Litter Supply as a Driver of Microbial Activity and Community Structure on Decomposing Leaves: a Test in Experimental Streams

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Succession of newly created landscapes induces profound changes in plant litter supplied to streams. Grasses dominate inputs into open-land streams, whereas tree litter is predominant in forested streams. We set out to elucidate whether the activity and structure of microbial communities on decomposing leaves are determined by litter quality (i.e., grass or tree leaves colonized) or whether changes during riparian succession affecting litter standing stocks on the stream bed play an overriding role. We used 15 outdoor experimental streams to simulate changes in litter supplies reflecting five stages of riparian succession: (i) a biofilm stage with no litter, (ii) an open-land stage characterized by grass litter inputs, (iii) a transitional stage with a mix of grass and tree litter, (iv) an early forested stage with tree litter, and (v) an advanced forested stage with 2.5 times the amount of tree litter. Microbial activities on tree (*Betula pendula*) and grass (*Calamagrostis epigejos*) litter were unaffected by either the quantity or type of litter supplied to the experimental streams (i.e., litter standing stock) but differed between the two litter types. This was in stark contrast with bacterial and fungal community structure, which markedly differed on grass and tree litter and, to a lesser extent, also among streams receiving different litter inputs. These patterns reveal distinct responses of microbial community structure and activity to the bulk litter available in streams but consistent responses to the litter type colonized.

Newly formed landscapes such as volcanic lava fields (1), glacier forefields (2), and postmining areas (3, 4) undergo ecosystem succession in three main stages distinguished largely by vegetation structure. Particularly relevant for streams draining these landscapes are changes in litter supply accompanying succession of the riparian plants. Open-land streams are initially deprived of plant litter until they begin to receive inputs from in-stream macrophytes and from forbs establishing in the riparian zone (5–7). Later, when woody riparian vegetation encroaches, large amounts of leaf litter and woody debris are delivered to the streams (8). Dominance of the riparian vegetation by either woody plants or forbs thus largely determines the nature and amounts of litter inputs to streams.

Characteristics of leaf species that can be expected to affect microbial communities colonizing leaf litter include concentrations of nutrients, especially nitrogen and phosphorus (9), and the composition of carbon compounds such as cellulose and lignin (10). An analysis across a broad range of plants showed that leaf litter of deciduous woody plants tends to have higher nitrogen and phosphorus concentrations (i.e., low C/N and C/P ratios) than graminoids (11), though this is not universally true (12). How these qualitative differences affect microbial decomposers and decomposition is not well-known. What is more, information on grass litter in streams is scarce, except for some studies on litter decomposition (7, 13), and direct comparisons with deciduous tree litter are lacking. Nevertheless, there is limited evidence that suggests that there are clear differences between microbial communities on deciduous tree and grass litter (14). Furthermore, a previous study found that a sediment bacterial community from a grassland stream differed in its capacity to grow on dissolved organic matter derived from deciduous tree or grass litter (15). Microbial communities colonizing decomposing leaves in terrestrial environments have been found to vary their enzyme expression patterns according to leaf chemical composition (16). Thus, changes in riparian vegetation during ecosystem succession could alter both leaf-associated microbial community structure and metabolic activities.

Tests for effects of litter quantity on stream microbial decomposers and decomposition are also scarce (17, 18), although there is reason to believe that litter quantity can play an important role. In particular, greater litter inputs increase the inoculum potential of litter-colonizing fungi (19), as shown by large increases in the density of aquatic hyphomycete spores in stream water shortly after autumn pulses of leaf inputs in temperate regions (20). These observations are partly supported by a whole-stream litter exclusion experiment where fungal spore densities in autumn and winter tended to be lower in the litter-depleted stream (17). Leaf decomposition did not respond in the same way in another field experiment designed to test for effects of litter quantity in streams by augmenting or depleting litter standing stocks (18). It is possible, however, that the manipulated stream reaches in that study were too short for significant effects to be detected. Thus, it remains unclear to what extent litter quantity affects microbial decomposers and decomposition.

As successional shifts from open-land to forest streams are not abrupt, there will be intermediate stages when streams receive a mix of litter types, including grass and tree litter. Although a number of studies comparing mixed-species and single-species litter have produced variable results, both positive and negative effects on litter decomposition have been detected (21). Much less is known about effects of litter mixing on microbial diversity and activity. Greater diversity of microbes was found in a forest in mixed-species litter compared to single-species litter (22), whereas fungal diversity in streams was unaffected by litter mixing (23).

In the experiment reported here, we aimed at elucidating the relative importance of the effect of leaf litter quality versus surrounding stream environmental conditions on litter-associated microbial communities. Specifically, we assessed effects of leaf litter type colonized by microbial communities (substrate directly used) versus the effects of litter quality and quantities deposited in stream beds (litter standing stock) on microbial community structure, biomass, and activities at different stages of experimentally simulated riparian succession. We hypothesized that litter-associated microbial communities change either gradually or abruptly along an experimental litter supply gradient reflecting different successional stages. Specifically, we hypothesized that microbial community structure, biomass, respiration, spore production, and various enzymatic activities associated with decomposing leaves are influenced by the following factors: (i) the input of any type of leaf litter to experimental streams, (ii) the presence specifically of leaf litter from trees, (iii) litter quality (grass versus tree litter), (iv) litter quantity, and (v) litter mixing (presence of both grass and tree litter). In addition, we expected the structure, biomass, and activities of the microbial communities to respond directly to the quality of the leaf litter on which they grow.

MATERIALS AND METHODS

Experimental design. We conducted the experiment in 15 outdoor experimental streams simulating sand-bed streams receiving allochthonous litter at five distinct stages of ecosystem succession: (i) an initial biofilm stage, (ii) an open-land stage characterized by graminoids, (iii) a transitional open-land and forested stage, (iv) an early forest stage, and (v) an advanced forest stage. The experimental streams (LWH, 4.0 by 0.12 by 0.12 m) were placed on tripods, 1 m tall, next to a recently created watershed (Chicken Creek; 51°36′ N, 14°16′ E) in eastern Germany (24). The experimental streams were filled with 4 cm of sand collected from a dry ephemeral stream reach of the watershed (25). They had open tops to ensure natural light intensities and other meteorological conditions. Well-oxygenated stream water was supplied from the watershed. It was collected in a subterranean tank before being delivered to the experimental streams (26). A pump maintained water flow at 1.7 ± 0.3 cm s^{-1} (mean $\pm$ standard deviation [SD]), corresponding to a water level of 1 cm. The water was partly recirculated through a pipe connecting the upstream and downstream ends of each stream. The concentrations of nutrients (especially phosphorus) in the groundwater feeding the experimental streams were low (see Table S1 in the supplemental material) (25). Dissolved organic carbon had high concentrations (8 to 12 mg liter^{-1}) but was old and hardly bioavailable (27).

To simulate shifts in leaf litter input into streams during five stages of riparian succession, the experimental streams were stocked with grass or tree litter (referred to as bulk litter below). Wood small-reed (*Calamagrostis epigejos* (L.) Roth) was chosen as the graminoid because it was dominant during the early successional stages in the Chicken Creek watershed. This species produces lower-nutrient litter than other graminoids (28). Silver birch (*Betula pendula* (L.) Roth) was chosen because it is a common

tree species on sandy soils in the region, where it can form extensive stands. Both types of litter were collected from single stands in autumn 2007. The carbon content of the litter was estimated by determining litter organic matter content as ash-free dry mass (AFDM) (combustion of ground leaf material for 4 h at 450°C) and assuming 50% C in the litter AFDM. Nitrogen and phosphorus concentrations in the litter were determined by the method of Ebina et al. (29). Lignin and cellulose concentrations were determined by the forage fiber method of Van Soest as described previously (30).

The simulation of successional litter inputs to the experimental streams included the following: (i) no litter addition (i.e., bare stream), (ii) 100 g m^{-2} of wood small-reed litter (ca. 20% of the stream bed covered), (iii) mix of 50 g m^{-2} of wood small-reed litter and 50 g m^{-2} of birch litter (ca. 60% cover), (iv) 100 g m^{-2} of birch litter (100% cover, single layer of leaves), and (v) 250 g m^{-2} of birch litter (100% cover, 2 or 3 leaf layers). One fifth of the litter was mixed into the sediment when filling the experimental streams to mimic the natural burial of litter in sandy sediments. The rest was distributed on the sediment surface. The litter was continuously submerged throughout the study.

Stream water suspensions containing natural microbial communities were poured in all experimental streams as a natural inoculum to boost microbial colonization. To obtain a diverse microbial inoculum, 24 g (dry weight) of mixed leaf litter (various local species) was collected in three local open-land and forest streams. To detach bacterial cells from the leaf surfaces, half of the litter was sonicated in an ultrasonic bath (3 min, 35-W output) (31) containing 8 liters of Chicken Creek water. The other half was aerated for 48 h in 8 liters of Chicken Creek water to induce sporulation of the fungi (aquatic hyphomycetes) growing in the leaves. Subsequently, the 15 experimental streams each received 500 ml of both suspensions. After inoculation, water was completely recirculated in the individual streams for 24 h to facilitate establishment of the added microorganisms. Subsequently, they were supplied with stream water from the watershed such that 5% of the water volume in the experimental streams was replaced during each cycle, resulting in an average water renewal time of 6 h.

To determine responses related to litter quality, each of the experimental streams also received 6 small litter bags (2-mm mesh size) containing either 18 pieces of wood small-reed litter (2 cm^2) or 18 discs of birch litter (2 cm^2), corresponding to 305 ± 37 and 157 ± 14 mg dry weight, respectively.

Sampling. Litter bags of each type were collected after 6, 8, and 10 weeks and processed within 12 h after retrieval. Birch leaf discs or pieces of wood small-reed were gently cleaned from mineral deposits with filtered (5-μm membrane filters, SMWP; Millipore, Zug, Switzerland) ground water from the Chicken Creek watershed. Six leaf pieces or discs were immediately used to measure respiration and then to determine fungal sporulation rates before they were frozen at −20°C and later freeze-dried to determine mass loss and ergosterol content as a measure of fungal biomass. Leaf mass was determined by drying leaf discs or pieces at 105°C and weighing them to the nearest 0.1 mg. Nine additional discs or pieces were used to determine microbial biomass, enzymatic activity, and community structure. Three discs were preserved in 2% paraformaldehyde buffered with 0.1% sodium pyrophosphate to determine bacterial biomass, three discs were stored at −20°C for later enzyme analyses, and three discs were shock-frozen in liquid nitrogen and subsequently stored at −80°C for molecular analyses.

Water and litter chemistry. Surface water (250 ml) was collected in each stream with a syringe, immediately filtered through prewashed membrane filters (cellulose acetate; 0.45-μm pore size; HA Millipore, Zug, Switzerland), frozen, and later analyzed for dissolved organic carbon (DOC) and nutrients. Conductivity, pH, and temperature were measured directly in the experimental streams with handheld WTW probes (Weilheim, Germany).

Microbial respiration. Aerobic respiration associated with leaf litter was measured in 50-ml glass bottles containing water from the experi-

mental streams and 6 leaf discs or pieces per sample. After temperature acclimation (1 h), the oxygen decline was recorded overnight (12 h) in the dark with an optical oxygen meter (Microx TX3; PreSens GmbH, Regensburg, Germany), and the leaf litter samples were subsequently dried (105°C, 12 h). To determine organic matter content, 10 leaf discs or pieces were combusted at 550°C for 4 h and weighed.

Extracellular enzyme activities. The potential activities of five enzymes involved in the acquisition of carbon (β-glucosidase [BG] and β-xylosidase [BX]), nitrogen (leucine aminopeptidase [LAP] and β-*N*-acetylglucosaminidase [NAG]), and phosphorus (alkaline phosphatase [AP]) were determined along with the potential activities of two enzymes involved in the degradation of lignin (phenol oxidase [PO] and phenol peroxidase [PP]). These enzyme activities were assessed with substrate analogues linked to fluorescent molecules (4-methylumbelliferone [MUB] or 7-amino-4-methylcoumarin [AMC]) or to 3,4-dihydroxyphenylalanine (L-DOPA) for the spectrophotometric assay of PO and PP (32). Fluorometric assays were performed as follows: 3 pieces of wood small-reed or 3 birch leaf discs were placed in 60 ml of tri(hydroxymethyl)aminomethane buffer (Tris, 0.1 mM, adjusted to pH 7.5 with HCl, autoclaved). The suspension was homogenized with a blender (1,700 W) (Ultra-Turrax; IKA Werk, Staufen im Breisgau, Germany) for 30 s. Each well of a 96-well microplate received 200 μl slurry and 50 μl of the substrate analogue (200 μM stock solutions, final concentration of 40 μM). Fluorescence or absorbance was measured after incubation at 10°C for 1.5 h (AP and NAG) or 4 h (BG, BX, LAP, PO, and PP). NaOH (0.5 M, 10 μl) was added before shaking the microplates and measuring fluorescence on a microplate reader (Tecan Infinite 200; Männedorf, Switzerland) at an emission wavelength of 445 nm and 450 nm, respectively. The excitation wavelength was 365 nm for both types of substrate. Absorbance in the PO and PP assays was measured at 460 nm. Background fluorescence or absorbance from the sediment and substrate analogues was subtracted by measuring sample and substrate controls. Quenching was assessed with a quench coefficient (0.48 to 1.00) calculated as the quotient of fluorescence of each individual sample spiked with a fluorescence standard (MUB or AMC) and the same standard added to buffer only.

Bacterial and fungal biomass. Bacterial abundance was determined by flow cytometry after detaching bacterial cells from leaves and sediments by treating the leaves and sediments with an ultrasonic probe three times for 20 s each time (31, 32). Briefly, the detached bacterial cells were separated from other particles by collecting them on top of Histodenz solution (33), staining with SYBR green I, and counting on a CyFlow space flow cytometer system (Partec, Görlitz, Germany) equipped with a 200-mW solid-state laser (light emission at 488 nm) and volumetric counting hardware (34). A conversion factor of 58 fg per cell was used to calculate bacterial biomass from abundance data (26).

Fungal biomass was determined by extracting and quantifying ergosterol, a lipid specific to fungi (35). Briefly, the leaves were freeze-dried and weighed before the lipids were extracted in alkaline methanol (80°C, 30 min) with stirring. The extract was cleaned and concentrated by solid-phase extraction (SPE) (Waters Sep-Pak, Vac RC, tC18, 500 mg; 36). The extraction efficiency was routinely monitored with external ergosterol standards (Fluka, Neu-Ulm, Germany). The extract eluted from the SPE cartridges was purified, and ergosterol was quantified on a high-performance liquid chromatograph (HPLC) consisting of two Jasco PU-980 (Tokyo, Japan) pumps, a Jasco AS-950 autosampler, a LichroSpher 100 RP-18 column (0.46 by 25 cm; Merck Inc., Darmstadt, Germany), and a Jasco MD 2010 Plus multiwavelength detector set at 282 nm. The column temperature was 33°C. A factor of 5.5 mg of ergosterol per g of fungal mass (dry weight) was used to convert ergosterol values to fungal biomass (37).

Fungal sporulation. Sporulation of aquatic hyphomycetes was induced by submerging six pieces of wood small-reed litter or birch leaf discs from each experimental stream in 47 ml filtered (5-μm membrane filters; Sartorius, Göttingen, Germany) Chicken Creek water kept at 10°C with constant shaking (38). Three milliliters of 37% formalin was added after 48 h to preserve the samples. One to 3 ml of the spore suspension was filtered on a membrane filter (5 μm; Millipore, Zug, Switzerland), the filter was placed on a microscope slide, and the spores of aquatic hyphomycetes were stained with 0.1% trypan blue in 60% lactic acid. About 200 spores were identified and counted in 10 to 30 microscopic fields on each filter at a magnification of ×200. Sporulation rates were converted to conidial production based on conidial mass (dry weight) determined for individual species (39).

Fungal and bacterial community fingerprints. Fungal and bacterial communities were also examined by automated ribosomal intergenic spacer analysis (ARISA). DNA from 3 frozen leaf discs or pieces was extracted as previously described for sediment samples (26). DNA was obtained by first mechanically disrupting cells with a bead beater, then enzymatically digesting, and finally purifying the DNA. Purified DNA was stored at −20°C. The intergenic region of rRNA genes was amplified using forward primer 1406F-FAM (5′FAM-TGY ACA CAC CGC CCG T-3′ where FAM stands for 6-carboxyfluorescein and Y is T or C) and reverse primer 23Sr (5′-GGG TTB CCC CAT TCR G-3′, where B is G, T, or C and R is G or A; Microsynth AG, Balgach, Switzerland) for bacteria and forward primer ITS1F-FAM (5′FAM-CTT GGT CAT TTA GAG GAA GTA A-3′) and reverse primer ITS4A (5′CGC CGT TAC TGG GGC AAT CCC TG-3′; Microsynth) for fungi (26). The resulting fragments were separated on a 3130XL capillary genetic analyzer (Applied Biosystems, Rotkreuz, Switzerland). The relative peak areas of the fragments (lengths ranging from 200 to 1,200 bp) were determined, and the profiles obtained from different samples were compared by using the interactive binning script *interactive_binner.r* implemented in the R software environment (40).

Data analysis. Linear mixed-effects models were fitted with the function *lme* of the package *nlme* (41) for the statistical software R (42) to test for differences among litter treatments (i.e., five simulated riparian succession stages) and litter types (wood small-reed versus birch) over time (i.e., three sampling dates). Litter treatment, litter type, and elapsed time were treated as fixed effects with the time variable centered on sampling date 3 after 10 weeks so that estimated intercepts represented the situation at the end of the experiment when effects were expected to be the largest. The experimental stream was treated as a random factor. Response variables (x) were transformed ($\ln(x+1)$) if quantile-quantile (QQ) plots and frequency histograms indicated that residuals did not meet assumptions required for parametric tests. Five linear contrasts reflecting our hypotheses (see above) were calculated to test for differences between specific combinations of litter treatments: treatment 1 versus treatments 2 plus 3 plus 4 plus 5 (contrast A), treatments 1 plus 2 versus treatments 3 plus 4 plus 5 (contrast B), treatment 2 versus treatment 4 (contrast C), treatment 3 versus treatments 2 plus 4 (contrast D), and treatment 4 versus treatment 5 (contrast E).

Nonmetric multidimensional scaling (NMDS) was performed on a matrix containing the relative abundances of each operational taxonomic unit (OTU) detected by ARISA. The goodness of fit of the NMDS is given by stress values. Stress values above 0.2 might indicate unreliable ordinations. The function *meta.mds* of the R package *vegan* (43) was used for this purpose. Calculations were based on Bray-Curtis distances and 1,000 permutations. Permutational multivariate analysis of variance (PERMANOVA) was performed with the function *adonis* in R. The significance of Bray-Curtis distances among centroids of treatment clusters within each community sampled at the same date was assessed among all treatments and also depending on the specific contrasts described above. Environmental factors were fitted in the ordination plot as vectors by using the function *envfit* of the R package *vegan*. The significance of the associations was determined by 1,000 random permutations.

RESULTS

Water and litter chemistry. The concentrations of DOC, NO_3^-, NH_4^+, and PO_4^{3-} were significantly higher after the start of the experiment (i.e., 1 day after inoculation) than 6 to 10 weeks later (see Table S1 in the supplemental material) (25). However, none

TABLE 1 Comparison of the two kinds of leaf litter used to stock experimental streams[a]

Species	Concn (mean ± 1 SD)					C/N/P ratio (atomic)
	Cellulose (mg g^{-1}AFDM)	Lignin (mg g^{-1}AFDM)	ADF (mg g^{-1}AFDM)	N (mg g^{-1} AFDM)	P (mg g^{-1} AFDM)	
Wood small-reed (*Calamagrostis epigejos*)	249.1 ± 24.6	44.4 ± 6.0	424.6 ± 14.8	2.52 ± 0.23	0.12 ± 0.04	891:10:1
Birch (*Betula pendula*)	147.7 ± 11.9	146.3 ± 9.5	298.5 ± 3.7	6.47 ± 0.44	1.10 ± 0.12	154:3:1

[a] Cellulose, lignin, nitrogen, and phosphorus concentrations of leaf litter used to stock experimental streams to mimic five stages of riparian succession at the start of the experiment. Concentrations are means ± 1 SD ($n = 4$). C/N/P ratios are also shown. ADF, acid detergent fiber (cellulose plus lignin plus ash).

of the physicochemical parameters varied among streams receiving different litter treatments (25).

At the start of the experiment, organic matter content of the leaf litter averaged 0.94 and 0.74 g g^{-1} litter (dry weight) for birch and wood small-reed litter, respectively. Birch litter had higher concentrations of nitrogen and phosphorus than wood small-reed litter (Table 1). The lignin concentration in birch litter was also higher than in wood small-reed litter, but the cellulose concentration was only half that in wood small-reed litter, resulting in higher acid detergent fiber (i.e., cellulose plus lignin plus ash) in wood small-reed litter. There were also substantial differences in the carbon-to-nitrogen, carbon-to-phosphorus, and nitrogen-to-phosphorus ratios between the two species (Table 1).

Extracellular enzyme activities. Experimental litter additions to streams had no effect on four of the five enzymes tested (see Table S2 in the supplemental material). The only exception was leucine aminopeptidase (LAP) ($F_{1,10} = 7.44$ and $P = 0.005$), which had a lower potential activity in the simulated open-land stages (no litter or wood small-reed litter only) compared to the streams receiving birch litter (for contrast B, $P = 0.043$). In contrast, all potential enzyme activities clearly differed between wood small-reed and birch litter (Fig. 1 and Table S2). P-acquiring and especially C-acquiring enzymes showed higher potential activities associated with wood small-reed litter compared to birch litter (Fig. 1A to D), whereas the opposite pattern was observed for LAP, an N-acquiring enzyme (Fig. 1E).

Alkaline phosphatase (AP) showed by far the highest potential activity among the enzymes tested (Fig. 1A), reflecting initially low C/P ratios of both litter types (Table 1). Values were consistently higher ($F_{1,60} = 14.7$ and $P < 0.001$) for wood small-reed litter (99.9 to 126.7 nmol MUB h^{-1} g^{-1} AFDM) than for birch litter (71.2 to 104.7 nmol MUB h^{-1} g^{-1} AFDM), though the differences were not large (Fig. 1A). Among the C-acquiring enzymes, potential activities of β-glucosidase (BG) were much higher on wood small-reed (17.1 to 26.4 nmol MUB h^{-1} g^{-1} AFDM) than on birch litter (2.3 to 3.1 nmol MUB h^{-1} g^{-1} AFDM; $F_{1,60} = 231.3$ and $P < 0.001$; Fig. 1B), and similar patterns were apparent for both β-xylosidase (BX) (0.9 to 1.7 and 7.1 to 9.6 nmol h^{-1} g^{-1} for birch and wood small-reed litter, respectively; $F_{1,60} = 237.6$ and $P < 0.001$; Fig. 1C) and β-*N*-acetylglucosaminidase (NAG) (4.0 to 6.3 and 12.7 to 15.3 nmol h^{-1} g^{-1}; $F_{1,60} = 54.3$ and $P < 0.001$; Fig. 1D). These results contrast with the potential activity of LAP, which was consistently higher on birch (9.9 to 18.1 nmol h^{-1} g^{-1}) than on wood small-reed litter (5.1 to 10.2 nmol h^{-1} g^{-1}; $F_{1,60} = 80.6$ and $P < 0.001$; Fig. 1E). The potential activities of phenol oxidase (PO) and phenol peroxidase (PP) were almost undetectable, showing no differences among litter types or experimental streams receiving different litter supplies.

Leaf mass loss. Overall litter mass loss within 10 weeks was

relatively low (26 to 31%) and differed only slightly between birch and wood small-reed litter ($F_{1,58} = 4.63$ and $P = 0.035$; Fig. 2; see Table S3 in the supplemental material). Furthermore, mass loss was similar among litter addition treatments mimicking riparian succession ($F_{4,10} = 0.28$ and $P = 0.88$).

Respiration and biomass and spore production. Microbial respiration differed greatly between birch (62.8 to 74.7 μg C g^{-1} AFDM h^{-1}) and wood small-reed litter (31.5 to 36.5 μg C g^{-1} AFDM h^{-1}; $F_{1,59} = 87.2$ and $P < 0.001$; Fig. 3A), whereas no differences were observed among the litter addition treatments ($F_{4,10} = 0.27$ and $P = 0.89$; see Table S3 in the supplemental material). Similar patterns were apparent for microbial biomass and fungal spore production (Fig. 3 and see Table S3). Average bacterial biomass was nearly twice as high on birch litter than on wood small-reed litter (0.77 to 1.06 and 0.41 to 0.55 mg C g^{-1}
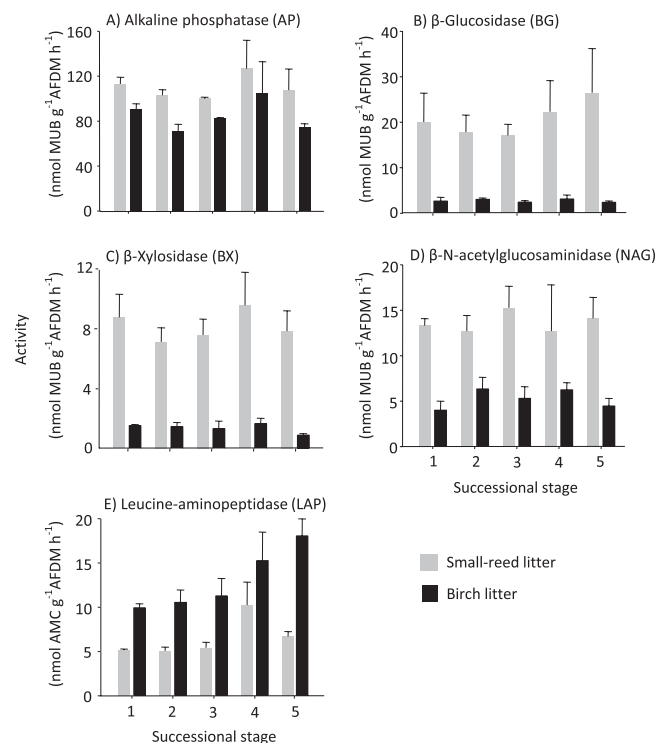

FIG 1 Potential enzyme activities of microbial communities associated with birch and wood small-reed litter exposed in experimental streams supplied with different types and amounts of leaf litter: (i) no litter, (ii) wood small-reed litter, (iii) mixed wood small-reed and birch litter, (iv) birch litter, (v) large amounts of birch litter. Bars depict means plus 1 standard error (SE) (error bars) calculated across streams ($n = 3$) based on aggregated values across sampling dates within each experimental stream.
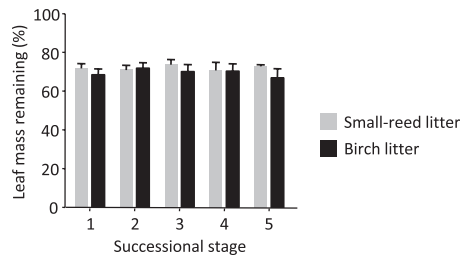
FIG 2 Leaf mass loss of wood small-reed and birch litter within 10 weeks in experimental streams mimicking five stages of riparian succession. Bars depict means plus 1 SE (error bars) calculated across 3 sampling dates in each of three replicate streams ($n = 3$).
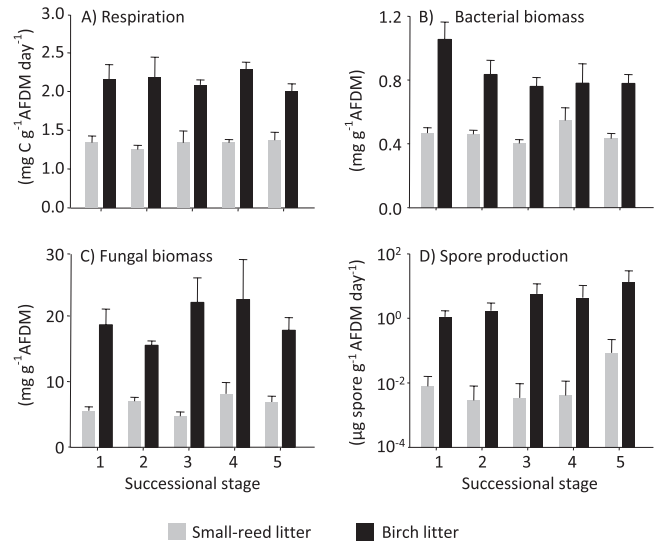

FIG 3 Respiration rate (A), biomass of bacteria (B) and fungi (C), and fungal spore production rate (D) associated with wood small-reed and birch litter in experimental streams mimicking five stages of riparian succession. Bars show means plus 1 SE calculated across 3 sampling dates (one sampling date after 8 weeks for sporulation rate) in each of three replicate streams ($n = 3$).

AFDM, respectively; $F_{1,60} = 112.0$ and $P < 0.001$), but there were no differences among litter addition treatments ($F_{4,10} = 1.73$ and $P = 0.22$; Fig. 3B). A significant interaction between litter type and sampling time ($F_{1,60} = 10.8$ and $P = 0.002$; Fig. 3B) was caused by differences in biomass associated with wood small-reed litter over time ($P = 0.008$). Average fungal biomass differed even 3-fold between litter types (15.6 to 22.6 and 4.8 to 8.2 mg cell dry mass g^{-1} AFDM on birch and wood small-reed litter, respectively; $F_{1,60} = 174.1$ and $P < 0.001$) and showed no differences among litter addition treatments ($F_{4,10} = 1.2$ and $P = 0.39$). Fungal biomass was 7 to 42 times greater than bacterial biomass (Fig. 3B and C). Similar to patterns of microbial biomass, fungal spore production after 8 weeks was 2 orders of magnitude higher on birch (0.95 to 10.8 µg day^{-1} g^{-1} AFDM) than on wood small-reed litter (2.7 to 76.3 ng day^{-1} g^{-1} AFDM; $F_{1,30} = 7.6$ and $P = 0.009$) after 8 weeks of litter decomposition (Fig. 3D). By week 10, however, spore production had declined (0.25 to 1.25 µg day^{-1} g^{-1} AFDM for birch litter; data not shown), resulting in a significant interaction between exposure time and litter type ($F_{1,30} = 4.9$ and $P = 0.03$). There was no difference in spore production on birch or wood small-reed litter among litter addition treatments ($F_{4,10} = 1.19$ and $P = 0.37$; Fig. 3D).

Bacterial and fungal community structure. Bacterial communities on wood small-reed and birch litter varied among litter addition treatments ($F_{4,30} = 2.73$ and 2.51, respectively, and $P = 0.001$ and 0.002, respectively) and sampling dates ($F_{2,30} = 17.1$ and 20.3, respectively, and $P = 0.001$ and 0.002, respectively,) but there was also a significant interaction between the two factors ($F_{8,30} = 1.74$ and 1.50, respectively, and $P = 0.008$ and 0.041, respectively; see Table S4 in the supplemental material). An important difference was also observed between litter types ($F_{1,80} = 6.77$ and $P = 0.001$; Fig. 4A) on each sampling date, though the communities sampled after 6 and 8 weeks were more similar between the two litter types than after 10 weeks (Fig. 4A). The cluster of the communities sampled after 6 and 8 weeks was clearly separated along the first NMDS axis from the communities sampled after 10 weeks. Additionally, the bacterial communities of both clusters were ordered along the second NMDS axis, reflecting the litter addition treatments. Specifically, structure of the communities was influenced by the presence of litter, regardless of the litter type (for contrast A, $F_{1,44} = 1.41$ and 1.47 and $P = 0.002$ and 0.001 for bacteria on birch and wood small-reed litter, respectively; Fig. 5A and C) and the presence of birch as opposed to wood small-reed litter (for contrast B, $F_{1,44} = 1.95$ and 1.60, respectively, and $P = 0.001$; Fig. 5A and C). Furthermore, the bacterial communi-

ties on both wood small-reed and birch litter differed, depending on litter quality (i.e., birch versus wood small-reed litter; for contrast C, $F_{1,44} = 1.42$ and 1.27 and $P = 0.001$; Fig. 5A and C) and litter quantity added to the experimental streams (for birch versus wood small-reed litter for contrast D, $F_{1,44} = 1.21$ and 0.94, respectively, and $P = 0.005$ and 0.032, respectively; Fig. 5A and C). Mixing wood small-reed and birch litter had no effect on the structure of the bacterial community compared to single-species litter treatments (for contrast E, $F_{1,44} = 0.65$ and 0.69 and $P = 0.41$ and 0.20, respectively; Fig. 5A and C).

Fungal communities also differed distinctly between litter types ($F_{1,70} = 2.06$ and $P = 0.002$) and sampling dates (6 and 8 weeks versus 10 weeks; $F_{2,26} = 7.73$ and $F_{2,24} = 4.51$ and $P = 0.001$ for birch and wood small-reed litter, respectively; Fig. 4B), as well as among litter addition treatments ($F_{4,26} = 1.56$ and $F_{4,24} = 2.28$ and $P = 0.019$ and 0.001, respectively; see Table S5 in the supplemental material). Overall, however, these differences were less clearly defined than for the bacterial communities (Fig. 4B). Specifically, composition of the fungal communities colonizing both wood small-reed and birch litter was different when birch litter was added to the streams (for contrast B, $F_{1,37} = 1.82$ and $F_{1,39} = 1.40$ and $P = 0.024$ for both litter types). Moreover, communities on wood small-reed litter were also different in streams deprived of litter or receiving any kind of litter (for contrast A, $F_{1,37} = 1.62$ and $P = 0.034$; for communities sampled after 6 and 8 weeks, Fig. 5B and D) as well as when litter quality differed (birch versus wood small-reed; for contrast C, $F_{1,37} = 1.77$ and $P = 0.022$; Fig. 5B and D). The quantity of birch litter added to the experimental streams, or mixing of wood small-reed and birch litter, had no effects on the structure of the fungal communities.

In general, neither bacterial nor fungal communities were significantly related to potential enzyme activities. An exception was β-xylosidase activity, which was weakly correlated with the ordination of the bacterial community ($r^2 = 0.14$ and $P = 0.022$; Fig. 4A). Several physicochemical parameters were significantly linked
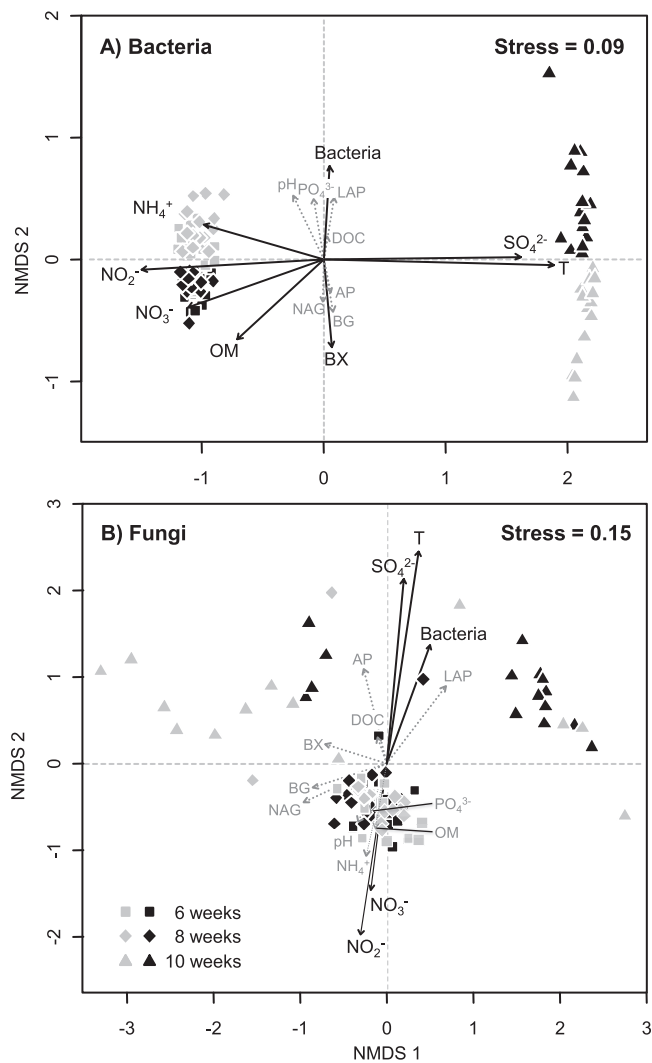
FIG 4 NMDS ordination of bacterial (A) and fungal (B) community structure inferred from ARISA profiles in relation to a suite of potential enzyme activities associated with birch and wood small-reed litter in experimental streams mimicking five stages of riparian succession. Different symbol shapes denote different sampling dates, and birch (black) and wood small-reed (gray) litter are distinguished by the color of the symbol. Black arrows indicate significant correlations of the ordination ($P \leq 0.05$), while potential extracellular enzyme activities and environmental variables and dashed arrows in gray denote variables that were not significantly correlated with the ordination. Abbreviations: T, water temperature; Bacteria, bacterial biomass; DOC, dissolved organic carbon; OM, sediment organic matter content; AP, alkaline phosphatase; BG, β-glucosidase; BX, β-xylosidase; LAP, leucine aminopeptidase; NAG, β-N-acetylglucosaminidase.

to microbial community structure (Fig. 4). The concentrations of NO_3^- and NO_2^- in stream water were correlated with the ordination of both the bacterial communities ($r^2 = 0.38$ and 0.62, respectively, and $P < 0.001$) and fungal communities ($r^2 = 0.17$ and 0.31 and $P = 0.013$ and < 0.001, respectively), with all arrows pointing in the direction of the first two sampling dates (6 and 8 weeks). Furthermore, the concentration of NH_4^+ and sediment organic matter content were significantly correlated with bacterial community structure ($r^2 = 0.28$ and 0.26, respectively, and $P < 0.001$), with arrows also pointing toward the first two sampling dates. In the ordinations with both bacteria and fungi, water tem-

perature ($r^2 = 0.98$ and $P < 0.001$ and $r^2 = 0.48$ and $P < 0.001$, respectively) and SO_4^{2-} concentration ($r^2 = 0.72$ and $P < 0.001$ and $r^2 = 0.99$ and $P < 0.001$, respectively) were almost exactly opposite to the N species, pointing toward the last sampling date (10 weeks). Finally, bacterial biomass was also significantly correlated with bacterial and fungal community structure ($r^2 = 0.16$ and 0.17 and $P = 0.007$ and 0.012, respectively).

DISCUSSION

Effects of litter standing stock on microbial activities. A remarkably clear finding of our experiment is that the simulated successional changes of either plant litter quality or quantity supplied to the experimental streams had little direct effect on litter-associated microbial activities. No differences among streams mimicking different successional stages were detected in terms of bacterial or fungal biomass, fungal spore production rate, microbial respiration, potential activities of all but one of the microbial extracellular enzymes tested, or litter decomposition rate. This general absence of effects is strong evidence suggesting that microbial activities on a given substrate are independent of the quality or quantity of the bulk litter present in a stream. In comparative field studies, litter decomposition rates have been found to vary between forest and pasture streams (44, 45). However, uncertainty in those comparative investigations about variation in environmental variables other than litter quality and quantity prevent unequivocal attribution of the observed effects to litter inputs. This uncertainty was overcome in the present study conducted in simplified but well-controlled experimental streams.

One particular enzyme activity associated with birch litter deviated from the general pattern in that it showed a response to differences in litter additions to the streams. The observed elevated potential activity of leucine aminopeptidase (LAP) in the simulated forest streams containing birch litter suggests increased efforts of microbial communities to acquire nitrogen under these conditions. Dissolved nitrogen concentrations were invariably low in all experimental streams, suggesting that N pools in the litter were important to sustain microbial activity. Furthermore, greater N demands would be expected for litter providing higher-quality carbon, such as birch, compared to the low-quality wood small-reed litter in the streams simulating early riparian succession stages. This difference in requirements could have been crucial in eliciting the greater effort to acquire N from birch litter in our simulated forest streams.

Effect of substrate quality on microbial activities. The observed lack of bulk litter effects in our experimental streams contrasts sharply with consistently large differences in microbial biomass, respiration, and fungal sporulation associated with either wood small-reed or birch litter, independent of the surrounding bulk litter. This pattern reflects distinct quality differences between the two litter types, including higher lignin and nutrient concentrations but lower cellulose and acid detergent fiber contents of birch (Table 1). In comparisons of deciduous tree leaves, high lignin content has often been found to limit both decomposition rate (46) and fungal performance on leaf litter submerged in streams (10). However, in our experiment, it is unlikely that lignin affected microbial decomposition to a significant extent, not only because microbial activity was much greater on the higher-lignin species (birch) but also because potential activities of lignin-degrading enzymes (i.e., phenol oxidase and peroxidase) were weak or nonexistent on either litter type. This might indicate that de-
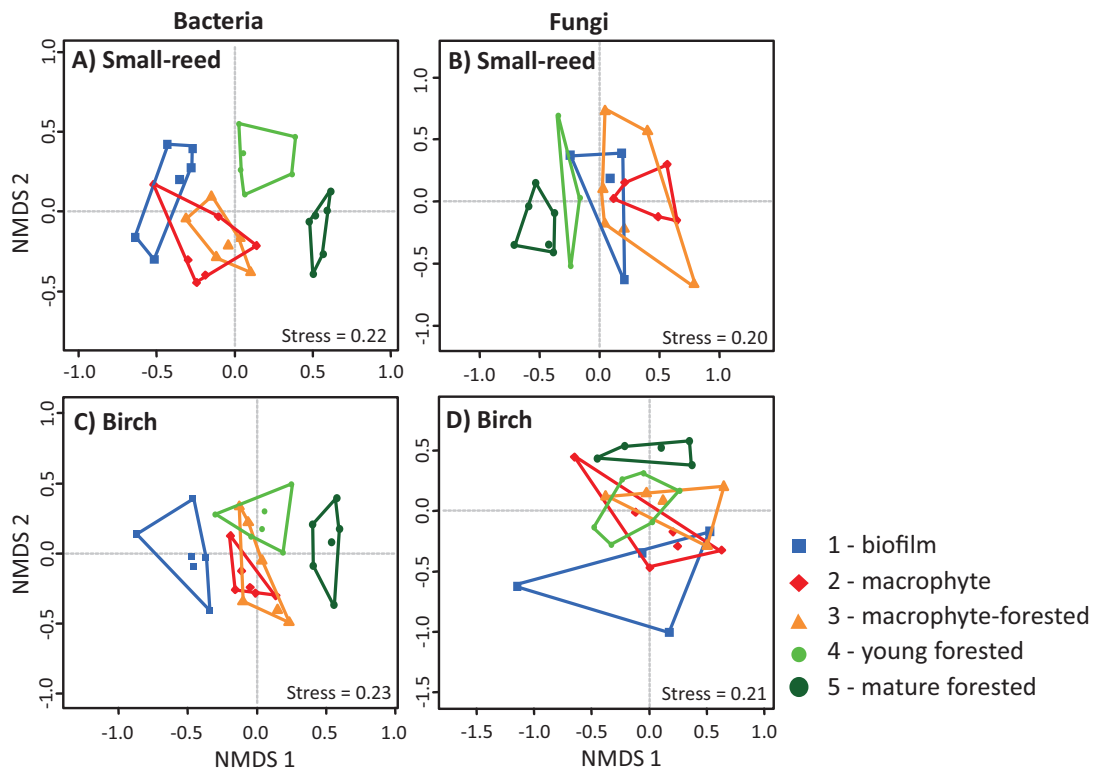
FIG 5 NMDS ordination of bacterial (A and C) and fungal (B and D) communities inferred from ARISA profiles in wood small-reed litter (A and B) and birch litter (C and D) sampled after 6 and 8 weeks from experimental streams mimicking five stages of riparian succession.

composition of both litter types was in an early phase dominated by polysaccharide degradation (47, 48), in accordance with the relatively small proportion of litter mass that was lost by the end of the experiment (Fig. 2). Despite the similar loss of mass, the observed higher fungal and bacterial biomass, as well as respiration, suggests a more effective use of birch litter by the microbial community.

Litter nutrient contents appeared important in affecting microbial activities on both litter types. In particular, 3- to 100-fold-higher potential activities of alkaline phosphatase compared to the other measured enzymes imply that microbial communities on both litter types could have been particularly short of phosphorus (23). Since total dissolved P concentrations in the streams were low, water was unlikely to act as a sufficient nutrient source to compensate for the relative scarcity of phosphorus in the litter. This could lead to phosphorus limitation of the litter-associated microbial communities, as is evident in the higher P concentration and lower potential activities of alkaline phosphatase in birch compared to wood small-reed litter.

Similarly, the C/N ratio of wood small-reed litter was much greater than that of birch litter, pointing to lower nitrogen availability in wood small-reed litter, given that the relative microbial demand compared to the litter C/N ratio is greater for nitrogen than for carbon. Consequently, reduced excretion of the N-acquiring enzyme by the microbial communities on wood small-reed litter was unlikely due to reduced N limitation but to overall lower metabolic activities of these communities. This was also indicated by lower fungal and bacterial biomass, fungal spore production, and microbial respiration observed on wood small-reed

litter (see above). Extracellular enzymes are energetically and nutritionally costly; therefore, the production of nutrient-acquiring enzymes is expected to increase with increasing availability of labile carbon (49). Greater investment in C-acquiring enzymes indicates that labile C was in shorter supply than N on wood small-reed litter, whereas the opposite was true for birch litter (Fig. 1B to E). Thus, overall, it appears that microbial extracellular enzyme production, growth, and reproduction ultimately depend on specific aspects of litter quality that vary in relative importance.

Effect of litter quality on microbial community composition. The observed discrepancies in microbial activities and performance on the two litter types were also reflected in the composition of the fungal and bacterial communities (Fig. 4). This finding seems to conflict with data from field studies on stream microbial communities on decomposing leaves (50, 51) but can be reconciled by considering the stark difference in litter chemistry and structure of wood small-reed and birch leaves (Table 1) compared to two different leaf species of deciduous trees (maple and oak [50]; alder and sycamore [51]). Because the two litter types were not sterilized before use in our experiment, they could have been a source of microbial community dissimilarity observed between the two litter types. However, the dried litter was colonized by terrestrial microbial communities, which tend to be quickly outcompeted by aquatic microbes within days after litter submergence, at least the fungi (52). In view of the large quality differences between wood small-reed and birch litter, it is remarkable, however, that communities varied even more strongly over time than between litter types, specifically between 8 and 10 weeks of litter exposure in the streams. This is particularly evident for the

bacteria (Fig. 4A) and, in part, could also be related to changes in litter chemistry during decomposition, as noted in other studies (48, 51). Alternatively, the shifts may reflect changes in species interactions both within and between the fungal and bacterial communities colonizing leaf litter in our experimental streams (53), which is supported by the observed strong declines in fungal spore production after 8 weeks.

Similar to microbial activities, litter quality and temporal dynamics during litter decomposition were more important than litter quantity for shaping microbial (especially bacterial) communities (Fig. 4). This result is consistent with previous observations of early microbial communities on leaf litter differing in quality (50–52). Nevertheless, in contrast to virtually all of the quantitative microbial variables discussed above (microbial biomass, potential enzyme activities, etc.), there were notable differences in fungal and bacterial community composition among the five successional stages. The observed disconnect between microbial community composition and metabolic activity (i.e., between community structure and function) parallels a previous field survey of sediment microbial communities (32). It suggests that microbial communities on decomposing litter are as effective at exploiting their substrate in streams simulating very early successional conditions (i.e., no litter or only wood small-reed litter present) as in streams receiving litter of higher quality and in greater amounts. This conclusion holds for both deciduous and graminoid leaf litter—and notwithstanding the fact that the type of litter colonized by the microbial communities strongly affects microbial biomass and metabolic activities.

ACKNOWLEDGMENTS

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Applied and Environmental Microbiology

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