Litter degradation rate and β-glucosidase activity increase with fungal diversity

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Abstract: Declining biodiversity is a critical component of global change owing to its influence on ecosystem functioning. Decomposition rate frequently increases with fungal species number, but the responses of extracellular enzymes to fungal species number have not been tested. To test the effect of biodiversity on decomposition and enzyme activities, quaking aspen (*Populus tremuloides* Michx.) litter was inoculated with mixtures of one, two, four, or eight fungi from a pool of 16 fungi that had been isolated from a boreal forest in Alaska. Total CO_2 release and the activities of β-glucosidase, which targets cellulose, and polyphenol oxidase, which targets lignin and other recalcitrant phenolic compounds, were observed across the range of species numbers in the mixtures. Total CO_2 release and β-glucosidase activity increased with number of species but were only weakly correlated with each other; polyphenol oxidase activity had no correlation with number of species or CO_2 release. The results indicate that, over 4 months, decomposition of labile carbon is positively correlated with number of species.

Résumé : Le déclin de la biodiversité est une composante cruciale du changement global étant donné son influence sur le fonctionnement des écosystèmes. Le taux de décomposition augmente fréquemment avec le nombre d'espèces de champignon mais la réaction des enzymes extracellulaires au nombre d'espèces de champignon n'a pas été testée. Pour tester l'effet de la biodiversité sur la décomposition et l'activité enzymatique, de la litière de peuplier faux-tremble (*Populus tremuloides* Michx.) a été inoculée avec des mélanges de un, deux, quatre ou huit champignons à partir d'un groupe de 16 champignons isolés dans une forêt boréale de l'Alaska. Les émissions totales de CO_2, ainsi que l'activité de la β-glucosidase qui s'attaque à la cellulose et celle de la polyphénol oxydase qui s'attaque à la lignine et aux autres composés phénoliques récalcitrants, ont été observées chez les différents mélanges d'espèces. Les émissions totales de CO_2 et l'activité de la β-glucosidase ont augmenté avec le nombre d'espèces mais elles étaient seulement faiblement corrélées l'une avec l'autre. L'activité de la polyphénol oxydase n'était pas corrélée avec le nombre d'espèces ni avec les émissions de CO_2. Ces résultats indiquent que, sur une période de quatre mois, la décomposition du carbone labile est positivement corrélée avec le nombre d'espèces.

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Introduction

Humans are causing species numbers to decline in all of the world's ecosystems (Chapin et al. 2000; Ehrlich and Pringle 2008). Moreover, declines in species diversity alter ecosystem services like net primary productivity, decomposition, and nutrient retention (Hooper et al. 2005). In a recent review of 20 diversity experiments, 19 exhibited relationships between diversity and various types of ecosystem function (Schwartz et al. 2000). In addition to the effects of species numbers, the presence or absence of individual species influences ecosystem functions (Cardinale et al. 2006). Thus, a reduction in species diversity or an alteration in species composition can lead to larger scale consequences.

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Most biodiversity studies have manipulated plant diversity; fewer have altered microbial diversity. Among those studies that have manipulated microbes, decomposition rates often increase (Tiunov and Scheu 2005; Setälä and McLean 2004), sometimes do not respond (Hedlund and Öhrn 2000; Dang et al. 2005), and occasionally decline (Janzen et al. 1995; Toljander et al. 2006) with increasing decomposer diversity. These previous studies have focused on overall rates of decomposition rather than on the turnover of particular carbon pools, such as recalcitrant versus labile carbon.

Multiple mechanisms can lead to biodiversity effects on a specific ecosystem function, such as decomposition. Positive effects of biodiversity may be caused by niche partitioning, the sampling effect, or facilitation. Negative effects can also occur, for example, as a result of competition or inhibition.

Niche partitioning is one mechanism that could generate biodiversity effects on ecosystem processes. If species vary in traits related to resource use, then greater numbers of species should lead to increased use of resources by the community as a whole (Tilman et al. 1997). For example, if microbes target different substrates, then communities that are more diverse should decompose heterogeneous substrates more efficiently (Loreau 2001).

Alternatively, more diverse communities may display faster decomposition rates because they are more likely to

contain species that are strong decomposers. This mechanism is known as the sampling effect (Loreau and Hector 2001). It is difficult to tease these two mechanisms apart in decomposition studies.

A third mechanism that can link ecosystem functioning to diversity is facilitative interactions. Facilitative interactions occur when species facilitate and enhance the function of another species, as in mutualism, for example. In the case of fungi, a lignin-decomposing species might facilitate the activity of a cellulose-decomposing species, because lignin tends to form a protective barrier around cellulose in plant litter. We know that fungi vary in their ability to produce lignin- versus cellulose-targeting enzymes (Trigiano and Fergus 1979; Hatakka 1994; Novotoný et al. 2004). Communities that harbor a greater number of species are more likely to contain combinations of species that form facilitative relationships.

Negative interactions occur when one organism reduces the functioning of another. In the presence of a competitor, an organism might divert resources from decomposition to production of defensive compounds or compounds that stabilize and physically protect organic matter from enzymes or to growth. Among multiple species, such tradeoffs in resource allocation can have substantial negative effects on one or more specific functions. It is possible that both positive and negative interactions occur simultaneously. For this reason, random assemblages of diverse fungi may respond idiosyncratically in ways that show no relationship with the total number of species.

Microbial diversity can control decomposition through any of these mechanisms. However, it is difficult to tease these mechanisms apart among decomposers.

The present study investigates the influence of fungal species diversity on three measures of decomposition: the release of CO_2, the activity of an enzyme that targets lignin (i.e., polyphenol oxidase), and the activity of an enzyme that targets cellulose (i.e., β-glucosidase). Rates of CO_2 release represent the integrated rate of mineralization of all organic compounds in plant litter. Activities of ligninases and cellulases indicate the potential breakdown of the two most abundant phytochemicals, lignin and cellulose, in particular. I hypothesized that the release of CO_2 and the activities of lignin- and cellulose-targeting enzymes would increase with fungal species diversity (i.e., richness) owing to niche partitioning, the sampling effect, or facilitative interactions. To test this hypothesis, a taxonomically broad representation of decomposer fungal species was isolated and a laboratory microcosm experiment was then conducted in which the numbers of fungal species was isolated.

Materials and methods

Isolation of fungi

Fungi were collected from a boreal forest near Delta Junction, Alaska (63°55′N, 145°44′W), in the region described by Treseder et al. (2004). This area is dominated by black spruce (*Picea mariana* (Mill.) BSP) forests that are prone to stand-replacing fires; fire scars are first colonized by quaking aspen (*Populus tremuloides* Michx.) before spruce stands return.

Individual fungi were isolated from sporocarps and fungal-colonized substrates. From an initial collection of more than 100 isolates, 16 were chosen that represent broadly a diversity of morphologies, growth rates, substrates, and taxonomies (Table 1). The morphological characteristics considered when selecting these fungi included fruiting structure, clamp connections, growth rate, culture shape, texture, and color.

To isolate these fungi into axenic culture, sporocarp tissue and colonized substrates were plated on malt yeast agar (MYA: 5 $g \cdot L^{-1}$ malt extract, 5 $g \cdot L^{-1}$ yeast extract, 20 $g \cdot L^{-1}$ agar) amended with selective antibiotics (6 $mg \cdot L^{-1}$ 2-phenyl-phenol and 8 $mg \cdot L^{-1}$ benomyl). These antibiotics facilitated isolation by inhibiting the growth of bacteria and lower fungi (Carey and Hull 1989).

Two isolates previously cultured from soil at these sites were also included, *Penicillium* sp. and the Mortierellaceae. These two isolates had been cultured on minimal nutrient agar containing 500 $mg \cdot L^{-1}$ KH_2PO_4; 150 $mg \cdot L^{-1}$ $MgSO_4 \cdot 7H_2O$; 50 $mg \cdot L^{-1}$ $CaCl_2 \cdot 2H_2O$; 20 $mg \cdot L^{-1}$ ferric EDTA; 0.1 $g \cdot L^{-1}$ thiamine HCl; 12 $g \cdot L^{-1}$ agar; 130 $\mu g \cdot L^{-1}$ streptomycin; 10 $mg \cdot L^{-1}$ chlortetracycline; and either 250 $mg \cdot L^{-1}$ $(NH_4)_3PO_4$, 3 $g \cdot L^{-1}$ malt extract, and 10 $g \cdot L^{-1}$ glucose for *Penicillium* sp., or 1 $mg \cdot L^{-1}$ malt extract, 1 $g \cdot L^{-1}$ bovine serum albumin, and 10 $g \cdot L^{-1}$ tannic acid for *Mortierella* sp. (Allison et al. 2009).

These 16 isolates were identified to the nearest taxonomic group based on ecology, morphology, and gene sequence (Table 2). Following isolation, fungal cultures were maintained on unamended MYA. DNA was extracted from these cultures using a kit (Ultra Clean Plant DNA Kit, Mo Bio Laboratories, Carlsbad, California), amplified using polymerase chain reaction, and then sequenced by Agencourt (Agencourt Bioscience Corporation, Beverly, Massachusetts). BLAST (McGinnis and Madden 2004) matched 18S and internal transcribed spacer sequences, separately, with GenBank accessions (Benson et al. 2007, Table 2). The 16 isolates included 11 basidiomycetes and 5 other fungi (Table 1).

Experimental design

To assemble experimental communities, the 16 isolates were randomly assigned to treatments of 1, 2, 4, or 8 species; replication levels were chosen to ensure that each species appeared with equal frequency within each treatment level. There were 16, 48, 64, or 42 species combinations in the 1-, 2-, 4-, or 8-species treatments, respectively. The experimental unit in this study was a specific combination of species. This design permits inferences to be made about the relationship between diversity and function, although it is not possible to draw conclusions about specific species combinations because these communities were not replicated.

Litter was collected and allowed to partially decompose in the same habitat where fungi were collected (Treseder et al. 2004). The aspen litter substrate had been collected at senescence, air-dried, placed in 1 mm mesh nylon bags, and affixed to the soil surface in a spruce forest for 1 year beginning in September 2005. The partially decomposed litter was collected in September 2006 and was then dried overnight at 60 °C, ground to a coarse powder (<0.5 mm grains), and sterilized by exposure to 2.5–3.0 Mrad γ irradi-

Table 1. Traits of the 16 fungal isolates in monoculture.

Species	BG (μmol C·(g litter)$^{-1}$·h^{-1})[a]	PPO (μmol C·(g litter)$^{-1}$·h^{-1})[a]	Total CO_2 (mg CO_2-C·(g litter)$^{-1}$)[a]	Phylogenetic group[b]	Habitat[c]	White or brown rot[d]
Coprinus sp.	0.0 (1)	4.7 (1)	33.6±4.3 (2)	bas, ag	Wood, dung, humus	Brown
Lyophyllum sp.	0.2 (1)	0.9 (1)	27.6±15.9 (2)	bas, ag	Moss, black spruce	Brown
Collybia cirrhata	0.03 (1)	0.1 (1)	10.9±5.3 (2)	bas, ag	Humus, leaves, bas	
Flammulina populicola	3.7±2.1 (2)	0.1±0.06 (2)	5.3±4.8 (2)	B, ag	*Populus* spp.	White
Agrocybe praecox	0.56 (1)	4.0 (1)	0.42 (1)	bas, ag	Wood, leaves	White
Pholiota carbonaria	0.2±0.02 (2)	0.7±0.6 (2)	0.2±0.1 (2)	B, ag	Charred wood and soil	
Trametes versicolor	2.6 (1)	0.4 (1)	24.0±0.8 (2)	bas, pp	Wood	White
Polyporus varius	0.1 (1)	0.9 (1)	18.6±8.1 (2)	bas, pp	Decaying *Populus* spp.	White
Ceriporiopsis sp.	0.3±0.02 (2)	0.2±0.3 (2)	0.4±0.1 (2)	bas, pp	Advanced-decay trees	White
Marchandiomyces sp.	1.1±0.9 (2)	0.4±0.2 (2)	22.9±11.3 (2)	bas	Lichens	
Rhizoctonia sp.	1.2±1.1 (2)	21.9±0.3 (2)	0.6±16.6 (2)	bas	pp	
Penicillium sp.	1.0±0.1 (2)	0.8±0.7 (2)	22.3±17.8 (2)	asc	Leaves, bas	
Ascomycete	5.1±2.8 (2)	17±13.4 (2)	12.8±15.4 (2)	asc	Cosmopolitan	
Ambomucor sp.	0.12±0.1 (2)	1.0±1.5 (2)	16.5±3.9 (2)	zyg	Peat	
Mortierellaceae	2.3±2.2 (2)	1.3±0.01 (2)	2.6±2.1 (2)	zyg	Leaves	
Hyphomycete	0.2±0.1 (2)	0.3±0.1 (2)	18.4 (1)	hyp		

[a]Enzyme activities (BG, β-glucosidase; PPO, polyphenol oxidase) and decomposition (total CO_2 released) by each species in monoculture is reported with the mean ± SE (*n*).
[b]Of the 16 fungi, most were basidiomycetes (bas), and these basidiomycetes included agarics (ag) and polypores (pp); in addition, ascomycetes (asc), zygomycetes (zyg), and a hyphomycete (hyp) were represented.
[c]Habitat includes a list of the substrates that are found at the collection site (Delta Junction, Alaska) on which the species have been observed to grow.
[d]Of the basidiomycetes, two are known to cause brown rot by preferentially degrading cellulose and five cause white rot by preferentially targeting lignin.

ation in a self-shielded gamma irradiator equipped with a CS-137 ion source (Mark I; Shepherd and Associates).

Microcosms

Microcosms were 40 mL glass vials with rubber septa (No. SB36-0040; I-Chem, Rochester, New York). One gram of coarse sand (particle size 0.4–0.8 mm) was added to each vial, and the vials were autoclaved at 120 °C for 40 min to sterilize. In a sterile laminar flow hood, 47 ± 0.2 mg (mean ± SE) of sterile aspen litter was added to each microcosm vial.

Microcosms were inoculated with an inoculum of fungal culture growing on MYA that had been homogenized in a liquid growth medium. To prepare the inoculum, first, a 0.125 cm^3 cube of fungal culture was added to a 1.5 mL Eppendorf tube and the mass of the cube was recorded. This culture cube was homogenized with 0.5 mL of a growth medium in the Eppendorf tube using a pestle. The liquid growth medium contained 500 mg·L^{-1} KH_2PO_4, 150 mg·L^{-1} $MgSO_4·7H_2O$, 50 mg·L^{-1} $CaCl_2·2H_2O$, 20 mg·L^{-1} ferric EDTA, and 0.1 g·L^{-1} thiamine HCl (Allison et al. 2009). After homogenizing the sample, 0.5 mL of the homogenate was diluted with enough of the growth medium (approximately 14.5 mL) to reach a final concentration of 0.67% culture in medium (*v*/*v*).

For each of the 176 species combinations, 1, 0.5, 0.25, or 0.125 mL of inoculum from each of the 1, 2, 4, or 8 component species (1 mL total) were combined in a separate Eppendorf tube. Thus, a total of 1 mL inoculum was prepared for each species combination, of which 0.6 mL was used to inoculate each microcosm vial. Each microcosm vial received 4 mg of total fungal culture that was transferred from Eppendorf tubes to vials in the 0.6 mL aliquot of the mixture liquid inoculum. The final moisture content of microcosms was 57% (*w*/*w*). All combinations of species were grown in a single microcosm with the exception of monocultures. Each isolate was grown in two vials, and the mean of the two monoculture vials was used in the analysis.

An additional 0.1 mL of aliquot was transferred from monoculture inoculum to a Petri plate with MYA. These plates were used to confirm the viability and lack of contamination of each individual isolate at the time that the multispecies mixtures were prepared. In addition to the inoculated experimental vials, eight control vials were created, which contained sterile growth media in place of inoculum. The median concentration of CO_2 in these control vials at each sampling time was subtracted from CO_2 concentrations measured in the experimental vials to control for contamination and abiotic CO_2 efflux. When enzyme activities were measured at the end of the study, the median values in these control vials were similarly subtracted from the values measured in the inoculated vials.

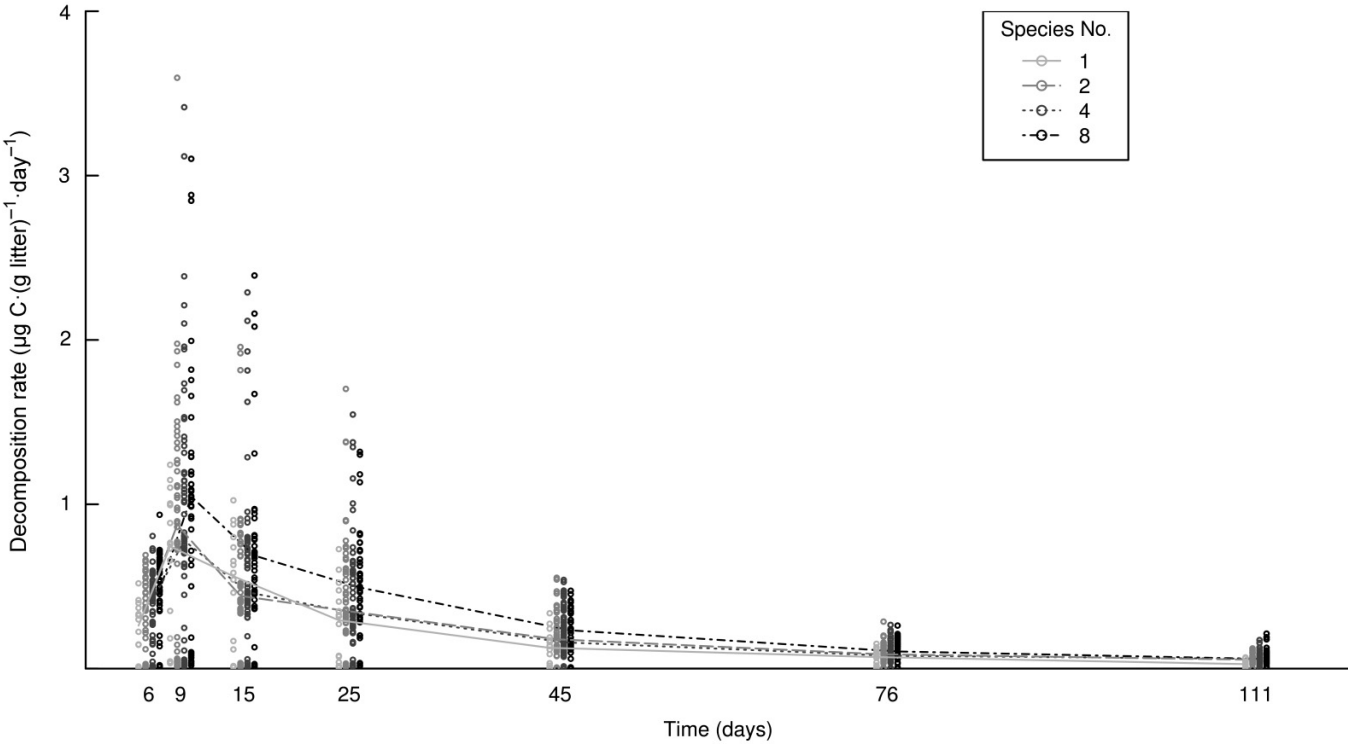
Decomposition rates

The concentration of CO_2 in microcosm vials was measured at seven time points: 6, 9, 15, 25, 45, 76, and 111 days. At each sampling, 10 mL of gas was removed from the vial headspace and injected into an EGM-4 infrared gas analyzer (PP Systems, Amesbury, Massachusetts) to measure CO_2 concentration. After sampling, vials were placed under a laminar flow hood, their caps removed, and

Table 2. Closest matches to the internal transcribed spacer (ITS) and 18S sequences for each isolate from a search of the GenBank database using BLAST.

Isolate	ITS hit	Acc. No.	% Identity	Match length (bp)	18S hit	Acc. No.	% Identity	Match length (bp)
Coprinus sp.	*Coprinus* sp.	AJ890441	97	609	*Psathyrella gracilis*	DQ851582	99	689
	Unknown basidiomycete	AJ246160	97	577	*Pleurotopsis longinqua*	DQ851580	99	689
Lyophyllum sp.	*Lyophyllum* sp.	DQ182502	96	613	*Lyophyllum* sp.	DQ457628	98	713
	Uncultured Trichlomataceae	DQ273423	97	594	*Clitocybe candicans*	AY771609	98	713
Collybia cirrhata	*Collybia cirrhata*	AF274382	99	568	*Collybia tuberosa*	AY77106	99	715
	Collybia cirrhata	AF274381	99	568	*Lepista irina*	AY705948	99	715
Flammulina populicola	*Flammulina populicola*	AF044193	99	727	*Flammulina velutipes*	AY665781	99	713
	Flammulina populicola	AF047873	98	717	*Cylindrobasidium laeve*	AF518576	99	706
Agrocybe praecox	Uncultured fungus	AJ875383	99	641	*Pholiota squarrosa*	DQ465337	99	688
	Agrocybe praecox	AY818348	99	639	*Agrocybe smithii*	DQ115779	99	688
Pholiota carbonaria	*Pholiota carbonaria*	DQ182502	94	483	*Nivatogastrium nubigenum*	DQ459373	99	687
	Uncultured *Pholiota*	DQ273423	91	504	*Pholiota squarrosa*	DQ465337	99	687
Trametes versicolor	*Trametes versicolor*	AY840582	99	579	*Pycnoporus sanguineus*	EU000253	98	674
	Trametes versicolor	AY840580	99	579	*Trametes versicolor*	AY336751	98	674
Polyporus varius	*Polyporus varius*	AF516578	89	146	*Polyporus squamosus*	AY705963	90	271
	Polyporus varius	AF516575	94	97	*Polyporus squamosus*	AF026573	90	271
Ceriporiopsis sp.	*Ceriporiopsis pannocincta*	AY219361	99	694	*Ceriporiopsis pannocincta*	AB084590	99	694
	Fungal endophyte	DQ979767	99	581	*Ceriporia viridans*	AB084592	99	694
Marchandiomyces sp.	*Marchandiomyces aurantiacus*	AY583324	98	177	*Marchandiomyces aurantiacus*	AF289661	98	691
	Uncultured basidiomycete	EU003059	99	172	*Marchandiomyces corralinus*	DQ915464	98	695
Rhizoctonia sp.	*Rhizoctonia* sp.	AF407006	91	133	*Clitopilus prunulus*	AY771607	98	716
	Rhizoctonia sp.	AF407009	90	124	*Lyophyllum* sp.	DQ457628	98	716
Penicillium sp.	*Penicillium soppii*	AF033488	98	534	*Penicillium* sp.	AY960110	99	715
	Penicillium kojigenum	AF033489	96	535	*Eladia saccula*	AB031391	99	715
Ascomycete	Fungal endophyte	DQ979548	100	441	Uncultured fungus	EF674423	99	639
	Leaf litter ascomycete	AF502609	97	407	Ascomycete sp	EF638693	98	639
Ambomucor sp.	*Ambomucor seriatoinflatus*	AY743664	98	568	*Ambomucor seriatoinflatus*	AY743664	98	728
	Unidentified fungus	DQ093732	98	515	*Rhizomucor variabilis*	AF113435	97	728
Mortierellaceae	*Zygomycete* sp.	AY590795	99	498	*Mortierella parvispora*	AY129549	99	667
	Uncultured fungus	AF504850	98	524	*Mortierella alpina*	AJ271630	99	666
Hyphomycete	Uncultured fungus	DQ865807	90	280	*Paecilomyces* sp.	DQ401104	99	709
	Uncultured fungus	DQ865793	90	280	Acremonium-like hyphomycete	AB108787	99	709

Fig. 1. Decomposition rate of aspen litter by combinations of one, two, four, or eight species over the course of 4 months. Total decomposition increased with number of species (also see Fig. 2*a*). Each circle represents one of the 16, 48, 64, or 42 replicate microcosms in the 1-, 2-, 4-, and 8-species treatments, respectively. Lines connect the medians within treatments at each sampling time.



the headspace was allowed to equilibrate with the ambient air for at least 5 min. Decomposition was calculated as the total CO_2 released over the course of the experiment in each vial minus the median CO_2 level in the control vials. Total CO_2 released was divided by the initial mass of the litter in each vial and decomposition is reported in units of milligrams CO_2-C respired per gram of dry litter. The maximum CO_2 concentration observed in a single vial was 39 mg C·g^{-1} (3.9%), and the highest mean concentration at any sampling time was 9 ± 0.5 mg C·g^{-1}. It is assumed that the vials remained aerobic throughout the experiment because the CO_2 accumulation was insufficient to remove the 21% oxygen that was present in the vial headspace after each measurement when the vials were allowed to equilibrate with the atmosphere.

Enzyme activities

After the last sampling time, colorimetric assays (Allison and Jastrow 2006) were used to determine the activities of the cellulose-degrading enzyme β-glucosidase (BG) and the lignin-targeting enzyme polyphenol oxidase (PPO). First, 10 mL of 50 mmol·L^{-1} acetate buffer (pH 5.0) was added to each microcosm vial, and the vials were swirled on a culture rotating machine at 90 r/min for 1 h to create a homogenized slurry of buffer, litter, sand, and fungi (hereafter referred to as "homogenate"). For each enzyme assay, 50 μL of homogenate was combined with 150 μL of an enzyme substrate solution in a well of a 96-well plate. The enzyme substrates used were 5 mmol·L^{-1} *p*-nitrophenyl-β-glucopyranosidase to assay BG activity and 50 mmol·L^{-1} pyrogallol + 50 mmol·L^{-1} EDTA to assay PPO activity, both in acetate buffer (pH 5.0; Allison and Jastrow 2006). For each microcosm, seven analytical replicates (homogenate and enzyme substrate) were run. Alongside these seven analytical replicates, seven controls (homogenate and buffer instead of homogenate and enzyme substrate) were run to correct for absorbance of the homogenate. On each plate, six substrate controls (enzyme substrate and buffer instead of enzyme substrate and homogenate) were run to correct for absorbance of the substrate.

After combining the enzymes in the homogenate with the target substrate compounds, the reactions incubated at 20 °C for 2–4 h on a rotary shaker that was set just slow enough to prevent the sample from escaping from the wells. At the end of the BG assay, addition of 5 μL of 1 mol·L^{-1} NaOH terminated the reaction and developed color from the *p*-nitrophenyl products. The amount of *p*-nitrophenol produced (for BG) or pyrogallol consumed (for PPO) was calculated from absorbance at 405 nm by the reaction mixture using empirically determined extinction coefficients for each substrate. Enzyme activities are reported in units of micromoles of substrate transformed per milligram of litter per hour.

Data analysis

To test the effect of species number on respiration and enzyme activity, regression analyses were conducted with number of species as the predictor variable and CO_2 respiration, BG activity, or PPO activity as the response variable. Linear and log-linear regressions were conducted, and results from the model that explained most of the variation are presented (Setälä and McLean 2004). Significantly positive relationships between predictor and response variables

would indicate that species number is correlated to the functional response.

Next, an analysis of variance (ANOVA) was conducted with 16 predictor variables indicating the presence or absence of each of the 16 isolates plus number of species as a covariate and with CO_2 respiration, BG activity, or PPO activity tested separately as the response variable. The effects of individual species on CO_2 efflux or enzyme activity would support the conclusion that species identity affects function. Unlike investigations of plant diversity effects on biomass, it was not feasible to quantitatively assess the relative importance of sampling effect and complementarity. Such an assessment would have required a measurement of the contribution of individual species within a mixture to CO_2 respiration, BG activity, or PPO activity (Loreau and Hector 2001).

Finally, Pearson's correlations tested for relationships between BG or PPO activity and CO_2 respiration.

Enzyme and CO_2 data from two leaky microcosm vials and enzyme data from 24 misplaced microcosm vials were omitted. The misplaced vials represented a random sample across diversity levels. Because the CO_2 respiration, BG activity, and PPO activity data and residuals were not normally distributed, all variables were rank transformed to normality prior to analysis using the rntransform function in using the R program (Aulchenko et al. 2007). All analyses were performed using the lm function in R (R Development Core Team 2007).

Results

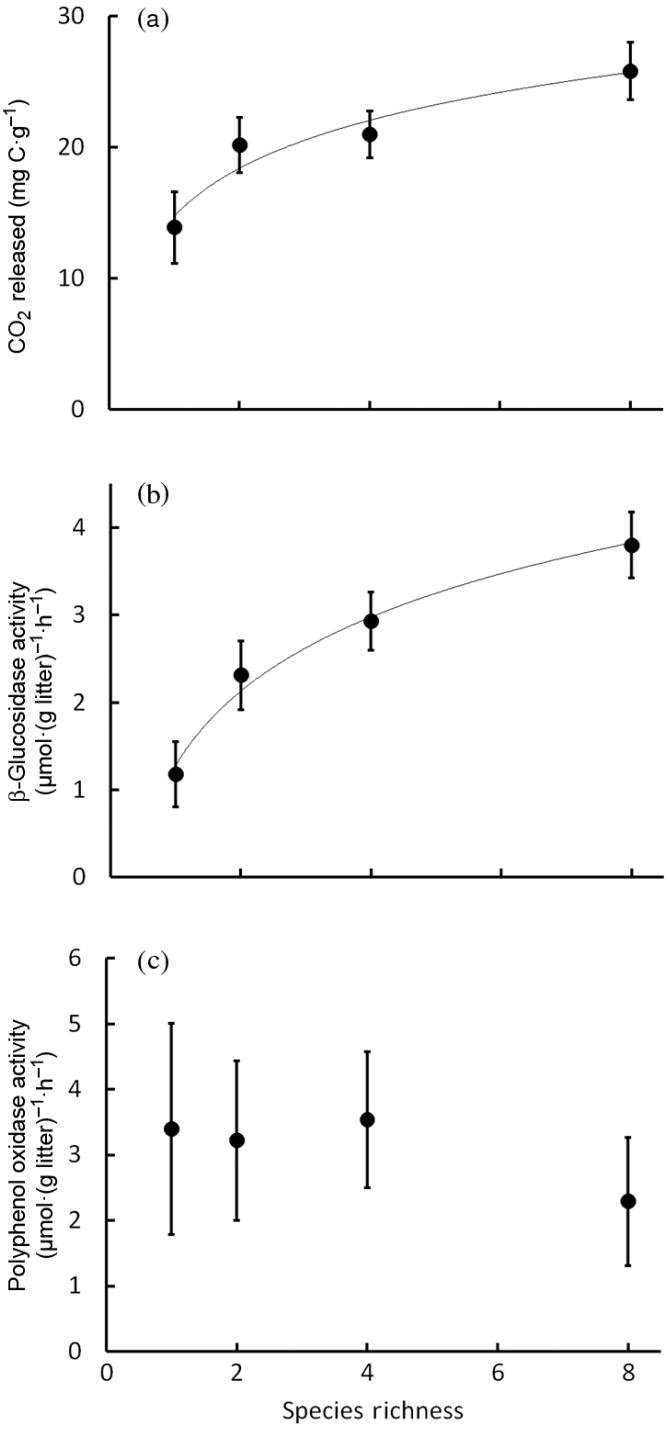
Decomposition rates initially increased with time, and the median rates of decomposition in all treatments were greatest from days 6 to 9 (Fig. 1); decomposition rates subsequently declined. Total CO_2 released increased as a log-linear function of species diversity ($P = 0.002$, $r^2 = 0.05$; Fig. 2*a*) as did BG activity ($P < 0.001$, $r^2 = 0.14$; Fig. 2*b*). In these cases, diversity effects were stronger at lower levels of diversity but were still evident at higher levels of diversity. In contrast, PPO activity did not significantly change as a function of species numbers (Fig. 2*c*).

Decomposition rate was not significantly affected by the presence of any single species (Fig. 3*a*). However, BG activity was significantly higher in microcosms that contained one of three isolates: *Trametes versicolor* (L.:Fr.) Quél. ($P = 0.019$), *Flammulina populicola* Redhead & Petersen ($P = 0.009$), or the unidentified ascomycete ($P < 0.001$; Fig. 3*b*). Moreover, the presence of the unidentified ascomycete had a significant and negative effect on PPO ($P = 0.022$; Fig. 3*c*). Emissions of CO_2 were very weakly correlated with BG activity ($P = 0.07$, $r^2 = 0.014$), but there was no correlation with PPO activity ($P = 0.6$, $r^2 = 0.005$).

Discussion

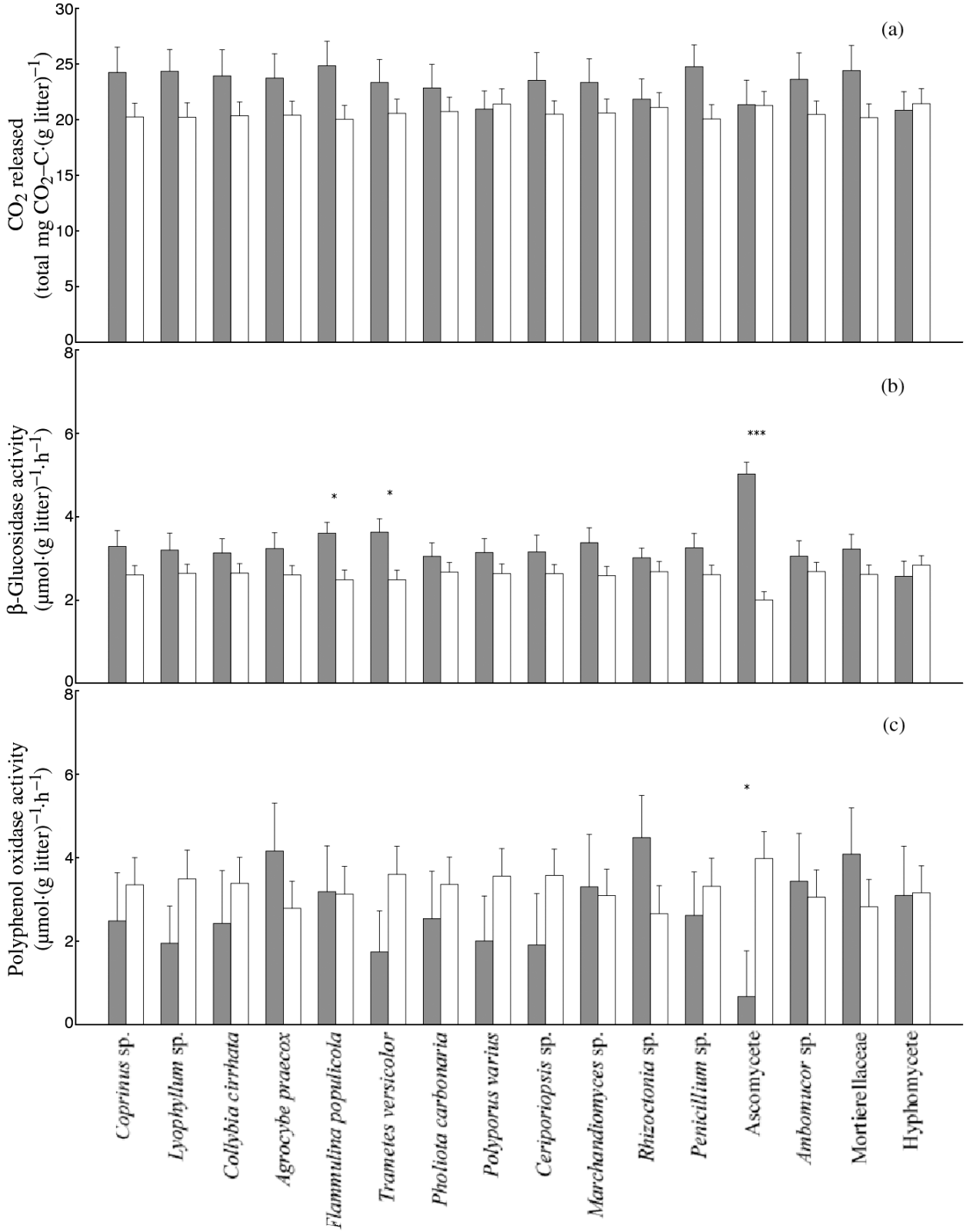
Total CO_2 released and activity of cellulose-targeting BG increased as log-linear functions of the number of fungal species present. These responses supported the initial hypothesis that fungal diversity should have a positive impact on decomposition and cellulose-targeting enzymes. In contrast, there was no demonstrable link between diversity and PPO activity, so the hypothesis was rejected in the case of lignin-targeting enzymes.

Fig. 2. Numbers of fungal species alter two components of ecosystem functioning. Number of species number has a positive effect on (*a*) total decomposition (log-linear regression, $P = 0.002$) and (*b*) β-glucosidase activity (log-linear regression, $P < 0.001$); in contrast, there was no effect of species number on (*c*) polyphenol oxidase activity (no significant correlation). Symbols are means, and error bars are SEs of untransformed data; statistics were conducted on rank-normal transformed data.



Positive relationships between the number of microbial species and the rate of decomposition have been previously

Fig. 3. The effect of the presence (shaded bars) or absence (open bars) of each species on (*a*) decomposition, (*b*) β-glucosidase activity, and (*c*) polyphenol oxidase (PPO) activity. Bars are means, and error bars are SEs. Statistics were conducted on rank-normal transformed data; asterisks indicate a significant effect of species presence: ***, $P < 0.001$; *, $P < 0.05$.



demonstrated in experimental microcosms (Setälä and McLean 2004; Robinson et al. 1981; Tiunov and Scheu 2005). Among 15 studies of effects of decomposer diversity on decomposition (measured as CO_2 release, O_2 uptake, or litter mass loss), nine report positive relationships (Jiang et al. 2008). The results of the present study are similar to those of Setälä and McLean (2004), who report a log-linear response of CO_2 release to species number. In both studies, species effects were stronger at lower levels of diversity and the relationship began to level off at higher levels of diversity.

To test the importance of facilitation and complementarity, Tiunov and Scheu (2005) compared the effect of species number on decomposition of cellulose filters to the effect of species number on decomposition of soil organic matter. They found that respiration of cellulose increased fivefold, in direct proportion to the number (one through five) of species present. In contrast, respiration of carbon from soil increased <20% across the gradient of species numbers (Tiunov and Scheu 2005). Their finding indicates that the increased respiration by diverse communities results from interspecific facilitation rather than resource partitioning.

In this study, the effect of fungal species diversity on the activity of extracellular enzymes involved in decomposition was evaluated. Extracellular enzymes control decomposition of polymers, such as cellulose and lignin (Sinsabaugh and Moorhead 1994). The increase in activity of BG with species number in the present study indicated increased use of cellulose at higher levels of species diversity. This response is consistent with the facilitative interactions observed on cellulose (Tiunov and Scheu 2005). It could also have been generated by the sampling effect by an increase in cellulase production owing to an increase in fungal biomass under high diversity or by complementarity of diverse isozymes (i.e., forms with different amino acid sequences) of BG produced by different species.

In contrast to cellulase activity, PPO activity was much more variable among replicate microcosms within treatments. Because of this high variability, a large effect of species number on polyphenol oxidase activity would be required to produce a significant relationship between species number and activity of this enzyme. There was no evidence that species number had an effect on PPO activity. Species identity may be a more important determinant of PPO activity in mixtures than the number of species per se; only overall presence or absence effects were tested in this study. Interactions among species such as facilitation and inhibition are also possible determinants of PPO activity in multispecies mixtures.

Microbial communities are sensitive to global change (Allison and Martiny 2008). In the field sites from which I collected my isolates, nitrogen fertilization decreased fungal species richness by approximately 25% (Allison et al. 2007). In contrast, experimental warming nearly doubled richness (Allison and Treseder 2008). My findings indicate that these changes in community composition might accentuate or mitigate any direct effects of nitrogen deposition or global warming on litter decomposition and cellulase activity. Thus, fungal communities may form a feedback on climate change by mediating production of greenhouse gases. Loss of species diversity would reduce the return of CO_2 from terrestrial ecosystems to the atmosphere through decomposition.

Only a subset of species that I isolated from these field sites were included in the present study, but these species represent the dominant groups at this site (Allison et al. 2007, 2008). Basidiomycetes are the most abundant fungi in boreal forest soils (O'Brien et al. 2005; Allison et al. 2008), although ascomycetes are also relatively prevalent in litter layers of soil (Lindahl et al. 2007). My study used species from basidiomycetes and ascomycetes as well as endemic hyphomycetes and zygomycetes.

The enzymes BG and PPO target cellulose and lignin, which represent the two most abundant compounds in plant litter. We used these enzymes as indices of the breakdown of each compound. However, enzyme activities do not directly quantify the decomposition rates of lignin and cellulose in situ. Rather, they measure activity under optimal conditions for a simple proxy substrate in a well-mixed and homogenized solution of enzymes and substrates. In addition, other enzymes, such as peroxidases, can target lignin. The lack of response of PPO to fungal diversity may not necessarily rule out sensitivity of lignin turnover to community composition.

In this study, the rate of decomposition and cellulase activity increased with fungal species diversity; considering the similar response of these variables, it is surprising that they are so weakly correlated. In contrast to decomposition and cellulase activity, the high variability of PPO activity was much larger than any effect of fungal diversity. It follows that more rapidly decomposed substrates are most sensitive to the effects of fungal diversity. Declining biodiversity may slow the return of carbon from terrestrial ecosystems to the atmosphere through decomposition. However, reduced carbon uptake by terrestrial ecosystems could also arise if reduced decomposition rates reduce the availability of nutrients for plant growth.

Conclusion

The latest report of the Intergovernmental Panel for Climate Change (IPCC 2007) encouraged investigations such as the present study on the effects of biodiversity loss on global change. My results indicate that conservation of microbial biodiversity may be as important as conservation of plant diversity, if the ecosystem service of organic matter decomposition is to be maintained (Schwartz et al. 2000; Ehrlich and Pringle 2008). Biodiversity effects on production and decomposition have consequences for the concentration of CO_2 in the atmosphere, and CO_2 and other global change agents can in turn alter the structure of decomposer communities (Allison and Martiny 2008).

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References

Allison, S.D., and Jastrow, J.D. 2006. Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. Soil Biol. Biochem. **38**(11): 3245–3256. doi:10.1016/j.soilbio.2006.04.011.

Allison, S.D., and Martiny, J.B.H. 2008. Colloquium paper: resistance, resilience, and redundancy in microbial communities. Proc. Natl. Acad. Sci. USA, **105**(Suppl 1): 11 512 – 11 519. doi:10.1073/pnas.0801925105. PMID:18695234.

Allison, S.D., and Treseder, K.K. 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Glob. Change Biol. **14**(12): 2898–2909. doi:10.1111/j.1365-2486.2008.01716.x.

Allison, S.D., Hanson, C.A., and Treseder, K.K. 2007. Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. Soil Biol. Biochem. **39**(8): 1878–1887. doi:10.1016/j.soilbio.2007.02.001.

Allison, S.D., Czimczik, C.I., and Treseder, K.K. 2008. Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Glob. Change Biol. **14**(5): 1156–1168. doi:10.1111/j.1365-2486.2008.01549.x.

Allison, S.D., LeBauer, D.S., Ofrecio, M.R., Reyes, R., Ta, A.M., and Tran, T.M. 2009. Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. Soil Biol. Biochem. **41**(2): 293–302. doi:10.1016/j.soilbio.2008.10.032.

Aulchenko, Y.S., Ripke, S., Isaacs, A., and van Duijn, C.M. 2007. GenABEL: an R library for genome-wide association analysis. Bioinformatics, **23**(10): 1294–1296. doi:10.1093/bioinformatics/btm108. PMID:17384015.

Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., and Wheeler, D.L. 2007. GenBank. Nucleic Acids Res. **35**: D21–D25. doi:10.1093/nar/gkl986. PMID:17202161.

Cardinale, B.J., Srivastava, D.S., Emmett Duffy, J., Wright, J.P., Downing, A.L., Sankaran, M., and Jouseau, C. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature (Lond.), **443**(7114): 989–992. doi:10.1038/nature05202. PMID:17066035.

Carey, J.K., and Hull, A.V. 1989. A selective medium for the isolation of wood-rotting basidiomycetes. Int. Biodeterior. **25**(5): 373–376. doi:10.1016/0265-3036(89)90016-X.

Chapin, F.S., III, Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E., Mack, M.C., and Díaz, S. 2000. Consequences of changing biodiversity. Nature (Lond.), **405**(6783): 234–242. doi:10.1038/35012241. PMID:10821284.

Dang, C.K., Chauvet, E., and Gessner, M.O. 2005. Magnitude and variability of process rates in fungal diversity–litter decomposition relationships. Ecol. Lett. **8**(11): 1129–1137. doi:10.1111/j.1461-0248.2005.00815.x.

Ehrlich, P.R., and Pringle, R.M. 2008. Colloquium paper: where does biodiversity go from here? A grim business-as-usual forecast and a hopeful portfolio of partial solutions. Proc. Natl. Acad. Sci. USA, **105**(Suppl. 1): 11 579 – 11 586. doi:10.1073/pnas.0801911105. PMID:18695214.

Hatakka, A. 1994. Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. FEMS Microbiol. Rev. **13**(2–3): 125–135. doi:10.1111/j.1574-6976.1994.tb00039.x.

Hedlund, K., and Sjögren Öhrn, M. 2000. Tritrophic interactions in a soil community enhance decomposition rates. Oikos, **88**(3): 585–591. doi:10.1034/j.1600-0706.2000.880315.x.

Hooper, D.U., Chapin, F.S., III, Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J., and Wardle, D.A. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecol. Monogr. **75**(1): 3–35. doi:10.1890/04-0922.

Intergovernmental Panel on Climate Change (IPCC). 2007. Climate change 2007: the physical science basis. Contribution of Working Group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, and New York.

Janzen, R.A., Dormaar, J.F., and McGill, W.B. 1995. A community-level concept of controls on decomposition processes: decomposition of barley straw by *Phanerochaete chrysosporium* or *Phlebia radiata* in pure or mixed culture. Soil Biol. Biochem. **27**(2): 173–179. doi:10.1016/0038-0717(94)00164-V.

Jiang, L., Pu, Z., and Nemergut, D.R. 2008. On the importance of the negative selection effect for the relationship between biodiversity and ecosystem functioning. Oikos, **117**(4): 488–493. doi:10.1111/j.0030-1299.2008.16401.x.

Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Högberg, P., Stenlid, J., and Finlay, R.D. 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytol. **173**(3): 611–620. doi:10.1111/j.1469-8137.2006.01936.x. PMID:17244056.

Loreau, M. 2001. Microbial diversity, producer–decomposer interactions and ecosystem processes: a theoretical model. Proc. R. Soc. B: Biol. Sci. **268**(1464): 303–309. doi:10.1098/rspb.2000.1366.

Loreau, M., and Hector, A. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature (Lond.), **412**(6842): 72–76. doi:10.1038/35083573. PMID:11452308.

McGinnis, S., and Madden, T.L. 2004. BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Res. **32**: W20–W25. doi:10.1093/nar/gkh435. PMID:15215342.

Novotný, Č., Svobodová, K., Erbanová, P., Cajthaml, T., Kasinath, A., Lang, E., and Šašek, V. 2004. Ligninolytic fungi in bioremediation: extracellular enzyme production and degradation rate. Soil Biol. Biochem. **36**(10): 1545–1551. doi:10.1016/j.soilbio.2004.07.019.

O'Brien, H.E., Parrent, J.L., Jackson, J.A., Moncalvo, J.-M., and Vilgalys, R. 2005. Fungal community analysis by large-scale sequencing of environmental samples. Appl. Environ. Microbiol. **71**(9): 5544–5550. doi:10.1128/AEM.71.9.5544-5550.2005. PMID:16151147.

R Development Core Team. 2007. R: a language and Eenvironment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Robinson, C.H., Dighton, J., Frankland, J.C., and Coward, P.A. 1981. Nitrogen and carbon dioxide release by interacting species of straw-decomposing fungi. Soil Biol. Biochem. **13**(1): 1–10.

Schwartz, M.W., Brigham, C.A., Hoeksema, J.D., Lyons, K.G., Mills, M.H., and van Mantgem, P.J. 2000. Linking biodiversity to ecosystem function: implications for conservation ecology. Oecologia (Berl.), **122**(3): 297–305. doi:10.1007/s004420050035.

Setälä, H., and McLean, M.A. 2004. Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. Oecologia (Berl.), **139**(1): 98–107. doi:10.1007/s00442-003-1478-y.

Sinsabaugh, R.L., and Moorhead, D.L. 1994. Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. Soil Biol. Biochem. **26**(10): 1305–1311. doi:10.1016/0038-0717(94)90211-9.

Tilman, D., Lehman, C.L., and Thomson, K.T. 1997. Plant diver-

sity and ecosystem productivity: theoretical considerations. Proc. Natl. Acad. Sci. USA, **94**(5): 1857–1861. doi:10.1073/pnas.94.5.1857. PMID:11038606.

Tiunov, A.V., and Scheu, S. 2005. Facilitative interactions rather than resource partitioning drive diversity-functioning relationships in laboratory fungal communities. Ecol. Lett. **8**(6): 618–625. doi:10.1111/j.1461-0248.2005.00757.x.

Toljander, Y.K., Lindahl, B.D., Holmer, L., and Högberg, N.O.S. 2006. Environmental fluctuations facilitate species co-existence and increase decomposition in communities of wood decay fungi. Oecologia (Berl.), **148**(4): 625–631. doi:10.1007/s00442-006-0406-3.

Treseder, K.K., Mack, M.C., and Cross, A. 2004. Relationships among fires, fungi, and soil dynamics in Alaskan boreal forests. Ecol. Appl. **14**(6): 1826–1838. doi:10.1890/03-5133.

Trigiano, R.N., and Fergus, C.L. 1979. Extracellular enzymes of some fungi associated with mushroom culture. Mycologia, **71**(5): 908–917. doi:10.2307/3759281.