Supporting Information

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SI Methods

Study Area. The study was conducted at the Harvard Forest LTER site in Petersham, MA (42°50′ N, 72°18′ W). The forest is composed of mixed hardwoods, predominantly red maple (*Acer rubrum*), striped maple (*Acer pensylvanicum*), American beech (*Fagus grandifolia*), American chestnut (*Castanea dentata*), red oak (*Quercus rubra*), black oak (*Quercus velutina*), and white birch (*Betula papyrifera*). Soils at the site belong to the Gloucester series (fine loamy, mixed, mesic, Typic Dystrochrepts). Average total annual precipitation is 1,100 mm, and air temperatures range from −25 °C to 32 °C, with an annual mean of around 7 °C.

Abiotic Manipulation. Within the Harvard Forest LTER site, twenty-four 3 × 3 m forest floor plots were manipulated, with each assigned randomly to one of four abiotic treatments: control (C), warming (W), nitrogen addition (N), or warming and nitrogen addition (W+N). All abiotic experimental treatments were established in 2006 and had run continuously until the beginning of the study in September 2013. Plots with nitrogen addition (N and W+N treatments) had ∼5 g nitrogen·m^{-2}·y^{-1} applied during the growing season (May–October) in the form of an aqueous solution of NH_4NO_3. This fertilization rate was about eight times that of ambient nitrogen deposition at the Harvard Forest, which has been calculated at 0.66 g·m^{-2}·y^{-1}. Plots within the warmed treatments (W and W+N) were heated constantly to 5 °C above ambient temperature by heating cables buried 10 cm below the surface of each plot spaced 20 cm apart. The levels of warming and nitrogen deposition reflect those predicted by worst-case climate scenarios for the year 2100. Mean soil temperatures in September, October, and December were ∼16.17, 12.63, and 6.36 °C, respectively, in unheated plots and were 21.31, 17.48, and 11.91 °C, respectively, in heated treatments.

Biotic Manipulations. *Phanerochaete velutina* and *Rescinicium bicolor* were subcultured onto 2 × 2 × 1 cm beech (*Fagus sylvatica*) woodblocks following Crowther et al. (1). Both species are common throughout European, Asian, and North American temperate and boreal woodlands. Two interacting species were used, because interaction zones between competing macrofungi are hotspots of invertebrate and microbial activity. Isopods (Carolina Biological Supply Company) were maintained within plastic containers and were starved for 1 d to clear gut contents before they were added to experimental mesocosms.

Biotic manipulations were established at the start of September 2013. Within each plot, four circular PVC pipes (diameter: 20 cm; height: 20 cm) were installed as enclosures for the biotic treatment. Enclosures were submerged into the topsoil to a depth of ∼10 cm to prevent migration of macroinvertebrates or fungal cords. Communities within each enclosure then were standardized: Leaf litter was cleared to expose topsoil, fungal cords were removed by hand (without disturbing the soil organic layer), and existing macrofauna were removed from the soil surface using a vacuum pump. Treatments (see *Methods*, *Study Design*) then were established, and litter was replaced by hand. Finally, stainless steel wire mesh (2 mm) was affixed by hose clamps to the top of each enclosure to prevent entry of aboveground macrofauna. After biotic manipulation, the experiment was allowed to run for 90 d throughout the fall. In late November 2013, the plots were destructively harvested. All isopods were extracted using a portable shop vacuum cleaner. Survival rates of introduced isopods did not vary significantly across treatments, ranging from 62.5 to 100% in all plots. Three soil cores (5 cm depth) then were extracted for molecular and enzymatic analyses, and wood blocks were removed to estimate wood decay.

Soil Enzyme Analyses. Enzyme analyses were carried out according to methods outlined previously (2, 3). Activities of the hydrolytic enzymes, cellobiohydrolase (CBH), acid phosphatase (PHOS), *N*-acetyl-β-glucosaminidase (NAG), and β-glucosidase (BG) were assayed using the methylumbelliferyl (MUB)-linked substrates β-D-cellobioside, phosphate, *N*-acetyl-β-D-glucosaminide, and β-D-glucopyranoside, respectively. The hydrolytic enzyme leucine aminopeptidase (LAP) was assayed using a 7-amido-4-methylcoumarin (AMC)-linked substrate, L-leucine. Activities of the oxidative enzymes phenol oxidase (OX1 and OX2) and peroxidase (PER1 and PER2) were assayed using the substrates L-DOPA (25 mM), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS; 10 mM), L-DOPA+H_2O_2 (0.3% hydrogen peroxide), and 3,3′,5,5′-tetramethylbenzidine (TMB+H_2O_2) (TMB substrate, 5 mM) (4), respectively.

Briefly, ∼1.0 g of field-moist soil was homogenized in 125 mL sodium acetate buffer (50 mM, pH = 4.6, average pH of the soil) for 30 s using a Magic Bullet (Homeland Housewares LLC). Sample homogenate (200 μL) was transferred to 96-well microplate, followed by the addition of 50 μL substrate. Microplates were incubated for 15 min to 18 h, depending on the enzyme substrate. After incubation, fluorescence was measured at excitation wavelength of 360 nm and an emission wavelength of 450 nm (hydrolytic enzymes), and absorbance was measured at 450 nm (OX1, PER1, PER2) or 420 nm (OX2) on a Biotek HT plate reader (Biotek). All enzyme assays were done with 16 replicate wells per sample and were corrected for background fluorescence or the absorbance of substrate (negative control). For hydrolytic enzymes, the conversion of fluorescence was based on measurements of the standard MUB (10 μM) (BG, CBH, NAG, PHOS) or AMC (10 μM) (LAP). Conversion of OX1 and PER1 was determined based on an empirically determined extinction coefficient of 7.9/μmol used in other studies (2, 3). Conversion of PER2 (TMB substrate), and OX2 (ABTS substrate) was determined based on an empirically determined extinction coefficient of ε_{450} = 59,000 M/cm (5) and ε_{420} = 18,460 M/cm (6), respectively. Final enzyme activity was expressed as micromoles of substrate converted per hour per gram of litter dry mass (μmol·h^{-1}·g^{-1}).

DNA Extraction and Quantitative PCR Analysis. The abundance of fungal and bacterial genetic markers was estimated using quantitative PCR. Although this common approach can provide variable results across soil types, because DNA extraction biases can amplify biomass estimates in certain soils relative to others, it has been shown to provide robust estimates of microbial biomass for samples collected from equivalent soil types (7). Total DNA was isolated from soil samples (0.3 g fresh mass) using phenol-chloroform extraction combined with the addition of $CaCl_2$, followed by purification using the GeneClean Turbo Kit (Biogenic) following Baldrian et al. (8). DNA was stored at −20 °C before further analysis. Three independent DNA extractions were performed for each sample. The fungal/bacterial rDNA copy ratio (F/B DNA ratio) was calculated based on quantitative PCR using 1,108 forward and 1,132 reverse universal primers targeting the bacterial 16S rDNA gene and ITS1/qITS2* universal primers for fungi (8). Quantitative PCR was performed in triplicate reactions as previously described.

Wood Decay. The interactive effects of isopod activity, warming, and nitrogen addition on fungal-mediated wood decomposition rates were explored by determining changes in density of the fungal-colonized wood blocks. Total mass loss is generally used to estimate wood decomposition (9). However, to focus specifically on fungal-mediated decomposition rather than on the loss of large sections of wood resulting from direct invertebrate feeding or environmental weathering, we estimated changes in wood density (dry mass/volume) over the course of our experiment, following Crowther et al. (2, 10). Fungal-mediated mass loss (in grams) then was calculated by scaling the initial volume of wood by the final density. Within-site variation in wood decomposition is generally high in this region because of the highly heterogeneous nature of temperate forest ecosystems (9). However, by using wood blocks cut from the same tree branch and colonized by identical fungal isolates (one wood block colonized by *R. bicolor* and one by *P. velutina* in each chamber), we were able to minimize variability and focus specifically on the effects of our experimental manipulations.

1. Crowther TW, et al. (2013) Top-down control of soil fungal community composition by a globally distributed keystone consumer. *Ecology* 94(11):2518–2528.
2. DeForest JL (2009) The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and l-DOPA. *Soil Biol Biochem* 41:1180–1186.
3. Saiya-Cork KR, Sinsabaugh RL, Zak DR (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biol Biochem* 34(9):1309–1315.
4. Johnsen AR, Jacobsen OS (2008) A quick and sensitive method for the quantification of peroxidase activity of organic surface soil from forests. *Soil Biol Biochem* 40:814–821.
5. Josephy PD, Eling T, Mason RP (1982) The horseradish peroxidase-catalyzed oxidation of 3,5,3′,5′-tetramethylbenzidine. Free radical and charge-transfer complex intermediates. *J Biol Chem* 257(7):3669–3675.
6. Floch C, Alarcon-Gutiérrez E, Criquet S (2007) ABTS assay of phenol oxidase activity in soil. *J Microbiol Methods* 71(3):319–324.
7. Baldrian P, et al. (2013) Estimation of fungal biomass in forest litter and soil. *Fungal Ecol* 6:1–11.
8. Baldrian P, et al. (2012) Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME J* 6(2):248–258.
9. Bradford MA, et al. (2014) Climate fails to predict wood decomposition at regional scales. *Nat Clim Chang* 4:625–630.
10. Crowther TW, Boddy L, Jones TH (2011) Outcomes of fungal interactions are determined by soil invertebrate grazers. *Ecol Lett* 14(11):1134–1142.

Table S1. *P*- and *F*-values for fixed effects [warming, nitrogen, isopods, and (fungal) cords] and second-order interaction terms within linear mixed-effects models for fungal biomass, fungal:bacterial ratio, hydrolytic enzymes, oxidative enzymes, and wood decomposition

	Fungal biomass		Bacterial biomass		Hydrolytic enzymes		Oxidative enzymes		Wood decomposition	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Warming	0.001	0.970	4.891	0.042	0.534	0.475	0.800	0.384	2.535	0.131
Nitrogen	1.553	0.231	0.340	0.568	6.101	0.025	0.255	0.620	4.738	0.045
Isopods	6.609	0.013	1.560	0.217	0.106	0.746	0.839	0.364	5.209	0.036
Cords	17.032	0.000	0.743	0.393	13.780	0.000	17.741	0.000	—	—
Warming*nitrogen	3.925	0.049	0.069	0.797	4.643	0.034	0.125	0.728	10.367	0.005
Warming*cords	1.874	0.177	0.000	0.991	1.281	0.263	1.432	0.237	—	—
Warming*isopods	0.669	0.417	0.391	0.534	1.282	0.263	0.629	0.431	0.649	0.432
Nitrogen*cords	5.566	0.022	2.001	0.163	4.653	0.036	0.623	0.433	—	—
Nitrogen*isopods	2.231	0.141	3.731	0.059	1.074	0.305	0.140	0.709	0.554	0.468
Cords*isopods	3.762	0.018	0.081	0.776	4.284	0.044	0.122	0.728	—	—

Third- and fourth-order interaction terms were all nonsignificant ($P > 0.05$), and their removal reduced AIC scores by at least a value of 3.