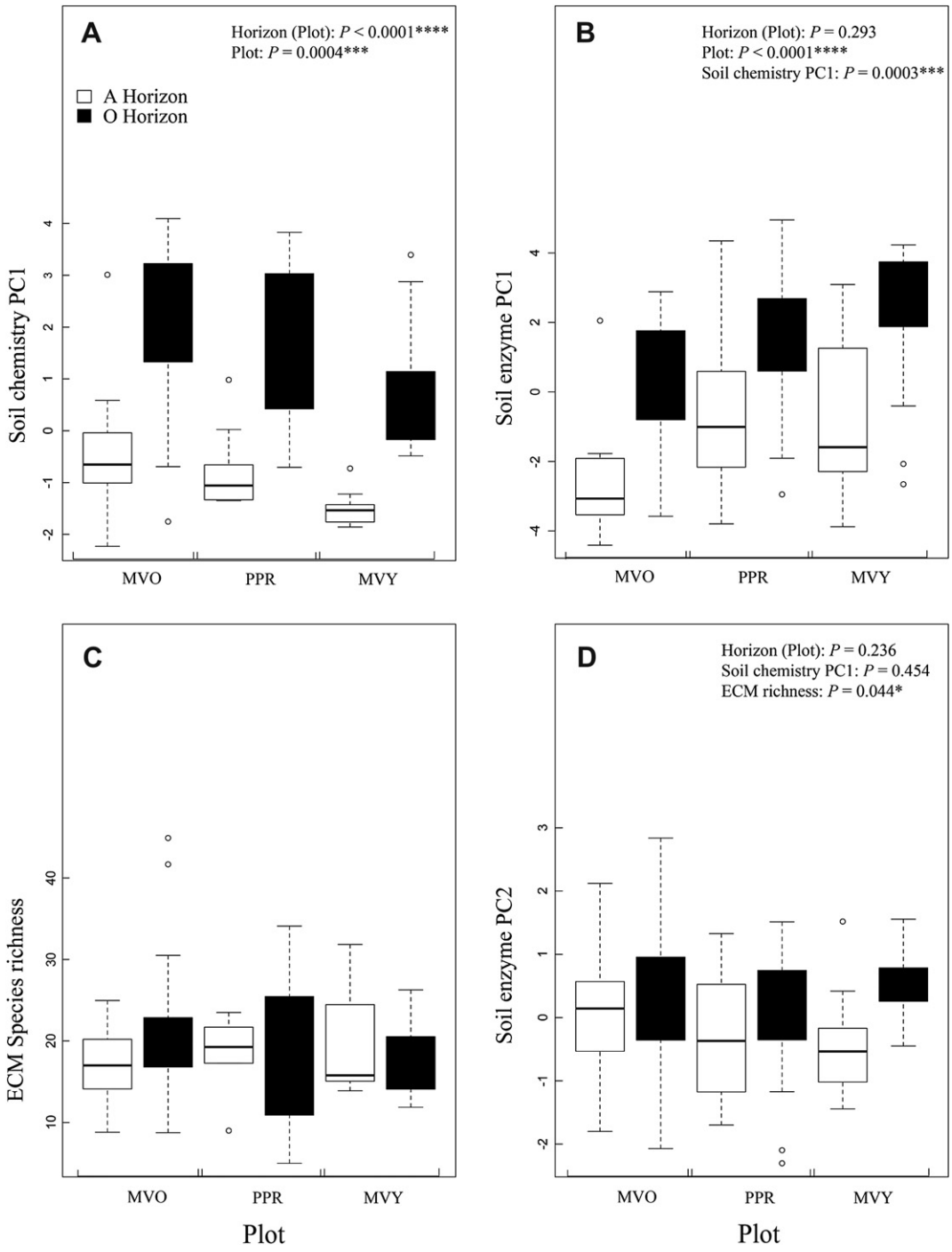

Fig. 1. Soil chemistry, soil enzyme activity, and ECM species richness in different soil horizons measured at plots in Point Reyes. The first principal component in soil chemistry characteristics (Soil chemistry PC1) was associated with total soil C, total soil N, and percent soil moisture. The first principal component in soil enzyme activity (Soil enzyme PC1) was associated with carbohydrate- (CBH, BG, AG, BX), chitin- (NAG), and organic phosphorus- (AP) targeting enzymes, as well as a phenolic-targeting enzyme (PER). The second principal component in soil enzyme activity (Soil enzyme PC2) was associated with protease (LAP) and oxidase (PPO, PER) activity. Statistics represent results from multiple regression analysis using only those factors that showed significant correlation in single regression analyses. Species richness is based on Chao1 estimates. Bars show the range of values and mid point is the average of 15–39 samples. Error bars represent 1 SD.

regression analyses, all these factors were significantly correlated with ECM community composition (Table 2). However, community composition was similar across soil horizons ($P = 0.168$). By contrast, saprotrophic communities varied significantly among horizons ($P = 0.001$), as well as by plot and spatial distance among samples. Multiple regression showed that variation in saprotroph community composition among horizons was driven by differences in resource abundance (Table 2).

3.4. Effects of fungal community structure and resource abundance on soil enzyme activity

Soil enzymes targeting labile C-, N- and P-rich molecules were positively associated with soil resource abundance (Fig. 1B). The first PCA score in soil enzymes (PC1) was significantly higher in the O horizon compared to the A horizon (Table 3, $P < 0.0001$). This principal component was associated with carbohydrate- (CBH, BG,

Table 2
Statistics predicting SAP and ECM species richness and community composition. Single factor statistics are from single regression analyses or Mantel tests. Multiple regression was performed only with variables that showed significant correlation with enzyme activity in single regression analyses.

	R^2	Slope (SE)	t/F ratio	Mantel r	P value
ECM richness					
Single factor					
Plot	0.003	−0.03 (0.07)	−0.39		0.698
Horizon (within plot)	0.004	0.01 (0.06)	0.17		0.868
Soil chemistry PC1	0.09	0.06 (0.03)	1.95		0.058
Soil chemistry PC2	1.74×10^{-5}	−0.001 (0.05)	−0.27		0.979
ECM basal area (m^2)	5.4×10^{-5}	−0.01 (0.42)	−0.03		0.976
Spatial distance (m)				−0.008	0.489
SAP richness					
Single factor					
Plot	0.08	−0.15 (0.08)	−1.92		0.062
Horizon (within plot)	0.003	−0.004 (0.05)	−0.82		0.935
Soil chemistry PC1	0.001	0.008 (0.03)	0.23		0.818
Soil chemistry PC2	0.06	−0.10 (0.06)	−1.60		0.118
ECM basal area (m^2)	2.22×10^{-5}	−0.006 (0.37)	−0.02		0.986
Spatial distance (m)				0.06	0.056
ECM community composition					
Single factor					
Plot	0.16		6.77		<0.0001****
Horizon (within plot)	0.04		1.15		0.182
Soil chemistry PC1	0.09		2.56		<0.0001****
Soil chemistry PC2	0.08		2.38		<0.0001****
ECM basal area (m)	0.06		1.75		0.021*
Spatial distance (m)				0.46	0.0001***
Multiple regression					
Plot	0.18		3.90		<0.0001****
Soil chemistry PC1	0.09		3.75		<0.0001****
Soil chemistry PC2	0.06		2.47		<0.0001****
ECM basal area (m^2)	0.05		2.29		0.0003***
Soil chemistry PC2*Plot	0.06		2.04		<0.0001****
ECM basal area (m^2)*Plot	0.09		1.95		0.0002***
SAP community composition					
Single factor					
Plot	0.17		7.71		<0.0001****
Horizon (within plot)	0.07		2.05		0.0002***
Soil chemistry PC1	0.065		4.88		0.0001***
Soil chemistry PC2	0.062		4.62		0.0001***
ECM basal area (m^2)	0.045		1.31		0.09
Spatial distance (m)				0.41	0.0001***
Multiple regression					
Plot	0.15		2.58		<0.0001****
Horizon (within plot)	0.03		0.99		0.361
Soil chemistry PC1	0.08		2.63		0.0002***
Soil chemistry PC2	0.05		1.72		0.016*

Significance levels are indicated by **** $P < 0.0001$, *** $P < 0.001$, * $P < 0.05$.

AG, BX), chitin- (NAG), and organic phosphorus- (AP) targeting enzymes, as well as a phenolic-targeting enzyme (PER). These enzymes were positively correlated with soil chemistry PC1 ($P < 0.0001$), which represented soil resource availability to fungi (percent soil moisture, total C, and total N). Multiple regression analysis showed that horizon effects on these soil enzymes were driven by soil chemistry PC1 (Fig. 1B, Table 3). Soil enzyme PC1was also significantly correlated with saprotroph community composition ($P = 0.001$), which varied among soil horizons. However, these enzymes were not correlated with ECM community composition, ECM richness, or saprotroph richness (Table 3).

In contrast to PC1 in soil enzymes, the second PCA score in soil enzymes was not associated with saprotrophic community composition ($P = 0.538$). Instead, soil enzyme PC2 was significantly correlated with ECM species richness (Fig. 1D, Table 3). This PCA score was associated with LAP, PPO, and PER activity in soils (Supplementary Table 3). When compared to variation in individual enzymes, richness was positively correlated with the activity of LAP and PER in soils (Fig. 2). Multiple regression analysis showed that ECM richness was the best predictor of soil enzyme PC2, LAP, and PER activity (Fig. 1D, Table 3, Supplementary Fig. 4). ECM richness was not correlated with any other soil enzyme. However, saprotroph richness was significantly correlated with phosphatase activity in soil (Supplementary Fig. 4).

3.5. Relationship between soil and ECM tip enzyme activity

Enzyme activity on ECM root tips was low relative to bulk soil enzymes (Supplementary Fig. 5); we calculated that between 2.7 thousand and 1.34 million individual tips were needed to reach activity levels found in each soil core, depending on the enzyme (Supplementary Table 4). ECM tip enzyme activity varied little among plots and horizons. Only ECM tip BG, AG, and PER varied among plots, while PER and NAG varied among horizons (Supplementary Fig. 5). Unlike soil enzymes, ECM tip enzymes were not related to any soil chemistry parameter, ECM richness, or ECM community structure (Table 3). Consequently, ECM tip enzymes were not correlated with the activity of extracellular enzymes measured in bulk soil (PC1: $R^2 = 0.007$, $P = 0.567$; PC2: $R^2 = 0.012$, $P = 0.501$). There was also no relationship between ECM species on the root tip and the activity of tip enzyme PC1 or PC2 (Table 3).

4. Discussion

ECM communities can vary depending on the season, chemical composition of soil, and barriers to dispersal (Courty et al., 2008; Cox et al., 2010; Kennedy et al., 2009; Peay et al., 2010). We found that variation in these communities is correlated with activity of extracellular enzymes in bulk soil. The activity of oxidative (peroxidase, PER) and proteolytic (leucine amino-peptidase, LAP) enzymes increased with increasing ECM richness in soil (Fig. 2). We observed this despite the fact that ECM richness showed no spatial structure across the sampling site and was not related to any environmental parameter. By contrast, LAP and PER activities were not correlated with the diversity of saprotrophs in soil (Fig. 2). These observations lend support to our original hypothesis that

Table 3
Statistics predicting soil and ECM root tip enzyme activity. Single factor statistics are from single regression analyses or Mantel tests. Multiple regression was performed only with variables that showed significant correlation with enzyme activity in single regression analyses.

	R^2	Slope (SE)	t	Mantel r	P value
Soil enzyme PC1					
Single factor					
Soil ECM richness	0.04	0.31 (0.25)	1.23		0.226
Soil ECM community composition				0.03	0.114
Soil SAP richness	0.06	−0.33 (0.20)	−1.63		0.110
Soil SAP community composition				0.14	0.001**
Soil chemistry PC1	0.25	0.72 (0.15)	4.71		<0.0001****
Soil chemistry PC2	0.04	0.52 (0.31)	1.67		0.099
Horizon (within plot)	0.25	0.24 (0.06)	4.03		<0.0001****
Plot	0.08	0.22 (0.09)	2.45		0.017*
Spatial distance (m)				0.0004	0.444
Multiple regression	0.44				
Soil chemistry PC1		0.19 (0.05)	3.82		0.0003***
Horizon (within plot)		0.18 (0.17)	1.06		0.293
Plot		0.34 (0.08)	4.37		<0.0001****
Soil enzyme PC2					
Single factor					
Soil ECM richness	0.10	0.91 (0.44)	2.08		0.044*
Soil ECM community composition				0.03	0.196
Soil SAP richness	5.5×10^{-7}	-1.0×10^{-3} (0.37)	−0.01		0.996
Soil SAP community composition				−0.02	0.538
Soil chemistry PC1	0.07	0.15 (0.07)	2.21		0.031*
Soil chemistry PC2	0.01	−0.11 (0.12)	−0.93		0.356
Horizon (within plot)	0.05	0.27 (0.11)	2.45		0.017*
Plot	3.0×10^{-3}	−0.07 (0.15)	−0.46		0.647
Spatial distance (m)				−0.02	0.666
Multiple regression	0.15				
Soil ECM richness		0.43 (0.21)	2.08		0.044*
Soil chemistry PC1		−0.04 (0.05)	−0.76		0.454
Horizon (within plot)		0.23 (0.19)	1.21		0.236
ECM tip enzyme PC1					
Single factor					
Soil ECM richness	0.05	−0.26 (0.17)	−1.54		0.147
Soil ECM community composition				0.06	0.120
Tip ECM species	0.19	0.14 (0.31)			0.980
Soil chemistry PC1	0.01	0.28 (0.30)	0.45		0.131
Soil chemistry PC2	0.02	0.60 (0.31)	0.91		0.370
Horizon (within plot)	0.05	−2.52 (2.34)	−1.08		0.286
Plot	0.04	5.29 (3.10)	1.70		0.093
Spatial distance (m)				0.01	0.403
ECM tip enzyme PC2					
Single factor					
Soil ECM richness	0.05	−0.15 (0.09)	−1.54		0.295
Soil ECM community composition				0.08	0.058
Tip ECM species	0.30	−0.05 (0.17)			0.386
Soil chemistry PC1	2.0×10^{-3}	0.07 (0.18)	−0.30		0.131
Soil chemistry PC2	4.0×10^{-3}	0.29 (0.18)	−0.42		0.680
Horizon (within plot)	0.03	−3.36 (2.37)	−1.42		0.120
Plot	0.003	1.54 (3.16)	0.49		0.628
Spatial distance (m)				-9.8×10^{-5}	0.453

Significance levels are indicated by **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

ECM communities control degradation of recalcitrant organic N in soil. Soil resource availability and community structure of saprotrophic fungi were correlated with carbohydrate- and organic P-targeting enzymes (Table 3), supporting the hypothesis that saprotrophs are primarily responsible for cycling this fraction of organic matter through soils.

The principal component representing oxidative and proteolytic enzymes was only weakly correlated with ECM diversity (soil enzyme PC2, Table 3) and given the number of independent correlations conducted, this relationship could have resulted from chance alone. The weak correlation may also be due to a lack of relationship between ECM diversity and PPO activity; only LAP and PER activity were positively correlated with ECM diversity (Supplementary Fig. 4). Direct experimental tests of ECM diversity effects on these enzymes are necessary to clarify and quantify the role of ECM fungi in the decay process. However, production of proteolytic and oxidative enzymes by ECM fungi in soil is consistent with the concept that ECM fungi act as decomposers as a consequence of mining soils for nutrients (the Coincidental Decomposer Hypothesis, Talbot et al., 2008). Between 83% and 90% of ECM fungi tested are able to decompose protein or a phenolic polymer, respectively, when growing vegetatively in pure culture (Talbot and Treseder, 2010). By contrast, only 17–54% of ECM fungi tested can produce enzymes that break down large C-rich biopolymers like cellulose, pectin, or lipids. ECM fungi tend to take up small organic N molecules that are abundant in soils, have high N content, and

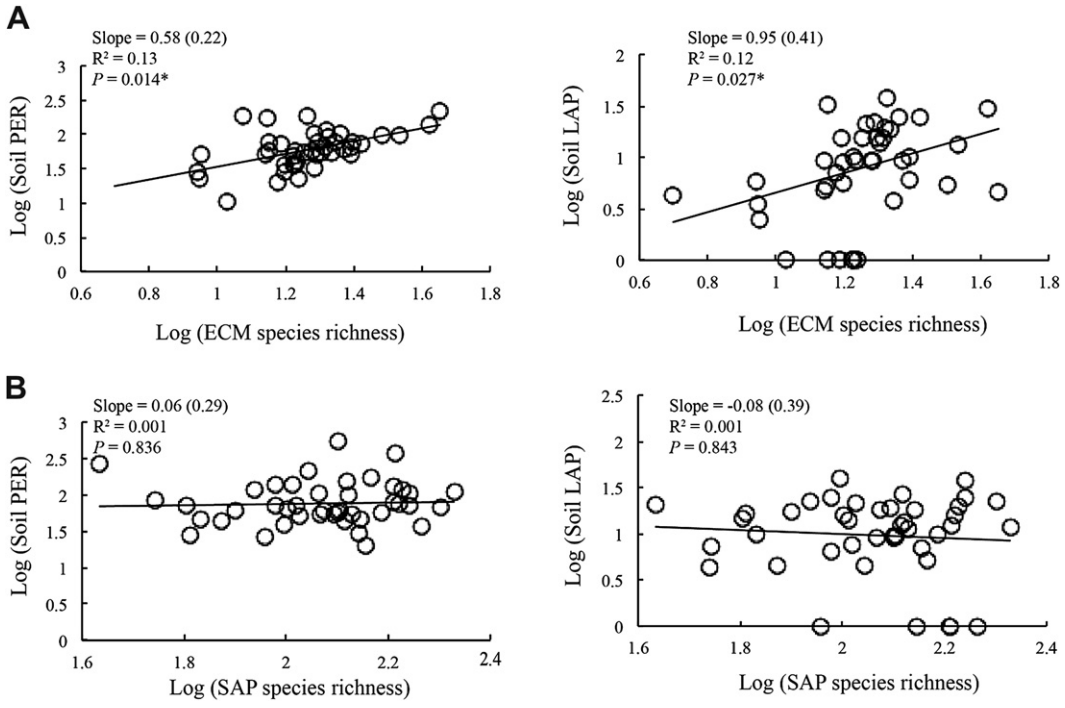

Fig. 2. Relationships between soil extracellular enzyme activity and species richness of ECM fungi (A) and saprotrophic fungi (B). Points represent soil enzyme activity measured in each soil horizon (O horizon and A horizon) in each soil core collected at each sample point. Lines show linear regression against ECM or saprotrophic species richness based on Chao1 estimates. Statistics represent results from single regression analysis.

have an acyclic C backbone (Talbot and Treseder, 2010), suggesting that decomposer activities of ECM fungi in soil are targeted toward releasing labile N from complex, recalcitrant organic matter. The relationship between protease and oxidative enzyme activity and ECM diversity indicates that ECM communities may play an important role in cycling C and nutrients through this fraction of soil organic matter.

Increases in protease and oxidase activity with ECM richness up to 44 species (Fig. 2) is surprising, given that effects of plant and animal diversity are typically observed for functions that are narrowly distributed among taxa, or when the initial diversity of the community is very low (Hooper et al., 2005; Schimel, 1995). Nevertheless, our results are similar to those of Rineau and Courty (2011), who found that the diversity of root tip enzymes was positively correlated with taxonomic diversity of ECM species in root samples that hosted under 50 ECM species. By contrast, effects of saprotrophic diversity on soil C cycling have only been observed when less than a dozen species are present (Setala and McLean, 2004). In our study, saprotrophic richness was higher than ECM richness (125 saprotroph species/sample vs. 17 ECM species/sample), which may explain lack of correlation between saprotroph diversity and labile C-targeting enzymes. However, saprotroph diversity was significantly correlated with acid phosphatase activity in bulk soils (Supplementary Fig. 4). This correlation, as well as the relationship between ECM diversity and LAP activity, suggests that resource partitioning or facilitation among fungi may be greater when mining for nutrients, compared to when targeting more C-rich substrates in soil.

ECM control of proteolytic and oxidative enzyme activity may explain variation in the activity of these enzymes that is often observed across soils in other studies. LAP, PPO, and PER activities are often co-correlated, and the magnitude of variation in these activities is typically higher and more difficult to model than that of other hydrolases (Sinsabaugh, 2010). When regressed against climate and soil chemistry variables, LAP, PPO, and PER activities are most strongly associated with soil pH both within and across biomes (Sinsabaugh et al., 2008). ECM diversity and community composition can vary with soil pH at both local and regional scales (Cox et al., 2010; Jonsson et al., 1999), and so may partly account for relationships between soil pH and protease or oxidase activity observed in these studies. It is unclear if the relationship between ECM diversity and soil protease or oxidase activity holds at higher levels of spatial and biological complexity, such as at the biome scale or across plant species ranges. ECM communities at these scales are often more diverse than within a stand, showing high beta-diversity (on the order of several hundred species per km^2) in a variety of habitats (Peay et al., 2010; Tedersoo et al., 2010b). If larger ECM communities contain more functionally redundant species, the role of ECM diversity in controlling soil enzyme activity may be negligible. In order to develop predictive global models of ECM contributions to organic N cycling, an important next step will be to assess taxonomic-functional diversity relationships of these ECM communities at larger geographic scales.

Soil enzyme activity also varied with resource availability (i.e. carbon, nitrogen, and water; soil chemistry PC1). However, the magnitude of effect varied by the type of enzyme. Soil enzyme PC1 tracked soil chemistry PC1, which was higher in the upper organic soil horizon compared to the lower mineral horizon (Fig. 1A). This result is consistent with our hypothesis that resource availability to saprotrophs (e.g. soil C content) would correlate positively with the activity of C-targeting hydrolases. Indeed, other studies have found correlations between total soil C and hydrolase activity both within and across biomes (Sinsabaugh et al., 2008). Soil chemistry had less of an effect on protease and oxidase activity (soil enzyme PC2; Table 3). This result is consistent with other studies showing little correlation between hydrolases and oxidases in soil (Sinsabaugh, 2010), indicating that these two enzyme pools are likely controlled by different factors.

Enzyme activity on ECM root tips was very low relative to enzyme activity in bulk soils, supporting the hypothesis that ECM root tips may make only small contributions to soil C and nutrient breakdown on the ecosystem-level. This is result is also consistent with functional compartmentalization in gene expression observed between ECM tips and foraging mycelium (Wright et al., 2005). We calculated that between 2.7 thousand and 1.3 million root tips were needed to reach enzyme activity levels observed in bulk soil samples. Courty et al. (2008) found that in an old-growth oak forest, a maximum of 900 ECM tips were obtained in six cores (each of which were twice as large as our cores) pooled together after collection during the growing season (Courty et al., 2008). We found fewer root tips than this in our soil cores, making it unlikely that ECM root tips contributed substantially to total rates of soil C and nutrient processing at Point Reyes. This may explain the lack of correlation between ECM tip enzyme and soil enzyme activity and suggests that ECM rhizosphere activity may not drive ECM contributions to decomposition on larger scales.

4.1. Conclusions

The role of ECM and saprotrophic fungi in soil organic matter breakdown has been a matter of debate (Baldrian, 2009; Cullings and Courty, 2009; Talbot et al., 2008). Our study is the first to link ECM and saprotrophic communities independently to the processing of C and nutrients in whole soils at multiple spatial scales. Carbon and nutrient exchange between ECM fungi and their host plants constitutes a significant ecosystem flux in resources between above and belowground. If ECM fungi contribute to organic nutrient degradation in soil, it is estimated that they could modify the soil C cycle by an order of magnitude (Orwin et al., 2011). Several soil C models have begun to incorporate fungal species diversity as a control over decay rates (Treseder et al., 2011), yet it is not clear how these modifications affect model predictions of the global carbon balance (Talbot and Treseder, 2011). An important step toward resolving this issue will be to identify the contribution of different ECM and saprotrophic species to C and organic nutrient uptake directly in field samples from a variety of ecosystems.

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Appendix A. Supplementary material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2012.10.004.

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