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Functional guild classification predicts the enzymatic role of fungi in litter and soil biogeochemistry

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ABSTRACT

Linking community composition to ecosystem function is a challenge in complex microbial communities. We tested the hypothesis that key biological features of fungi - evolutionary history, functional guild, and abundance of functional genes – can predict the biogeochemical activity of fungal species during decay. We measured the activity of 10 different enzymes produced by 48 model fungal species on leaf litter in laboratory microcosms. Taxa included closely related species with different ecologies (i.e. species in different "functional guilds") and species with publicly available genomes. Decomposition capabilities differed less among phylogenetic lineages of fungi than among different functional guilds. Activity of carbohydrases and acid phosphatase was significantly higher in litter colonized by saprotrophs compared to ectomycorrhizal species. By contrast, oxidoreductase activities per unit fungal biomass were statistically similar across functional guilds, with white rot fungi having highest polyphenol oxidase activity and ectomycorrhizal fungi having highest peroxidase activity. On the ecosystem level, polyphenol oxidase activity in soil correlated with the abundance of white rot fungi, while soil peroxidase activity correlated with the abundance of ectomycorrhizal fungi in soil. Copy numbers of genes coding for different enzymes explained the activity of some carbohydrases and polyphenol oxidase produced by fungi in culture, but were not significantly better predictors of activity than specific functional guild. Collectively, our data suggest that quantifying the specific functional guilds of fungi in soil, potentially through environmental sequencing approaches, allows us to predict activity of enzymes that drive soil biogeochemical cycles.

1. Introduction

Fungi are engines of carbon and nutrient recycling from dead organic matter to other live organisms (in the case of nutrients like nitrogen (N) and phosphorus (P)) or to the atmosphere (in the case of carbon(C)). One gram of soil contains as many as several hundred fungal species (Taylor et al., 2010), yet it has been challenging to understand the role of this species diversity in maintaining the diversity of biochemical decay mechanisms in natural ecosystems. This difficulty arises because of our inability to isolate and culture many species from the environment (Bridge and Spooner, 2001), the limitations of extrapolating functional studies of species in culture to field settings (Anderson and Cairney, 2004), and the biases associated with functional metagenomic data collected directly from the environment (Lombard et al., 2011). Despite its inherent challenge, systematically quantifying the decomposition functions of taxa in these communities is an important step towards obtaining more accurate predictions of the global carbon cycle, as fungal diversity and community composition can affect the rates of these processes (Setala and McLean, 2004; Srivastava et al., 2009; Fukami, 2010; LeBauer, 2010) and landscape-level C cycle models that

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incorporate explicit microbial processes can improve predictions of soil C balance (Wieder et al., 2013; Averill et al., 2014).

Assessing the biogeochemical function of many fungal species found in natural communities may be possible if we can identify key biological features that control decomposition capabilities broadly across these organisms. The first approach that may prove useful in this regard is using the evolutionary relationship between species to predict activity (Revell et al., 2008). Recent studies have found that closely related microbial taxa can have similar capacities to utilize resources in natural systems (McGuire et al., 2010; Treseder et al., 2011; Peay et al., 2012), suggesting conservation of these traits throughout the evolutionary history of the species (Zanne et al., 2005; Swenson et al., 2007). If we can approximate a species' function by its phylogenetic relatedness to well-characterized taxa, placing sequence data collected directly from natural fungal communities into a phylogenetic context may prove valuable for predicting community-level biogeochemical cycles.

An interesting characteristic of fungi, however, is that the phylogenetic relatedness of fungal taxa can be confounded with widely differing strategies for resource capture. These “guilds” of fungi have unique methods of decay (Hibbett and Donoghue, 2001; Tedersoo et al., 2010) and are polyphyletic across the Ascomycota and Basidiomycota (Hibbett and Donoghue, 1998, 2001; Tedersoo et al., 2010). The wide diversity of lifestyles, or functional traits, across these guilds can be observed within a single clade (such as the Boletales or the Agaricales), suggesting that functional guild constitutes another biological feature of fungi that could predict their activity in ecosystems. Traditional studies of decomposer fungi have focused on the guild of free-living, saprotrophic fungi that break down C compounds in plant litter and convert them into resources for growth. Within the broad saprotroph guild are more narrowly defined, “specific” functional guilds that use different biochemical mechanisms of decay. For example, white rot fungi (that target lignin in plant tissue) and brown rot fungi (that target cellulose and other cell wall polysaccharides) produce different catalysts for the breakdown of cellulose and lignin during wood decay (Worrall et al., 1997). In addition to saprotrophs, guilds of fungi that engage in a mycorrhizal lifestyle (obtaining C directly through symbiosis with plant roots) can also act as decomposers. Many species of ectomycorrhizal fungi have the genetic capability to produce extracellular enzymes (Bödeker et al., 2009), can release a variety of extracellular enzymes in culture (Talbot and Treseder, 2010; Pritsch and Garbaye, 2011; Burke et al., 2014), and can acquire nutrients from complex soil organic matter in microcosms (Bending and Read, 1995a,b). Soil enzyme activities can correlate positively with the diversity of ectomycorrhizal species (Talbot et al., 2013, 2014), and recent observations indicate that ectomycorrhizal fungi can produce activity that is equal to or higher than some saprotrophic fungi in culture (Burke et al., 2014). Nevertheless, the importance of ectomycorrhizal fungi in decay *in situ*, relative to saprotrophs in general or the various specific functional guilds of saprotrophic decomposers, remains unclear. Determining the contribution of ectomycorrhizal enzyme production to total soil enzyme activity is important to predicting soil biogeochemistry on a landscape scale, because when potential decomposer activities of mycorrhizal fungi are incorporated into C cycle models, it significantly changes predictions of nutrient cycling and soil C storage (Orwin et al., 2011; Deckmyn et al., 2014; Lindahl and Tunlid, 2015).

A third approach to estimating the role of fungal species in ecosystem function is to quantify the abundance of genes coding for specific decomposer functions (e.g. synthesis of extracellular enzymes). While abundance of these “functional genes” (in terms of gene copy number) can correspond to the evolution of different functional guilds (Floudas et al., 2012; Kohler et al., 2015), substantial variation in extracellular enzyme production exists among species within these guilds (Ander and Eriksson, 1977) and the lines between functional guilds can be blurred based on extracellular enzyme activities (Burke et al., 2014) and functional gene abundances (Riley et al., 2014). Therefore, functional gene abundance may be a more accurate predictor of decomposer activity in fungi than phylogeny or functional guild. Indeed, decomposer metagenomes can correlate with biogeochemical activity in soil (Hofmockel et al., 2007; Baldrian et al., 2011) and can improve predictions of soil N cycles in the field (Graham et al., 2014). If the ecology of fungi in natural systems can be predicted broadly by the abundance of functional genes, metagenomics and metatranscriptomics may provide a relatively straight-forward, reliable way of predicting how different fungi within a community shape soil biogeochemical cycles.

We tested the role of phylogenetic relatedness, functional guilds, and functional gene abundance in predicting the physiological capability of fungi to act as decomposers by studying extracellular enzyme activity produced by 48 fungal taxa during decay of natural organic matter in the laboratory. These taxa included pairs of closely related ectomycorrhizal, white rot, brown rot, and other saprotrophic fungi dispersed across the fungal phylogeny (Table 1, Fig. 1, S1), many of which have fully sequenced genomes or publicly available models of functional gene abundances. We chose to measure extracellular enzyme activities that are widely employed in soil studies as a metric of biogeochemical activity by fungi, because it allowed us to directly test the hypothesis that organismal function in complex systems like litter and soil can be predicted by scaling up the activity of species in the lab, or potentially by the genetic architecture of a species. To test this hypothesis, we matched functional genes for enzyme families with known activities in the genome of each species to the actual enzyme activities observed for the species on leaf litter in culture. This allowed us to explore the concept that microbial function can be scaled from the genes-to-ecosystem level which, to our knowledge is the first attempt to link genomes to standard assays of soil biogeochemical function. We expected that extracellular enzyme activity would be best explained by abundance of functional genes (as gene copy numbers) in the fungal genome, because of the specific role that functional genes play in constraining the capability of fungi to break down different plant biopolymers. We also expected that both broad functional guild (saprotrophic vs. ectomycorrhizal) and specific functional guild of fungi (brown rot, white rot, ectomycorrhizal, other saprotroph) would be better predictors of extracellular enzyme activity compared to evolutionary lineage, with ectomycorrhizal fungi having highest capacity to target nutrient (N and P) rich compounds (due to direct acquisition of C from live roots) and saprotrophs degrading C-rich compounds to a greater extent than ectomycorrhizal fungi. In addition, we expected white rot fungi to produce the highest activity of polyphenol oxidases and peroxidases and white and brown rot fungi to have higher capacity to target C-rich compounds in litter compared to ectomycorrhizal fungi and other saprotrophs. To determine how well culture studies of fungi predict the activity of fungal species *in situ*, we tested whether the biological features of fungi that predicted enzyme activity in the laboratory decomposition experiment could predict enzyme activities measured previously in field soils where the species occur (Talbot et al., 2014).

2. Materials and methods

2.1. Fungal cultures and leaf litter

The 48 species of fungi included 15 species of ectomycorrhizal fungi, 10 species of white rot fungi, 13 species of brown rot fungi, and 9 saprotrophic species with unknown substrate preference

("other saprotrophs"). Species spanned the Agaricomycetes within the Basidiomycota, including 13 orders, 29 families, and 38 genera of basidiomycetes (Table 1). Additional species included *Dacryopinax* sp. (brown rot) and *Ustilago maydis* (pathogen/saprotroph) of the Basidiomycota, as well as three species in the Ascomycota; *Pyrenochaete cava* and *Penicillium ochrochloron* (saprotrophs), as well as *Cenococcum geophilum* (ectomycorrhizal). Assignment of individual taxa to functional guilds was based on common categorizations in the literature (Worrall et al., 1997; Hibbett and Donoghue, 2001; Larsson and Larsson, 2003; Huckfeldt and Schmidt, 2006; Schmidt, 2006; Lindner and Banik, 2008; Binder et al., 2010; Tedersoo et al., 2010; Garcia-Sandoval et al., 2011). Cultures of all taxa were obtained from either the U.S. Forest Service Center for Forest Mycology Research Culture Collection, Department of Tree-Microbe Interactions Culture Collection (INRA-Nancy), Max Plank Institute for Terrestrial Microbiology, individual research laboratories, or isolated from fruitbodies collected at the Cloquet Forestry Center in Cloquet, MN in October 2011 (Table 1). For the taxa with fully sequenced genomes, the isolates used were the fully functional dikaryotic parental cultures of these strains.

Leaf litter from *Populus tremuloides* was collected at the Cedar Creek Ecosystem Science Reserve in East Bethel, MN, in November 2011. *P. tremuloides* litter has a large influence on the global C cycle, as it is the most widely distributed tree in North America (Little and Viereck, 1971). Four large litter traps (4 × 2 m) made from 1-mm-mesh screening were installed above the forest floor in late October 2011 and litter was collected 1 month later. Litter was air-dried, *P. tremuloides* litter was isolated from the mix, and the *P. tremuloides* litter was autoclaved twice prior to the experiment.

Table 1
Fungal taxa included in litter decomposition study. Colors indicate specific functional guild (dark gray = white rot, gray = brown rot, light gray = other saprotroph, white = ectomycorrhizal).

Taxa	Phylum	Class	Order	Family	Guild	Collection #	Accession #	Source
Agrocybe aegerita	Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	WR	TJV-93-248-T	HQ384307	1
*Amanita muscaria**	Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	ECM	Koide BX008	FJ890038	2
*Amanita thiersii**	Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	BR	SKay4041	HQ539752	2
Anomoporia bombycina	Basidiomycota	Agaricomycetes	Polyporales	Fomitopsidaceae	BR	L-6621-Sp	GU187564	1
Armillariella mellea	Basidiomycota	Agaricomycetes	Agaricales	Physalacriaceae	PATH/WR	ATCC 1113	DQ338545	3
*Auricularia delicata**	Basidiomycota	Agaricomycetes	Auriculariales	Auriculariaceae	WR	SJ-205	AF291290	1
Boletinellus merulioides	Basidiomycota	Agaricomycetes	Boletales	Boletinellaceae	PATH/OTH	FP-105904	AY684153	1
Botryobasidium subcoronatum	Basidiomycota	Agaricomycetes	Cantharellales	Botryobasidiaceae	OTH	FP-151108	AY647212	1
Cenococcum geophilum	Ascomycota	Dothideomycetes	Incertae sedis	Incertae sedis	ECM	1.58	JN860134	4
Clitocybe glacialis	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	OTH	OKM-3074-T	AF261389	1
Coniophora olivacea	Basidiomycota	Agaricomycetes	Boletales	Coniophoraceae	BR	FP-104386-Sp	GU187572	3
*Coniophora puteana**	Basidiomycota	Agaricomycetes	Boletales	Coniophoraceae	BR	FP-70841-Sp	AEIT01000516	1
Coprinopsis atramentaria	Basidiomycota	Agaricomycetes	Agaricales	Psathyrellaceae	WR	DAOM 192254	DQ457661	3
*Cryptococcus neoformans**	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	PATH/ OTH	JEC21	BR000310	5
*Dacryopinax sp.**	Basidiomycota	Dacrymycetes	Dacrymycetales	Dacrymycetaceae	BR	DJM 731	AB472720	6
Entoloma sp.	Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	ECM	PL0033	GU384617	7
Fistulina hepatica	Basidiomycota	Agaricomycetes	Agaricales	Fistulinaceae	BR	FP-102523-T	DQ071727	1
*Fomitiporia mediterranea**	Basidiomycota	Agaricomycetes	Hymenochaetales	Hymenochaetaceae	WR	MF3/22	AEJJ01002640	8
*Fomitopsis pinicola**	Basidiomycota	Agaricomycetes	Polyporales	Fomitopsidaceae	BR	FP-58527-T	AY684164	1
Hericium americanum	Basidiomycota	Agaricomycetes	Russulales	Hericiaceae	WR	PGK 073	DQ411538	9
Hygrophoropsis aurantiaca	Basidiomycota	Agaricomycetes	Boletales	Hygrophoropsidaceae	OTH	HHB-12841-T	AY684156	1
Lepista nuda	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	OTH	FP-102530-Sp	DQ071713	1
Ossicaulis lignatilis	Basidiomycota	Agaricomycetes	Agaricales	Lyophyllaceae	BR	AHS-73598-Sp	AF042625	1
*Paxillus involutus**	Basidiomycota	Agaricomycetes	Boletales	Paxillaceae	ECM	PGK 0013	AY612815	9
Penicillium ochrochloron	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	OTH	ATCC 9112	AF033441	3
*Piloderma bicolor**	Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	ECM	VT-987	GU187591	1
*Pisolithus tinctorius**	Basidiomycota	Agaricomycetes	Boletales	Sclerodermataceae	ECM	M270	EU718148	4
*Pleurotus ostreatus**	Basidiomycota	Agaricomycetes	Agaricales	Pleurotaceae	WR	Fla-34-2	DQ071722	3
*Postia placenta**	Basidiomycota	Agaricomycetes	Polyporales	Fomitopsidaceae	BR	MAD-698-R	JQ700293	10
*Punctularia strigosozonata**	Basidiomycota	Agaricomycetes	Corticiales	Corticiaceae	WR	HHB-11173-Sp	AY586702	1
Pyrenochaeta cava	Ascomycota	Dothideomycetes	Pleosporales	Incertae sedis	OTH	PL0078	EU754198	7
Rhizopogon arctostaphyli	Basidiomycota	Agaricomycetes	Boletales	Rhizopogonaceae	ECM	378	EU726304	11
Rhizopogon salebrosus	Basidiomycota	Agaricomycetes	Boletales	Rhizopogonaceae	ECM	PR 1.1	GU931706	9
Rhizopogon vulgaris	Basidiomycota	Agaricomycetes	Boletales	Rhizopogonaceae	ECM	343	JF908636	9
Sebacina sp.	Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	ECM	FP-150589	DQ521412	1
*Serpula lacrymans**	Basidiomycota	Agaricomycetes	Boletales	Serpulaceae	BR	S7	AEQB01000388	12
Sistotrema raduloides	Basidiomycota	Agaricomycetes	Cantharellales	Hydnaceae	WR	FP-155449	AY647213	1
*Stereum hirsutum**	Basidiomycota	Agaricomycetes	Russulales	Stereaceae	WR	FP-91666-Sp	AEGX01000554	1
Suillus brevipes	Basidiomycota	Agaricomycetes	Boletales	Suillaceae	ECM	SB2	JN858079	11
Suillus pungens	Basidiomycota	Agaricomycetes	Boletales	Suillaceae	ECM	3a	JQ310819	9
Tapinella atrotomentosa	Basidiomycota	Agaricomycetes	Boletales	Tapinellineae	BR	ATCC 64500	GU187603	3
Tapinella panuoides	Basidiomycota	Agaricomycetes	Boletales	Tapinellineae	BR	RLG-12933-Sp	DQ071746	1
Thelephora terrestris	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	ECM	CHA	GQ267489	4
Tomentella sublilacina	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	ECM	LAP45	AY880929	4
*Trametes versicolor**	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	WR	FP-101664-Sp	AY684159	1
Tricholoma flavovirens	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	ECM	USTF97	EU186294	9
*Ustilago maydis**	Basidiomycota	Ustilaginomycetes	Ustilaginales	Ustilaginaceae	PATH/ OTH	521	FJ644528	13
*Wolfiporia cocos**	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	BR	MD-104-R	AF393081	1

*Full sequenced genome or gene model available from public database.

[1]US Forest Service Center for Forest Mycology Research Culture Collection (FPL); [2]A. Pringle, Harvard University; [3]J. Schilling, University of Minnesota; [4]F. Martin, Centre de Recherches INRA-Nancy; [5]J. Heitman, Duke University; [6]D. McLaughlin, University of Minnesota; [7]J. Talbot, Cloquet Forestry Center in Cloquet, MN; [8]R. Blanchette, University of Minnesota; [9]P. Kennedy, University of Minnesota; [10]D. Cullen, FPL; [11]T. D. Bruns, UC Berkeley; [12]O. Schmidt, University of Hamburg; [13]Max Plank Institute for Terrestrial Microbiology

2.2. Decomposition experiments

Sterilized whole leaves of *P. tremuloides* litter (1 g, about 3 leaves/sample) were placed on the surface of petri dishes (9 cm diameter) containing 15 mL of modified Melin-Norkrans (MMN) medium, similar to previously published studies of enzyme activity in ectomycorrhizal fungi (Burke et al., 2014). Eight replicate dishes were then inoculated with each species by taking plugs of 1-month old cultures on MMN media with a sterile cork borer (6 mm diameter) and placing plugs on the surface of the leaves. Four plugs were added to each plate. The plates were sealed with laboratory film and incubated for 100 days at room temperature (~21 °C) in the dark, following previous studies of litter fungi (Osono and Takeda, 2006). After incubation, all litter was harvested from plates for each species, initial inoculum plugs were removed, and a small piece of litter was used to extract and amplify fungal DNA. The remaining litter sample was placed into a coin envelope, flash-frozen in liquid N, lightly crushed with a mortar and pestle, and stored at −80 °C for analysis of fungal biomass and extracellular enzyme activities.

2.3. Fungal biomass and enzyme activity analyses

To determine potential activity of enzymes produced by each species on the litter, we assayed the activity of 10 different enzymes involved in litter C and nutrient cycling: cellobiohydrolase (CBH, an exocellulase), β-glucosidase (BG, which hydrolyzes cellobiose into glucose), α-glucosidase (AG, which is accessory to starch hydrolysis), 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), peroxidase (PER, including oxidases that degrade lignin), acid phosphatase (AP, which releases inorganic phosphate from organic matter), β-N-acetyl-glucosaminidase (NAG, which is accessory to chitin degradation), and leucine-amino peptidase (LAP, which breaks down polypeptides). Potential enzyme activities were assayed on a subsample of each litter sample that was stored frozen at −80 °C after harvest. Hydrolase assays employed fluorogenic substrates (methylumbelliferyl-linked) that were incubated with litter slurries to induce cleavage of specific glycosidic or amide bonds in the substrate, releasing methylumbelliferone or methylcoumarin, respectively, and inducing fluorescence. Oxidoreductase assays employed a colorimetric procedure whereby L-DOPA is oxidized in the presence of litter either with peroxide added (peroxidase assay) or without peroxide (polyphenol oxidase assay). These "maximum potential" activity assays were measured on a microplate reader following German et al. (2011). Assays were conducted at pH 4.5 to match litter pH in the field (Hobbie, 2005) and to select for extracellular enzymes (Sinsabaugh, 2010). Litter was homogenized in sodium acetate buffer using a Tissue Tearor (BioSpec Products, Inc., Barlesville, OK). To determine total growth of each species on litter over time, we quantified ergosterol in each sample as an estimate of fungal cell growth and activity (Bååth, 2001; Castro et al., 2002). Ergosterol analysis followed the procedure of Montgomery et al. (2000). Ergosterol concentrations are reported as μg ergosterol per mg litter.

2.4. DNA sequencing, phylogeny construction, and functional gene data collection

To confirm identity of each fungal species colonizing litter at the end of the decomposition experiment, we extracted DNA from litter fragments using the Extract-N-Amp Tissue PCR kit (Sigma—Aldrich). Extracts were PCR amplified using ITS1F & ITS4 primers (White et al., 1990) and PCR products were sequenced using single pass Sanger sequencing (Beckman Coulter Genomics, Danvers, MA, USA). Sequences were edited using Geneious software (Biomatters Ltd., New Zealand) and taxonomy was assigned by searching against GenBank using BLASTn. Only those samples without contaminants were used (total sample number = 173).

To determine phylogenetic relatedness among taxa, a phylogenetic tree was generated for all 48 species (Fig. 1) using a previously published multi-gene tree for Kingdom fungi (James et al.,

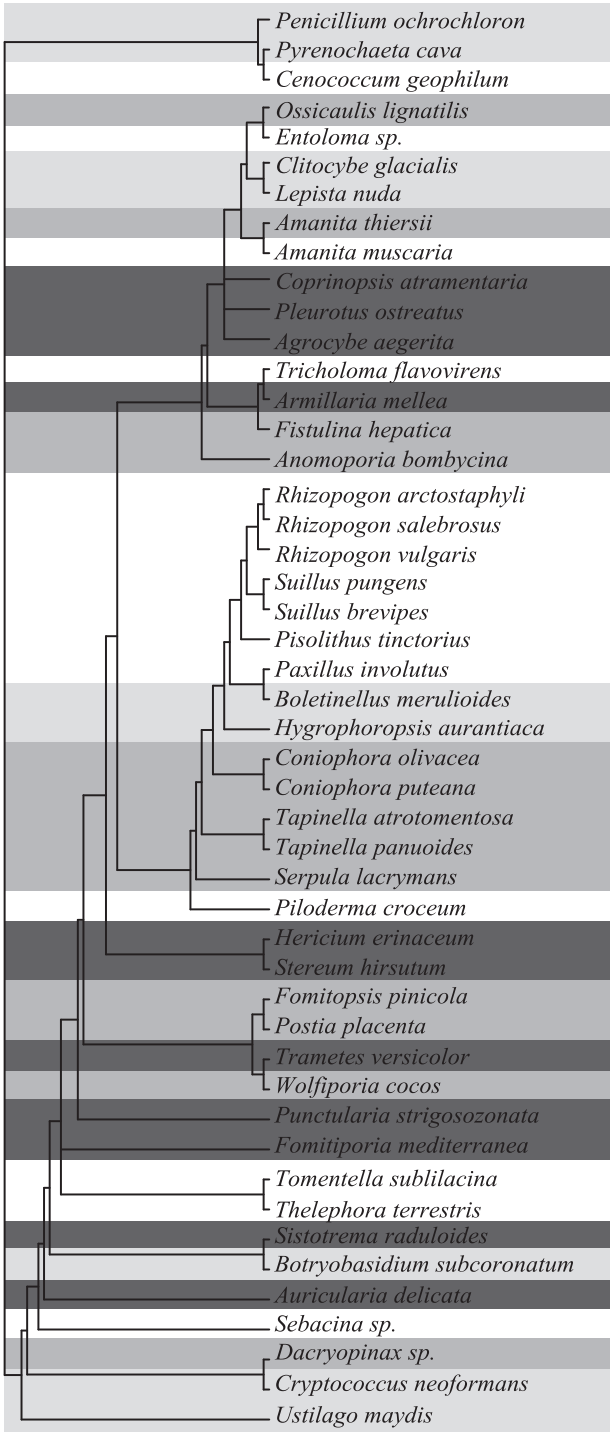

Fig. 1. Phylogram of pruned phylogeny containing only taxa in the litter decomposition study and shows ectomycorrhizal (white), general saprotroph (light gray), brown rot (gray) and white rot (dark gray) functional guilds.

2006) as a guide. One 28S gene sequence for each taxon in the decomposition experiment was downloaded from the SILVA rRNA database v. 115 (Quast et al., 2013). Additionally, a 28S gene sequence was downloaded from the SILVA database for each of 166 taxa included in the James et al. (2006) phylogeny. All sequences were aligned to the SILVA SEED LSU nucleotide alignment prior to download. A maximum-likelihood tree was constructed using the fast maximum-likelihood stepwise-addition algorithm in GARLI (Zwickl, 2006) with 19 positive constraints. Eight eukaryotic outgroup species were included from James et al. (2006). Branch lengths were calculated by the Grafen (Grafen, 1989) method with the compute.brlen command in the ape package in R (Paradis, 2011).

Copy numbers of gene families encoding extracellular enzymes and related substrate binding modules involved in decomposition of plant and microbial biopolymers (including plant cell wall molecules and fungal chitin) were obtained for individual taxa from the literature and from online databases. Glycoside hydrolase abundances and abundances of carbohydrate-binding modules (CBMs) were obtained from Kohler et al. (2015) and Riley et al. (2014) and oxidoreductase abundances were obtained from Kohler et al. (2015), Riley et al. (2014), Floudas et al. (2012), and the PeroxiBase peroxide gene database (http://peroxibase.toulouse.inra.fr/). Abundance of genes encoding 33 families of carbohydrate-active enzymes or modules and 12 families or superfamilies of oxidoreductases was obtained for 19 taxa used in the litter decay experiment. Functional genes were selected based on current understanding of catalytic mechanism and known activities of each gene family or superfamily in the CAZy database (Levasseur et al., 2013; Lombard et al., 2014), as well as searching the fluorogenic and colorimetric substrates used in assays of each enzyme activity within the Braunschweig Enzyme database (BRENDA, Schomburg et al., 2013). CAZymes included 23 glycoside hydrolases (GHs) and 10 carbohydrate binding modules (CBMs) that assist in the catabolism of crystalline cellulose (Lombard et al., 2014). Four broad groups of oxidoreductases were included that catalyze the oxidation of polyphenolic compounds (Burns et al., 2013). The first group (polyphenol oxidases) included families of general polyphenol oxidases that contribute directly to PPO activity measured by the L-DOPA assay (Table 2). Polyphenol oxidases include vanillyl alcohol oxidase (VAO) and the multifunction cytochrome P450s (P450), as well as multicopper oxidases (MCO). Although o-diphenol oxygen oxidoreductases (EC 1.10.3.1), di-oxygenases (EC 1.13.11), and mono-oxygenases (EC 1.14.18) are potentially active against the L-DOPA substrate used in enzyme assays (Kirby and Hollfelder, 2009), functional gene abundance was only available for p-diphenol oxygen oxidoreductases in the MCO family (EC 1.10.3.2). The second group (peroxidases) included four superfamilies of peroxidases that contribute directly to PER activity; dye-decolorizing peroxidases (DyP), heme-thiolate peroxidases (HTP), class I peroxidases (POD1) and class II peroxidases (POD2) (Table 2). The third group consisted of three families of metalloreductases hypothesized to catalyze Fenton reactions, which were included as predictors of both PER and PPO activity; 1,4-benzoquinone reductases (QRD), iron reductase domains (Cytb652), and cellobiose dehydrogenase (CDH). Lastly, three types of peroxide-generating oxidoreductases were included as predictors of PPO activity; glucose-methanol-choline oxidases (GMC), copper radical oxidases (CRO), and gluco-oligosaccharide oxidases (GOO). Functional gene abundances were obtained for 19 taxa across functional guilds. All genes coding for enzymes with activity towards a substrate (e.g. all CBH-coding genes) were summed to provide an additional metric of functional gene abundance for statistical analyses.

2.5. Predicting in situ soil decomposition activities from in vitro activities

To assess the ability of individual fungal traits observed in the laboratory to predict rates of decomposition in ecosystems, we tested for correlations between extracellular enzyme activity by individual taxa in the laboratory and enzyme activity in soil where these taxa occur, which was collected as part of a multi-scale study of the biogeography of soil fungi and their enzyme activities in pine forests across North America (Talbot et al., 2014). In this previous study, samples of the top 14 cm of soil were collected across nine major pine biomes within the United States and assayed for extracellular enzyme activity and fungal community composition ($n = 523$). Soils were kept on ice after removal from the field and transported to the nearest university within 8 h of collection. Soils were sieved through a 2 mm mesh to remove roots and small rocks and homogenized by hand. One subsample was preserved at 4 °C for DNA extraction and another subsample was preserved for extracellular enzyme analysis and characterization of soil chemistry (frozen at −80 °C). Soils were preserved within 48 h of field sampling. Abundance of different fungal genera in soils was calculated as sequence abundance based on 454-pyrosequencing of the ITS region of fungal DNA (Talbot et al., 2014). Sequences were assigned taxonomy by searching representative sequences from each OTU against a published fungal ITS database (Peay et al., 2013). Extracellular enzyme activity was measured in bulk soil from each sample following the methods outlined above.

2.6. Data analyses and statistics

All statistical analyses were performed on extracellular enzyme activities normalized to litter mass (nmol activity per g litter per hour) as well as enzyme activities normalized to ergosterol concentration on litter (nmol activity per μg ergosterol/mg litter per hour). Enzyme activities were highly correlated (Table S1), so to reduce the measured enzymes to a reasonable number of factors, we used a PCA on the log-transformed variables. After examining scree plots, we chose to retain the first two principal components, which explained 79.7% (PC1 69.2%, PC2 10.5%) of the variation in enzyme activities (per g litter).

To determine phylogenetic signal in fungal growth and extracellular enzyme activity, we calculated *K* statistic (Blomberg et al., 2003) for ergosterol concentration and activity of each enzyme. *K* statistic was calculated with a fully dichotomous tree using the multiPhylosignal command in the Picante package in R (Kembel et al., 2010). Because there was also significant phylogenetic signal to broad and specific functional guilds, we tested for phylogenetic effects in enzyme activities both across and within guilds.

To test for functional guild and functional gene effects on fungal growth and enzyme activities, we conducted generalized least squares regression with either functional guild classification or functional gene abundance as independent variable and extracellular enzyme activities or ergosterol concentration as dependent variable. We tested both broad functional guild (saprotrophic vs. ectomycorrhizal) and specific functional guild (white rot, brown rot, ectomycorrhizal, other saprotroph) effects on fungal growth and activity. To determine predictive power of functional gene abundance for enzyme activity, we performed linear regression with abundance of genes in each enzyme family or superfamily specific to an extracellular enzyme (Table 2) as independent variables and measured enzyme activity as dependent variable. To account for the non-independence of taxa from shared common ancestry in each type of regression, we fit a generalized least squares model to the data with Martin and Hansen's covariance

Table 2
Classes of extracellular enzymes included in gene models used for functional gene analysis. Target assayed enzymes were determined based on substrate specificity of known activities of Enzyme Commission numbers in each enzyme class.

Enzyme class/superfamily	Abbr.	Assayed enzyme	Known activities
Glycoside hydrolase 1	GH1	CBH, BG, BX, BGLU	β-glucosidase (EC 3.2.1.21); β-glucuronidase (EC 3.2.1.31); β-xylosidase (EC 3.2.1.37); exo-β-1,4-glucanase (EC 3.2.1.74)
Glycoside hydrolase 2	GH2	BGLU, NAG	β-glucuronidase (EC 3.2.1.31); exo-β-glucosaminidase (EC 3.2.1.165)
Glycoside hydrolase 3	GH3	CBH, BG, BX, NAG	β-glucosidase (EC 3.2.1.21); xylan 1,4-β-xylosidase (EC 3.2.1.37); β-N-acetylhexosaminidase (EC 3.2.1.52); glucan 1,4-β-glucosidase (EC 3.2.1.74)
Glycoside hydrolase 5	GH5	CBH, BG	Cellulose β-1,4-cellobiosidase (EC 3.2.1.91); exo-β-1,4-glucanase / cellodextrinase (EC3.2.1.74); β-glucosidase (EC 3.2.1.21)
Glycoside hydrolase 6	GH6	CBH	Cellobiohydrolase (EC 3.2.1.91)
Glycoside hydrolase 7	GH7	CBH	Reducing end-acting cellobiohydrolase (EC 3.2.1.176)
Glycoside hydrolase 8	GH8	BX	Reducing-end-xylose releasing exo-oligoxylanase (EC 3.2.1.156)
Glycoside hydrolase 9	GH9	CBH, BG, NAG	β-glucosidase (EC 3.2.1.21); cellodextrinase (EC 3.2.1.74); cellobiohydrolase (EC 3.2.1.91); exo-β-glucosaminidase (EC3.2.1.165)
Glycoside hydrolase 13	GH13	AG	Oligo-α-glucosidase (EC 3.2.1.10); α-glucosidase (EC 3.2.1.20);
Glycoside hydrolase 15	GH15	AG	Glucoamylase (EC 3.2.1.3)
Glycoside hydrolase 18	GH18	NAG	Chitinase (EC 3.2.1.14)
Glycoside hydrolase 20	GH20	NAG	β-hexosaminidase (EC 3.2.1.52)
Glycoside hydrolase 23	GH23	NAG	Chitinase (EC 3.2.1.14)
Glycoside hydrolase 30	GH30	BG, BX, BGLU	β-glucosidase (3.2.1.21); β-glucuronidase (EC 3.2.1.31); β-xylosidase (EC 3.2.1.37)
Glycoside hydrolase 31	GH31	AG	α-glucosidase (EC 3.2.1.20)
Glycoside hydrolase 35	GH35	NAG	Exo-β-glucosaminidase (EC 3.2.1.165)
Glycoside hydrolase 43	GH43	BX	β-xylosidase (EC 3.2.1.37)
Glycoside hydrolase 51	GH51	BX	β-xylosidase (EC 3.2.1.37)
Glycoside hydrolase 52	GH52	BX	β-xylosidase (EC 3.2.1.37)
Glycoside hydrolase 54	GH54	BX	β-xylosidase (EC 3.2.1.37)
Glycoside hydrolase 63	GH63	AG	Processing α-glucosidase (EC 3.2.1.106); α-glucosidase (EC 3.2.1.20)
Glycoside hydrolase 74	GH74	CBH	Oligoxyloglucan reducing end-specific cellobiohydrolase (EC 3.2.1.150)
Glycoside hydrolase 79	GH79	BGLU	β-glucuronidase (EC 3.2.1.31)
Cellulose binding module 1	CBM1	CBH, NAG	Cellulose-binding, chitin-binding
Cellulose binding module 5	CBM5	NAG	Chitin-binding
Cellulose binding module 12	CBM12	NAG	Chitin-binding
Cellulose binding module 13	CBM13	BX	Xylan-binding
Cellulose binding module 18	CBM18	NAG	Chitin-binding
Cellulose binding module 20	CBM20	AG	Starch-binding
Cellulose binding module 21	CBM21	AG	Starch-binding
Cellulose binding module 35	CBM35	BX	Xylan-binding
Cellulose binding module 50	CBM50	NAG	Xylan-binding
Cellulose binding module 63	CBM63	CBH	Cellulose-binding
Cytochrome P450	P450	PPO	Mixed-function oxidoreductases
Multicopper oxidases	MCO	PPO	Laccase (EC 1.10.3.2), Ferroxidase (EC 1.10.3.2), Laccase-like multicopper oxidase (EC 1.10.3.2)
Glucose-methanol-choline oxidases	GMC	PPO	Cellobiose dehydrogenase (EC 1.1.99.18); aryl-alcohol oxidase (EC 1.1.3.7/1.1.3.4); alcohol oxidase (EC 1.1.3.13); pyranose oxidase (EC 1.1.3.10)
Vanillyl alcohol oxidase	VAO	PPO	EC.1.1.3.38
Copper radical oxidases	CRO	PPO	Glyoxal oxidase (EC 1.1.3.−); galactose oxidase (EC 1.1.3.9)
Gluco-oligosaccharide oxidases	GOO	PPO	EC 1.6.5.7
1,4-benzoquinone reductase	QRD	PPO, PER	EC 1.6.5.2
Iron reductase domain	Cytb652	PPO, PER	Fe(III) reductase domain in cytochrome of spectral class “b”
Dye-decolorizing peroxidases	DyP	PER	EC 1.11.1.19
Heme-thiolate peroxidases	HTP	PER	EC 1.11.1.10; EC 1.11.2.1
Class II peroxidases	POD2	PER	Manganese peroxidase (EC 1.11.1.13); Lignin peroxidase (EC 1.11.1.14); Versatile peroxidase (EC 1.11.1.16)
Class I peroxidases	POD1	PER	EC 1.11.1.5; EC 1.11.1.11

Table 3
Statistical tests for effect of phylogenetic relatedness on fungal growth and extracellular enzyme activities in the litter decomposition experiment. Phylogenetic relatedness within "Broad functional guild" refers to phylogenetic signal within saprotrophic (SAP) versus ectomycorrhizal (ECM) fungi, while phylogenetic relatedness within "Specific functional guild" refers to phylogenetic signal within groups of fungi that employ different mechanisms of resource capture: WR = white rot, BR = brown rot, OTH = Other saprotroph, ECM = ectomycorrhizal. Phylogenetic signal is estimated with *K* statistic. Asterisks represent significance of non-random phylogenetic signal (****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). *P* value for activity of individual enzymes is Holm-corrected. Enzyme activities are expressed in units of nmol/mg litter or nmol/μg ergosterol/mg litter.

Enzyme	Across guilds		Broad Functional Guild				Specific Functional Guild							
			ECM		SAP		ECM		OTH		BR		WR	
	N	K	N	K	N	K	N	K	N	K	N	K	N	K
Ergosterol/mg litter	48	0.88	15	0.86	33	0.88	15	0.86	9	0.88	13	1.01	11	0.95
CBH/mg litter	48	1.09*	15	1.06	33	1.02	15	1.06	9	1.07	13	1.14	11	1.02
BG/mg litter	48	1.04	15	1.02	33	1.00	15	1.02	9	1.03	13	1.14	11	1.05
AG/mg litter	48	1.02	15	0.96	33	1.04	15	0.96	9	1.05	13	1.07	11	1.09
NAG/mg litter	48	1.01	15	1.03	33	1.04	15	1.03	9	1.04	13	1.04	11	1.01
BX/mg litter	48	1.18*	15	1.19	33	1.08	15	1.19	9	1.03	13	1.25*	11	1.06
AP/mg litter	48	0.98	15	0.85	33	1.00	15	0.85	9	0.92	13	1.10	11	1.06
BGLU/mg litter	48	1.18*	15	1.03	33	1.15*	15	1.03	9	0.98	13	1.25	11	1.08
LAP/mg litter	48	1.16*	15	1.02	33	1.09	15	1.02	9	1.08	13	1.16	11	1.02
PPO/mg litter	48	1.01	15	0.94	33	1.00	15	0.94	9	1.07	13	1.17	11	0.96
PER/mg litter	48	0.96	15	1.25	33	0.93	15	1.25	9	0.88	13	0.95	11	0.99
CBH/μg ergosterol	48	1.08	15	1.02	33	1.03	15	1.02	9	1.08	13	1.09	11	1.04
BG/μg ergosterol	48	0.99	15	0.99	33	1.03	15	0.99	9	1.04	13	1.17	11	1.06
AG/μg ergosterol	48	0.99	15	0.92	33	1.03	15	0.92	9	1.07	13	1.03	11	1.07
NAG/μg ergosterol	48	0.98	15	1.00	33	1.04	15	1.00	9	1.04	13	0.93	11	1.01
BX/μg ergosterol	48	1.15*	15	1.10	33	1.09	15	1.10	9	1.03	13	1.31*	11	1.07
AP/μg ergosterol	48	0.92	15	0.85	33	1.00	15	0.85	9	0.98	13	1.02	11	1.05
BGLU/μg ergosterol	48	1.14*	15	1.00	33	1.14*	15	0.98	9	0.97	13	1.20	11	1.08
LAP/μg ergosterol	48	1.11	15	1.06	33	1.01	15	1.06	9	1.01	13	1.03	11	0.99
PPO/μg ergosterol	48	0.97	15	0.98	33	0.98	15	0.98	9	0.95	13	1.06	11	0.94
PER/μg ergosterol	48	0.89	15	1.38	33	0.86	15	1.38	9	0.94	13	0.90	11	1.04

structure (Martins and Hansen, 1997) using the gls command in the nlme package (Pinheiro et al., 2007). Proportion of variation in enzyme activity explained by each independent variable was estimated by calculating pseudo R^2 values for each relationship with the r.squaredLR command in the R package "MuMIn", which uses results of the likelihood-ratio test to estimate improvement from a null model by a fitted model (Burnham and Anderson, 2002). For tests of functional guild and functional gene abundance, the null model used was the generalized linear model with data fit across the phylogeny using the Martin and Hansen's covariance structure. To test whether functional gene abundance predicted functional guild of fungi, we performed linear regression with functional gene abundances as independent variables and specific functional guild as dependent variable using the gls command in R. Holm's correction (Holm, 1979) was used in all analyses to account for multiple comparisons for an observation (i.e. individual enzyme activities or functional genes).

To determine the best predictor of individual enzyme activities, we compared generalized least squares regression models for phylogenetic relatedness, specific functional guild, and functional gene abundance using the log likelihood ratio test (Huelsenbeck and Crandall, 1997). For tests of specific functional guild vs. phylogenetic relatedness (Table 4), all 48 taxa were used in the analysis. For tests of functional gene abundances vs. specific functional guild vs. phylogenetic relatedness (Table 5), 19 taxa were used in the analyses based on the available functional gene abundance data. The functional gene used in each set of model comparisons for an enzyme was the gene that best explained the activity of that enzyme, based on the F-statistic from the gls regression of extracellular enzyme activity against all relevant gene families for that enzyme (Table S3).

Specific functional guild was generally the best predictor of extracellular enzyme activity produced across species in the laboratory-based litter decomposition experiment (Tables 4 and 5). We tested the utility of this fungal trait in predicting ecosystem-level activity by comparing measured enzyme activities in field soil to relative abundances of different specific fungal functional guilds in the soil. Relative abundances of fungal genera in soil were estimated by summing the proportion of total sequences in a soil sample that were assigned to taxa within a genus. Genera identified in soil were assigned to specific functional guilds using assignments reported in Tedersoo et al. (2014).

To determine the utility of specific functional guild classification in predicting ecosystem-level enzyme activity, we first conducted single-factor regressions with soil enzyme activity as the dependent variable and each environmental factor (soil chemistry, climate variables) or abundance of different specific functional guilds (white rot, brown rot, saprotroph, ectomycorrhizal) as the independent variable. Soil chemistry variables and climate data were collected in the previously published field study (Talbot et al., 2014), as described in the Supplementary Material. Then, we ran multiple regression analysis with those variables that showed significant correlation with soil enzyme activity in the single regression analyses. For all statistical models used in this study, we ensured normality and homogeneity of variance by log-transforming variables (i.e. enzyme activities) prior to fitting the models. Normality and homogeneity of variance was checked visually (as recommended, Garamszegi, 2014) and equality of variances was tested by the Fligner and Killeen test (Conover et al., 1981).

3. Results

There was a significant phylogenetic signal to some hydrolase enzymes across all 48 fungal species, as well as within the saprotrophic guild and specifically, within the brown rot fungi (Table 3, Fig. S2). β-xylosidase (BX) activity, cellobiohydrolase (CBH) activity, β-glucuronidase (BGLU) activity and leucine-amino peptidase (LAP) activity showed significant phylogenetic structure across guilds, but phylogeny explained 12% or less of variation in activity of these enzymes (Table 4). Only BGLU and BX showed phylogenetic structure within a guild (BGLU within saprotrophs: pseudo $R^2 = 0.05$, $P = 0.020$ (per unit litter); pseudo $R^2 = 0.05$, $P = 0.038$

Table 4
Statistical tests for effect of phylogenetic relatedness, broad functional guild, and specific functional guild on fungal growth and extracellular enzyme activities in the litter decomposition experiment. "Broad functional guild" refers to saprotrophic (SAP) versus ectomycorrhizal (ECM), while "Specific functional guild" refers to groups of fungi that employ different mechanisms of resource capture: WR = white rot, BR = brown rot, SAP = other saprotroph, ECM = ectomycorrhizal. Log likelihood (lnl) and Akaike Information Criterion (AIC) for fit are reported. Asterisks represent significance of regression (****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). P value for activity of individual enzymes is Holm-corrected. Log likelihood ratios (based on generalized least squares linear regression models) are shown for all possible model comparisons (n = 48).

Enzyme	*Phylogeny*		*Broad Functional group*			*Specific Functional group*			*Model comparisons (log likelihood ratio)*					
	Pseudo R^2	lnl	AIC	Pseudo R^2	F-ratio	lnl	AIC	Pseudo R^2	F-ratio	lnl	AIC	Phy vs. BF	Phy vs. SF	BF vs. SF	SF vs. Phy	SF vs. BF
Ergosterol/mg litter	2.35e-5	56.72	−107.45	0.99	10.24**	61.73	−115.47	0.99	5.37**	64.79	−117.58	10.02	16.14	6.11	−10.02**	−16.14****	−6.11*
CBH/mg litter	0.05	−121.82*	249.64	0.27	18.33**	−115.72	239.43	0.40	9.04**	−108.58	229.17	15.04	24.78	9.74	−15.04***	−24.78****	−9.74**
BG/mg litter	0.02	−110.25	226.50	0.31	20.50***	−102.60	213.19	0.39	9.11**	−99.33	210.67	17.56	24.08	6.52	−17.56****	−24.08****	−6.52*
AG/mg litter	0.01	−118.28	242.56	0.31	20.48***	−110.55	229.10	0.34	7.15**	−109.43	230.87	18.04	20.27	2.24	−18.04****	−20.27****	−2.24
NAG/mg litter	0.01	−118.31	242.62	0.39	28.38****	−107.80	223.59	0.43	10.43***	−105.97	223.94	23.41	27.06	3.65	−23.41****	−27.06****	−3.65
BX/mg litter	0.10	−113.87*	233.75	0.27	16.04**	−108.40	224.80	0.33	6.78**	−106.05	224.11	14.82	19.51	4.69	−14.82***	−19.51****	−4.69*
AP/mg litter	0.01	−117.04	239.89	0.31	20.74***	−108.45	224.90	0.35	7.51**	−107.05	226.10	18.12	20.91	2.79	−18.12****	−20.91****	−2.79
BGLU/mg litter	0.12	−116.54*	238.91	0.27	16.55**	−110.86	229.72	0.40	8.85**	−106.37	224.74	15.27	24.26	8.98	−15.27****	−24.26****	−8.98**
LAP/mg litter	0.04	−97.25*	200.51	0.06	2.83	−97.44	202.88	0.16	2.60	−94.68	201.36	2.96	8.47	5.51	−2.96	−8.47**	−5.51*
PPO/mg litter	3.04e-3	−68.86	143.71	0.04	1.94	−68.91	145.83	0.35	7.43**	−59.42	130.83	1.99	20.99	19.00	−1.99	−20.99****	−19.00****
PER/mg litter	7.91e-4	−53.09	111.98	6.54e-5	0.00	−53.20	114.40	0.08	1.25	−51.08	114.16	0.00	4.24	4.24	−0.00	−4.24*	−4.24*
CBH/μg ergosterol	0.05	−126.01	258.03	0.24	15.99**	−120.55	249.09	0.33	7.46**	−117.51	247.01	13.26	19.34	6.08	−13.26***	−19.34****	−6.08*
BG/μg ergosterol	5.12e-3	−108.36	222.54	0.26	16.18**	−101.36	210.71	0.31	6.14*	−99.83	211.67	14.77	17.82	3.05	−14.77***	−17.82****	−3.05
AG/μg ergosterol	3.39e-3	−120.36	246.72	0.32	20.53***	−111.82	231.63	0.33	6.54*	−111.47	234.94	18.28	18.97	0.70	−18.28****	−18.97****	−0.70
NAG/μg ergosterol	4.26e-3	−120.43	246.84	0.32	21.21***	−111.74	231.47	0.36	7.54**	−110.49	232.98	18.68	21.17	2.49	−18.68****	−21.17****	−2.49
BX/μg ergosterol	0.08	−114.00*	234.00	0.20	11.20*	−110.57	229.14	0.24	4.50	−109.21	230.41	10.62	13.35	2.73	−10.62**	−13.35***	−2.73
AP/μg ergosterol	1.08e-3	−122.65	251.09	0.24	14.05**	−116.02	240.03	0.26	4.70	−115.46	242.93	13.22	14.33	1.11	−13.22***	−14.33***	−1.11
BGLU/μg ergosterol	0.10	−120.65*	247.30	0.22	12.67**	−116.61	241.22	0.29	5.83*	−114.16	240.32	11.81	16.72	4.91	−11.81***	−16.72****	−4.91*
LAP/μg ergosterol	0.02	−116.47	238.94	0.07	2.25	−114.54	237.08	0.14	2.15	−112.16	236.32	3.44	7.27	3.83	−3.44	−7.27**	−3.83
PPO/μg ergosterol	5.14e-4	−80.01	165.86	2.84e-4	0.01	−80.07	168.14	0.18	2.97	−75.30	162.59	0.01	9.56	9.55	−0.01	−9.56**	−9.55**
PER/μg ergosterol	−4.70e-10	−77.63	161.25	0.08	3.76	−75.66	159.32	0.11	1.67	−74.82	161.64	3.93	5.62	1.68	−3.93*	−5.62*	−1.68

(per unit fungal biomass); BX within brown rot fungi: pseudo $R^2 = 0.04$, $P = 0.040$ (per unit litter); pseudo $R^2 = 0.06$, $P = 0.040$ (per unit fungal biomass)). No statistically significant phylogenetic signal was observed for polyphenol oxidase activity, peroxidase activity, or fungal growth on the litter across or within functional guilds (Table 3).

Both fungal biomass and activity of extracellular enzymes were significantly different among functional guilds of fungi. Ectomycorrhizal fungi produced less biomass on litter compared to saprotrophs (0.02–0.11 μg ergosterol/mg litter for ectomycorrhizal fungi vs. 0.02–0.38 μg ergosterol/mg litter for saprotrophs; Table 4). After accounting for phylogenetic distance among taxa, saprotrophs generally had significantly higher activities of labile C, N, and P degrading enzymes (CBH, BG, AG, NAG, AP, BX, and BGLU) compared to ectomycorrhizal fungi, per unit litter and per unit ergosterol (Fig. 2, Table 4, Fig. S3). This broad functional guild (saprotrophic vs. ectomycorrhizal) classification explained up to 39% of variation in enzyme activity across species, while specific functional guild (other saprotroph, brown rot, white rot, ectomycorrhizal) explained up to 43% (Table 4). White rot fungi produced the highest activities of all hydrolases and PPO activity per unit litter than any other fungal guild, yet were equal to ectomycorrhizal and brown rot fungi in PPO activity per unit fungal biomass (Fig. 2, Fig. S3).

PER activities were not significantly different among functional guilds of fungi (Table 4). However, white rot fungi had the highest PER activity per unit litter compared to other functional guilds, while peroxidase activity per unit fungal biomass was highest in ectomycorrhizal fungi (Fig. 2). When regression models were compared using log likelihood ratios, broad functional guild was generally a better predictor of enzyme activities than phylogenetic lineage, and specific functional guild was universally a better predictor of fungal biomass and enzyme activities than phylogeny. Specific functional guild was also generally a better predictor of enzyme activity and biomass than broad functional guild (Table 4).

Similar to recent studies (Floudas et al., 2012; Kohler et al., 2015), our analysis showed that the abundances of certain functional genes varied significantly among specific functional guilds (Table S3). After correcting for phylogenetic relatedness among taxa, white rot fungi had significantly higher abundance of total peroxidase genes than all other guilds (PER: $F_{(3,15)} = 8.60$, $P = 0.033$), including higher numbers of Class II peroxidase genes (POD2: $F_{(3,15)} = 11.80$, $P = 0.006$). White rot fungi also had the highest numbers of most glycoside hydrolase genes and genes coding for oxidases, with significantly higher abundances of genes coding for glucose-methanol-choline oxidases (GMC: $F_{(3,15)} = 7.71$, $P = 0.043$). Exceptions include genes coding for glycoside hydrolase 9, cellulose binding module 21 and laccase, which were highest in ectomycorrhizal fungal species (Table S3).

Across species with published functional gene models, certain enzyme activities correlated with abundance of genes coding for specific families or superfamilies of hydrolytic and oxidative enzymes (Table S4). After Bonferroni correction, CBH activity, β-xylosidase activity, and β-glucosidase activity correlated significantly with the abundance of glycoside hydrolase 3 (CBH: pseudo $R^2 = 0.46$, $P = 0.022$, BX: pseudo $R^2 = 0.39$, $P = 0.011$, BG: pseudo $R^2 = 0.42$, $P = 0.041$). In addition, PPO activity correlated with the total abundance of PPO genes, calculated as the sum of genes coding for the all oxidases showing activity towards L-DOPA (PPO-genes: pseudo $R^2 = 0.45$, $P = 0.002$). Specifically, PPO activity correlated positively with abundance of GMC oxidoreductases (GMC: pseudo $R^2 = 0.44$, $P = 0.014$). Within the GMC oxidoreductases, there was a positive relationship between PPO activity and aryl alcohol oxidase (AAO: pseudo $R^2 = 0.49$, $P = 0.006$) gene abundances. Abundance of genes coding for glycoside hydrolase 8

Table 5
Model comparisons for effect of phylogenetic relatedness, specific functional group, or functional gene abundance on extracellular enzyme activity in the litter decomposition experiment. Model comparisons are made between one predictor (column heading) against another (row in Trait column). Log likelihood (lnl) and Akaike Information Criterion (AIC) for fit are reported. Asterisks represent significance of regression (****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ŦP<0.1). P value for activity of individual enzymes is Holm-corrected. Log likelihood ratios (based on generalized least squares linear regression models) are shown for all possible model comparisons.

Trait	Multiple regression model	Individual models				Model comparisons (log likelihood ratio)		
	Pseudo R^2	N	F ratio	lnl	AIC	Phylogeny	Specific functional group	Functional genes
CBH/μg ergosterol	0.64	19						
Phylogeny				−48.43	102.86		18.13****	11.56***
Specific functional group			6.50**	−39.19	90.38	−18.49		−6.92
Functional gene (GH3)			12.74**	−42.65	93.30	−11.56	6.92**	
BG/μg ergosterol	0.54	19						
Phylogeny				−48.02	102.04		11.70***	10.35**
Specific functional group			3.36*	−42.17	96.34	−11.70		−1.36
Functional gene (GH3)			11.02**	−42.85	93.70	−10.35	1.36	
AG/μg ergosterol	0.45	19						
Phylogeny				−47.22	100.45		11.15***	2.39
Specific functional group			3.15Ŧ	−41.65	95.29	−11.15		−8.77
Functional gene (GH31)			2.03	−46.03	100.06	−2.39	8.77**	
NAG/μg ergosterol	0.60	19						
Phylogeny				−49.98	105.96		16.09****	8.32**
Specific functional group			5.26*	−41.93	95.87	−16.09		−7.77
Functional gene (GH3)			8.36*	−45.82	99.64	−8.32	7.77**	
BX/μg ergosterol	0.65	19						
Phylogeny				−48.23	102.46		19.69****	9.41**
Specific functional group			7.22**	−38.39	88.77	−19.69		−10.28
Functional gene (BX.genes)			9.75**	−43.53	95.05	−9.41	10.28**	
BGLU/μg ergosterol	0.45	19						
Phylogeny				−51.91	109.81		9.62**	5.92*
Specific functional group			2.65Ŧ	−47.09	106.19	−9.62		−3.70
Functional gene (GH2)			5.73*	−48.95	105.89	−5.92	3.70 Ŧ	
PPO/μg ergosterol	0.65	19						
Phylogeny				−35.14	76.29		18.18***	12.65***
Specific functional group			6.33**	−26.06	64.11	−18.18		−5.53
Functional gene (AAO)			14.38**	−28.82	65.64	−12.65	5.53*	
PER/μg ergosterol	0.52	19						
Phylogeny				−32.91	71.82		6.68**	4.27*
Specific functional group			1.69	−29.57	71.14	−6.68		−2.42
Functional gene (POD1)			3.85Ŧ	−30.78	69.56	−4.27	2.42	

and 9 tended to correlate negatively with activity of some hydrolases (Table S4). However, after correction for multiple tests, no other enzyme activities significantly correlated with the abundance of functional genes in any gene family. Functional gene abundances also did not significantly correlate with activity of any enzyme across species within specific functional guild (data not shown). While functional gene abundance was a significantly better predictor of nearly all enzyme activities than phylogeny, specific functional guild was a significantly better predictor of activity than functional gene abundance for CBH, AG, NAG, BX, and PPO (Table 5). Activities of BG, BGLU, and PER were predicted equally well by functional genes and specific functional guilds. Multiple regression models explaining enzyme activities that included both functional gene abundance and specific functional guild explained ~45–65% variation in enzyme activities for species with published genomes.

Enzyme activities produced by fungal species in culture collapsed into two principle components based on mechanism of catalysis (hydrolytic vs. oxidative), similar to enzyme activity in natural bulk soil (Fig. 3). The first principle component (enzyme PC1) was associated with variation in carbohydrate-, protein-, and phosphorus-targeting hydrolases, the second (enzyme PC2) with polyphenolic-targeting oxidoreductases (Tables S1 and S2). Principle component scores were similar for enzyme activities calculated per g litter and per unit ergosterol (Tables S1 and S2).

Extracellular enzyme production by fungal species in the litter culture experiment explained a portion of extracellular enzyme activity in soils where these taxa are found in nature. Most carbohydrases (CBH, BG, AG, NAG, BGLU) and oxidoreductases (PPO, PER) were positively correlated with relative abundance of white rot species in soil (Table 6). Abundance of brown rot species was correlated with AG and PPO activity, while abundance of other saprotrophs was significantly correlated with CBH, BG, and NAG activity. Relative abundance of white rot fungi in soil correlated positively with total soil resources (soil C: $R^2 = 0.01$, $P = 0.003$; soil N: $R^2 = 0.01$, $P = 0.001$; soil moisture: $R^2 = 0.02$, $P = 0.003$), as did the abundance of brown rot species (soil N: $R^2 = 0.01$, $P = 0.004$; soil moisture: $R^2 = 0.02$, $P < 0.0001$; soil CN ratio: $R^2 = 0.03$, $P < 0.0001$; soil pH: $R^2 = 0.01$, $P = 0.03$) and other saprotrophs (soil C: $R^2 = 0.04$, $P < 0.0001$; soil N: $R^2 = 0.02$, $P = 0.0002$; soil moisture: $R^2 = 0.01$, $P = 0.04$; soil CN ratio: $R^2 = 0.01$, $P = 0.03$). When these soil properties and other environmental parameters were included in multiple regression analysis, the best model to explain enzyme activities (determined through stepwise selection using AIC) almost always included abundance of at least one specific functional guild in soil (Table 6). Abundance of white rot fungi predicted activity of NAG and PPO, abundance of saprotrophs predicted BG activity, and soil peroxidase (PER) activity was negatively correlated with abundance of saprotrophs, but positively correlated with abundance of ectomycorrhizal species in both single regression ($P = 0.003$) and multiple regression analyses ($P = 0.01$).

4. Discussion

Extracellular enzymes commonly measured in soil are a metric of microbial activity that recycles carbon and nutrients through soil systems. We hypothesized that abundance of functional genes coding for these enzymes would be the best predictor of biogeochemical activity produced by fungi, given the limits it imposes on decay. In contrast to this hypothesis, we found that specific

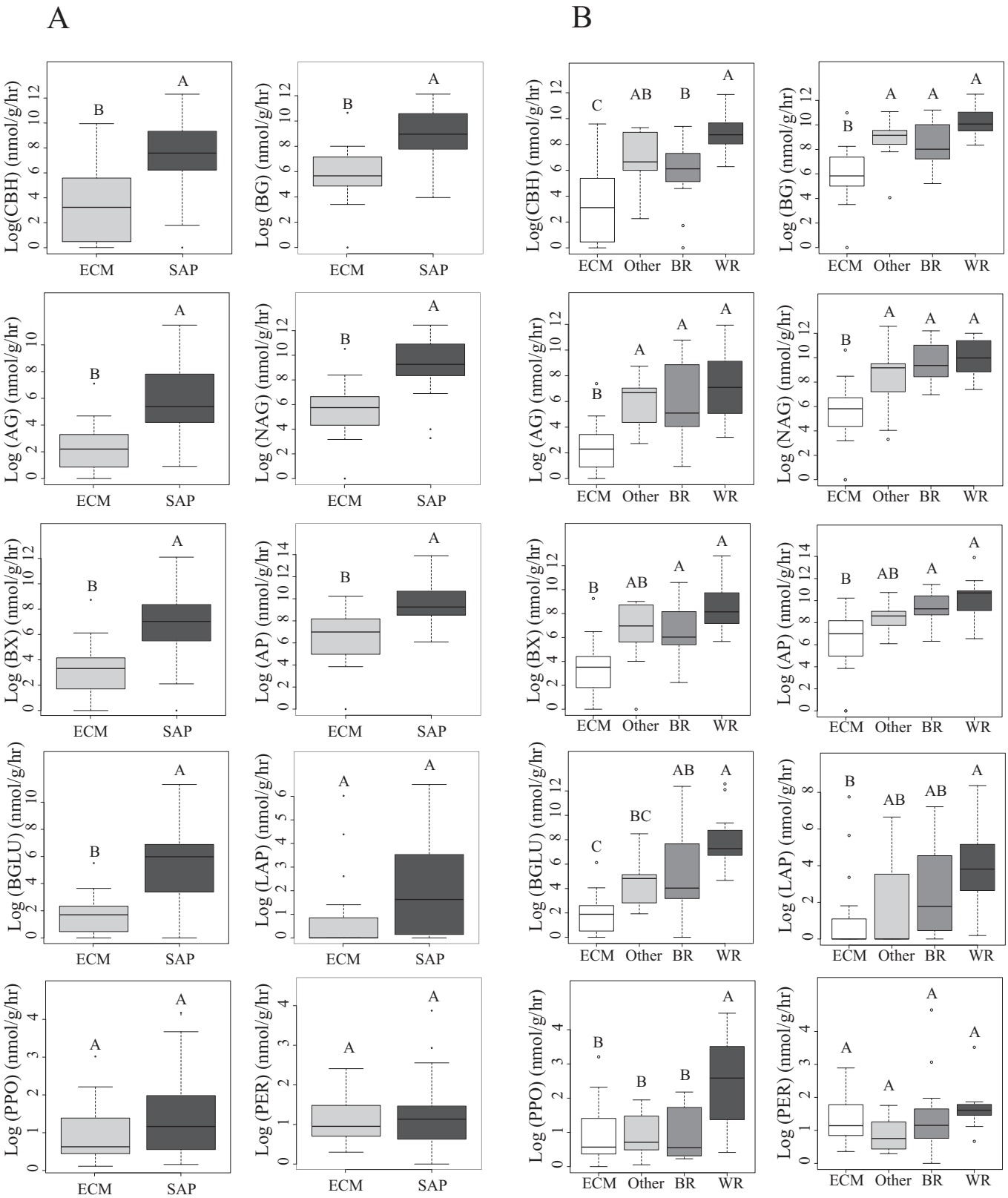

Fig. 2. Potential enzyme activity (nmol/g litter) on litter colonized by different functional guilds of fungi. Panel A represents enzyme activities by broad functional group. Panel B represents enzyme activities by specific functional group. Enzymes are designated by acronyms (CBH = Cellobiohydrolase, BG = β-glucosidase, AG = α-glucosidase, NAG = N-acetyl glucosaminidase, BX = β-xylosidase, AP = Acid phosphatase, BGLU = β-glucuronidase, LAP = Leucine aminopeptidase, PPO = Polyphenol oxidase, PER = Peroxidase). Letters represent Tukey groupings (n = 6–17 per group).

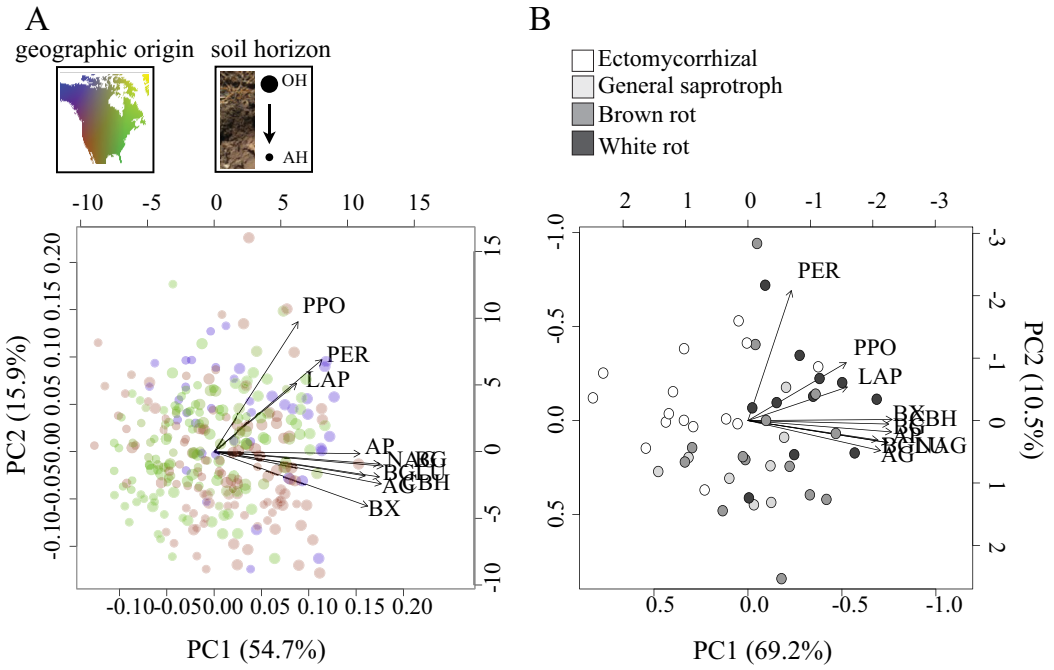

Fig. 3. PCA of enzyme activity in (A) bulk soil collected from various pine biomes in North America (Talbot et al., 2014) and (B) the laboratory-based litter decay experiment. Arrows represent loading factors for individual enzymes, points represent (A) individual soil samples or (B) individual fungal species. Point colors indicate (A) geographic location of soil samples, or (B) specific functional guild of species: white = ectomycorrhizal, light gray = general saprotrophs, gray = brown rot, dark gray = white rot.

functional guild of fungi was generally the best predictor of enzyme activity across species (based on log likelihood ratios), and that functional guild and functional gene abundances were both generally better predictors of enzyme activity compared to phylogenetic lineage of fungi. Saprotrophic fungi produced higher activities of C- and nutrient-degrading hydrolases in culture than ectomycorrhizal fungi, supporting the long-standing assumption that saprotrophs are principle decomposers of labile C in ecosystems (Baldrian, 2009). Specific functional guild was a better predictor of most hydrolase activity compared to broad functional guild (saprotrophic vs. ectomycorrhizal) or functional gene abundance, where white rot fungi consistently produced the highest activity of hydrolases compared to any other guild (Fig. 2 and S3). This observation is consistent with the main niche of white rot fungi as wood decomposers, as well as other studies showing faster hydrolysis of organic matter by white rot fungi compared to other fungal groups (Sun and Cheng, 2002). White rot fungi also produced the highest polyphenol oxidase and peroxidase activities per unit litter, consistent with their well-known role as lignin oxidizers (Kirk and Farrell, 1987). However, ectomycorrhizal fungi also produced high levels of polyphenol oxidase and peroxidase activity per unit fungal biomass, consistent with the emerging view that some ectomycorrhizal fungi can be powerful oxidizers of soil organic matter (Rineau et al., 2013; Tunlid et al., 2013; Bödeker et al., 2014). Prediction of enzyme activity by these specific functional guilds supports the emerging view that trait-based frameworks, rather than taxonomic assignments, are promising approaches for linking microbial community composition to ecological function (Crowther et al., 2014).

Although functional guild classification was the best predictor of fungal enzyme activities on litter, we observed large overlap in enzyme activities between both broad functional guilds and specific functional guilds, indicating a gradient in decomposer ability of species within a functional guild. While functional gene abundance generally did not predict measured enzyme activities as well as functional guild (Table 5), most enzymes had at least one gene that explained over 40% of variation in activity (Table S4). This suggests that abundance of specific functional genes may provide a relatively accurate estimate of enzyme activities by a species, especially in the absence of information about functional guild classification (Table S3). It is possible that gene copy number would explain a larger fraction of variation in enzyme activities among species in scenarios when resources are limiting, such as under low nutrient conditions, or if a greater diversity of species are examined. The enzyme activities we measured are commonly used in bulk soil analyses, yet our results may have been different if a wider suite of enzymes were measured or alternate assays were used to measure extracellular enzyme activity, as activity varies by method employed. For example, cellobiohydrolase activity measured with the fluorogenic substrate (MUB) assay could reflect repeated cleavage of terminal glucose units from cellulose fragments by β-glucosidase, rather than cellobiohydrolase activity per se. An additional possibility is that enzyme activity is regulated by factors that control transcription of these genes, rather than copy number of the functional genes themselves. Our results suggest that this may be the case, and that further research on how genomes control extracellular enzyme production is warranted.

Phylogeny was a generally poor predictor of enzyme activities across species (Tables 3–5). However, in high diversity soil communities, phylogeny could prove useful in predicting activity of fungi within a guild. In our study, a significant phylogenetic signal to enzyme activity was observed within guilds for two hemicellulases (Table 3). Including multiple fungal species within a genus may result in phylogeny having more predictive power for enzyme activity across a greater diversity of species within a guild. Phylogeny can also prove useful in assigning species to functional guilds within lineages (i.e. orders) where groups of species cluster phylogenetically based on trophic group.

There are well-known limitations to extrapolating culture studies to field studies. For example, factors such as abiotic conditions and species interactions (such as symbiosis and parasitism) likely occur in the field and are not represented in single-species

Table 6
Statistics predicting soil enzyme activity from samples collected in major pine biomes across the continental U.S. (Talbot et al., 2014). Statistics are generated from single-factor analysis with abundance of taxa in specific functional guild (white rot, brown rot, other saprotroph, or ectomycorrhizal) from litter decay experiment as independent variable. Independent variables in multiple regression analysis were selected by AIC in a stepwise algorithm. Soil chemistry and climate parameters included in multiple regression analysis were taken from Talbot et al. (2014) and are described therein. Sample size reflects number of soil samples for which enzyme activity data is available. Asterisks represent significance of regression (****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$).

Factor	Single factor regression				Multiple regression			
	df	R^2	Slope	t-value	df	R^2	Slope	t-value
CBH	503				473	0.47****		
White rot		0.02	0.17	3.51***				
Brown rot		−0.002	−0.04	−0.40				
Other saprotrophs		0.04	0.38	4.90****		0.003	0.11	1.82
Ectomycorrhizal		0.02	−0.23	−3.30**		0.003	−0.11	−2.01*
Total soil C (%)		0.43	1.39	19.18****		0.01	0.34	3.54***
Total soil N (%)		0.39	5.07	17.68****		0.006	0.91	2.47*
Total soil moisture (%)		0.18	6.65	10.46****				
Soil C:N		−0.002	0.08	0.30				
Soil pH		0.003	−0.97	−0.60				
Climate PC1		0.01	−0.03	−2.43				
Climate PC2		0.11	0.11	7.81****		0.03	0.06	5.56****
BG	502				471	0.60****		
White rot		0.03	0.15	4.31****				
Brown rot		0.00	−0.06	−1.00				
Other saprotrophs		0.04	0.27	4.71****		0.005	0.10	2.61**
Ectomycorrhizal		0.01	−0.12	−2.43*		0.003	−0.08	−2.19***
Total soil C (%)		0.50	1.09	22.33****		0.14	0.73	5.20****
Total soil N (%)		0.48	4.01	20.80****				
Total soil moisture (%)		0.25	5.65	12.89****		0.01	1.58	2.99**
Soil C:N		−0.002	0.09	0.47				
Soil pH		−0.0003	−0.40	−0.92				
Climate PC1		0.03	−0.03	−4.18****		0.06	−0.04	−8.20****
Climate PC2		0.10	0.08	7.66****		0.03	0.05	6.41****
AG	301				300	0.54****		
White rot		0.05	0.18	4.08****				
Brown rot		0.01	0.24	2.19*				
Other saprotrophs		0.002	0.09	1.25				
Ectomycorrhizal		−0.003	−0.004	−0.06				
Total soil C (%)		0.49	1.12	17.54****		0.13	0.39	9.34****
Total soil N (%)		0.52	4.51	18.38****				
Total soil moisture (%)		0.38	8.02	13.92****		0.01	1.01	2.96**
Soil C:N		−0.002	0.11	0.47				
Soil pH		0.004	−0.78	−1.51				
Climate PC1		0.03	−0.03	−3.37***		0.02	−0.03	−4.07***
Climate PC2		0.03	0.04	3.34***				
NAG	506				300	0.50****		
White rot		0.04	0.17	4.70****		0.003	0.06	2.05*
Brown rot		0.002	−0.10	−1.39				
Other saprotrophs		0.01	0.13	2.20*				
Ectomycorrhizal		0.002	−0.07	−1.41				
Total soil C (%)		0.47	1.10	21.05****		0.44	0.50	20.74****
Total soil N (%)		0.44	4.05	19.58****				
Total soil moisture (%)		0.23	5.72	12.46****				
Soil C:N		0.01	0.43	2.15*		0.004	−0.14	−2.21*
Soil pH		0.04	−2.08	−4.67****				
Climate PC1		0.01	−0.02	−2.52*		0.02	−0.02	−4.15****
Climate PC2		0.02	0.03	3.18**				
BX	317				302	0.41****		
White rot		0.01	0.09	1.74				
Brown rot		−0.001	0.10	0.84				
Other saprotrophs		0.01	0.13	1.68				
Ectomycorrhizal		0.003	−0.10	−1.44				
Total soil C (%)		0.38	1.07	13.86****				
Total soil N (%)		0.39	4.28	14.16****		0.06	1.29	5.85****
Total soil moisture (%)		0.32	8.03	12.14****		0.02	1.46	3.24**
Soil C:N		−0.003	0.06	0.25				
Soil pH		0.04	−2.15	−3.90***		0.008	−0.45	−2.30*
Climate PC1		−0.002	−0.01	−0.68				
Climate PC2		0.01	0.03	1.97*		0.004	−0.02	−1.74
BGLU	316				302	0.39****		
White rot		0.04	0.19	3.79***		0.001	0.02	1.27
Brown rot		0.003	0.18	1.39				
Other saprotrophs		−0.002	−0.04	−0.50				
Ectomycorrhizal		−0.003	−0.02	−0.28				
Total soil C (%)		0.33	1.05	12.43****		0.002	−0.02	−0.16
Total soil N (%)		0.38	3.46	13.93****		0.03	1.72	3.70***
Total soil moisture (%)		0.27	7.81	10.79****		0.001	0.62	1.27
Soil C:N		0.00	−0.27	−0.99				

Table 6 (*continued*)

Factor	Single factor regression				Multiple regression			
	df	R^2	Slope	t-value	df	R^2	Slope	t-value
Soil pH		0.001	−0.68	−1.16				
Climate PC1		0.02	−0.03	−2.98**		0.004	−0.02	−1.73
Climate PC2		0.01	0.03	2.13*		0.00	−0.01	−1.10
PPO	481				451	0.22****		
White rot		0.03	0.10	3.68***		0.009	0.03	2.52*
Brown rot		0.02	−0.17	−3.38***				
Other saprotrophs		−001	−0.02	−0.63				
Ectomycorrhizal		0.003	0.06	1.52				
Total soil C (%)		0.09	0.32	6.71****		0.01	0.10	2.99**
Total soil N (%)		0.11	1.43	7.87****				
Total soil moisture (%)		0.15	3.14	9.23****		0.02	0.85	3.73***
Soil C:N		0.02	−0.48	−3.37***		0.01	−0.21	−3.36***
Soil pH		0.00	0.32	1.01				
Climate PC1		0.01	−0.01	−2.27*		0.01	−0.02	−3.00**
Climate PC2		0.02	−0.03	−3.58***		0.02	−0.03	−3.87***
PER	476				449	0.35****		
White rot		0.02	0.07	3.47****		0.002	0.01	1.44
Brown rot		0.04	−0.16	0.19				
Other saprotrophs		−0.002	0.01	−5.17****		0.008	−0.03	−2.50*
Ectomycorrhizal		0.02	0.09	3.18**		0.008	0.03	2.51*
Total soil C (%)		0.17	0.34	9.81***		0.04	0.23	5.48****
Total soil N (%)		0.14	1.21	8.89***		0.03	−0.88	−4.89****
Total soil moisture (%)		0.29	3.26	13.96****		0.14	1.54	9.77****
Soil C:N		−0.002	−0.01	−0.07				
Soil pH		0.02	−0.78	−3.27**				
Climate PC1		−0.002	0.00	0.003				
Climate PC2		−0.001	−0.005	−0.87				

cultures (Hiscox et al., 2010; Crowther et al., 2011). As only 17% of known fungal species are culturable, it is possible that fungal species cultured from the environment were inactive in the original ecosystem (Bridge and Spooner, 2001). However, despite these limitations, we were able to explain a portion of variation in enzyme activity in field soils collected from across the continent based on lab measurements of enzyme activity and the relative abundance of different specific functional guilds in soil samples. Specifically, abundance of white rot, brown rot, and other saprotrophic fungi correlated positively with activity of carbohydrate-targeting hydrolases, while abundance of ectomycorrhizal fungi consistently correlated negatively with hydrolases (Table 6). Abundance of white rot fungi also correlated with PPO activity, while abundances of ectomycorrhizal fungi correlated positively with soil PER activity. When included in a model with resource availability, the models that best predicted enzyme activity almost always included abundance of a fungal functional guild (Table 6). White rot, other saprotrophic, and ectomycorrhizal fungi were significant predictors of soil polyphenol oxidase, hydrolase, and peroxidase activity, respectively, despite correlations with other soil parameters (Table 6). This suggests that while soil resources are a first-order predictor of soil enzyme activities — potentially because they select for specific fungal communities— explicitly including functional guilds of fungi responsible for driving some of this activity will improve predictions of soil C cycling in our landscape-level biogeochemical models.

Despite observed expansions in secreted protease families in some ectomycorrhizal fungi (Martin et al., 2008), we did not find that ectomycorrhizal fungi in our study generally produced higher protease (LAP) activity than saprotrophs. This may be due to the absence of fresh plant photosynthate, if labile plant C primes enzyme production by ectomycorrhizal fungi (Phillips et al., 2011). Leucine amino peptidase (LAP) has semi-broad catalytic capabilities, catalyzing the hydrolysis of various amino acid residues (including leucine) as well as amino acid amides and methyl esters (German et al., 2011). However, LAP is one of many peptidases that target litter proteins (Monod et al., 2002) and so may not reflect activity of the entire protease pool. In addition, secreted proteases may have functions beyond extracellular breakdown of litter protein, e.g. in intracellular ectomycorrhizal tissue developmental processes or fungal–plant interactions (Rao et al., 1998). Alternatively, the species in our study may have different LAP-producing capabilities than ectomycorrhizal fungi with expanded protease gene families (i.e. *Laccaria bicolor*).

Instead of N- and P-targeting hydrolases, we found that ectomycorrhizal fungi generally produced high levels of peroxidase activity (Fig. 2 and S3). These results are consistent with previous studies showing that many ectomycorrhizal fungi oxidize phenolic molecules in culture (Burke and Cairney, 2002). Recent results show that powerful redox capability may be restricted to a few ectomycorrhizal fungi (Kohler et al., 2015), such that there is a continuum of poor to strong decomposers within this guild. Nevertheless, it is unclear why this capacity is retained in certain ectomycorrhizal lineages. One widely invoked hypothesis is that ectomycorrhizal fungi release oxidases to obtain nutrients from phenolic soil organic matter (Bödeker et al., 2014). However, production of peroxidases can play a variety of roles in decay, such as catalyzing breakdown of fungal cell walls and other tissues, synthesis of recalcitrant fungal cell wall material (melanins), detoxifying the immediate environment, reducing oxidative stress caused by an increase in reactive oxygen species (ROS), converting plant biopolymers into more oxidized, recalcitrant components of soil organic matter (Sinsabaugh, 2010), and activating or repressing fungal signaling pathways (Scott and Eaton, 2008). Studies with wood rot fungi show that ROS production often increases during antagonistic interactions (van der Wal et al., 2013). As space can be a limiting resource for ectomycorrhizal fungi (Kennedy and Bruns, 2005; Fukami et al., 2010), we speculate that ectomycorrhizal fungi may have also retained the capacity to produce oxidoreductase activity for defense (i.e. degradation of competitor biomass).

Fungal oxidation of phenolic substrates in the presence of peroxide is often attributed to the activity of extracellular peroxidases, the most well-known of which are the class II peroxidases (Kirk and Farrell, 1987). Despite observed expansions in peroxidase

gene families coinciding with the evolution of white rot guilds (Floudas et al., 2012), we did not find that class II peroxidase gene copy number predicted peroxidase activity well within this guild. Similarly, class II peroxidase genes are present in the genomes of many ectomycorrhizal species (Bödeker et al., 2009; Kohler et al., 2015), yet we did not find that the number of sequences in class II peroxidase gene families predicted peroxidase activity either across or within functional guilds. After correcting for multiple comparisons, no functional gene was a significant predictor of peroxidase activity across taxa in different functional guilds. *Cenococcum geophilum* had particularly high peroxidase activity in our study (Fig. S1). This fungus synthesizes large amounts of intracellular melanin (Fernandez and Koide, 2013), which arise from the activity of various intracellular oxidoreductases, potentially including peroxidase (Bell and Wheeler, 1986; Butler and Day, 1998). Therefore, the high levels of peroxidase activity observed for this species may reflect intracellular oxidases released through the homogenization of litter samples for enzyme assays. However, Floudas et al. (2012) reported abundances of peroxidase-encoding genes that are specific to intracellular melanin peroxidases (for white rot and brown rot fungi) and these genes did not correlate with peroxidase activity in our study ($P = 0.965$, $n = 8$). For the ectomycorrhizal fungi in our study, peroxidase activity may either have been generated by intracellular or extracellular processes. Peroxidase activity has been observed in the culture filtrates of *C. geophilum* in the presence of soil humus (Heinonsalo et al., 2012), as well as in the culture filtrates of many other ectomycorrhizal fungi (Gramss et al., 1999), including *Piloderma* species (Heinonsalo et al., 2012), which had the second highest peroxidase activity of ectomycorrhizal fungi in our study. Whether generated intracellularly or extracellularly, the production of peroxidase activity by certain ectomycorrhizal fungi in soils is substantial (Table 6), and could be due in part to turnover of ectomycorrhizal hyphae and release of intracellular products into the soil matrix.

Peroxidase activity may be generated by mechanisms other than secretion of peroxidase enzymes, as the substrate used to detect peroxidase activity (DOPA, L-3,4-dihydroxyphenylalanine) is also subject to chemical oxidation by Mn and Fe species (Sinsabaugh, 2010). There is evidence that some species of ectomycorrhizal fungi grown in laboratory culture can produce enzymes and metabolites that interact with these metals to generate free-radical-based Fenton reactions, similar to brown rot fungi (Burke and Cairney, 1998; Rineau et al., 2012; Tunlid et al., 2013). Nevertheless, within the saprotrophs, we did not find that gene families coding for enzymes likely involved in the Fenton reaction (Fe-reducing glycopeptides, quinone reductases, cytochrome *b*562, cellobiose dehydrogenase) correlated with peroxidase production. Secondary metabolites that contribute to Fenton chemistry may be active in generating peroxidase activity by these species. Brown rot fungi possess expanded groups of reducing polyketide synthases, which have the potential to contribute to iron (III) reduction in the brown rot decay mechanism (Riley et al., 2014). If secondary metabolites like polyketides in ectomycorrhizal fungi contribute to Fenton reactions, it is possible that these functional genes could explain peroxidase activity generated by these species. Alternatively, oxidoreductase activity in these soil fungi may be more contingent on regulation of gene expression by substrate, than on the genomic architecture of a species.

Activity of enzymes in culture (per g litter) separated into two principle component axes describing hydrolase activity and oxidoreductase activity (Fig. 3). This separation of extracellular enzyme activity is a widely observed pattern in soil systems (Sinsabaugh, 2010; Talbot et al., 2013), suggesting that these different groups of enzymes are controlled by different environmental factors. To date, studies of extracellular enzyme activity in field soil have focused on understanding the abiotic factors that may give rise to this pattern, such as substrate availability and enzyme pools (Sinsabaugh et al., 2008; Sinsabaugh, 2010). However, when controlling for variation in abiotic variables, we found that extracellular enzyme activity in culture showed the same separation of hydrolase and oxidase activity observed in soil, indicating that these two groups of enzymes are regulated independently of one another. Results from our study suggest that while both hydrolase and oxidase activities in soil may be regulated by environmental conditions and soil resource availability, these activities are also regulated by the presence of specific fungal functional guilds.

5. Conclusions

Fungi are major decomposers in terrestrial ecosystems (Sinsabaugh, 2005) and so are directly responsible for cycling carbon (C) and nutrients through soils. Our observation that an ecological signal in enzyme activity (i.e. specific functional guild) emerges in fungi and scales to the level of forest landscapes and biomes is consistent with recent studies showing that models which incorporate different microbial metabolisms produce more accurate simulations of soil biogeochemical cycles (Wieder et al., 2013; Graham et al., 2014). While traditionally defined functional guilds were the best predictors of extracellular enzyme activity in our study, it is challenging to use this characterization to make inferences about decay in complex fungal communities. This challenge arises because the specific functional guild classification for most soil fungi is unknown, and because these guilds may constitute a continuum across the diversity of soil fungi (Riley et al., 2014). Functional gene abundances effectively delineated specific functional guilds of fungi, so future work focused on classifying fungi isolated from environmental samples into functional guilds based on functional genes could prove useful in predicting the role of species in biogeochemical cycles.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.05.006.

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