



Does mixing litter of different qualities alter stream microbial diversity and functioning on individual litter species?

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We examined effects of leaf litter quality and species mixing on microbial community diversity and litter processing in a forested headwater stream. Single- and mixed-species litter from dominant tree species (*Liriodendron tulipifera*, *Acer rubrum*, *Quercus prinus*, *Rhododendron maximum*) were incubated in a southern Appalachian headwater stream. Litter carbon-to-nitrogen ratios (C:N), mass loss, microbial respiration, and microbial community diversity were analyzed on individual litter species after incubation. Initial C:N varied widely among individual litter species, and these differences persisted throughout the 50-day incubation period. Litter C:N of the recalcitrant species *R. maximum* remained higher than that of all other litter species, and C:N of *R. maximum* and *L. tulipifera* increased when both species were present together in a mixture. Although mass loss of individual species was generally unaffected by mixing, microbial respiration was greater on *A. rubrum* and *Q. prinus* litter incubated with *R. maximum* compared to either species alone. Enhanced resource heterogeneity, which was experimentally achieved by litter mixing low- and higher-quality litter species, resulted in apparent shifts in microbial community diversity on individual litter species. Responses of bacterial and fungal community diversity to litter mixing varied among individual litter species. Our results suggest that changes in tree species composition in riparian forests and subsequent changes in litter resource heterogeneity could alter stream microbial community diversity and function. As bacteria and fungi are important decomposers of plant litter in aquatic ecosystems, resource-dependent changes in microbial communities could alter detrital processing dynamics in streams.

Rapid losses of species worldwide have raised concerns about how ecosystems will function as biodiversity declines (Tilman et al. 2001, Loreau et al. 2002, Hooper et al. 2005). Changes in biodiversity can affect fundamental ecosystem processes, such as primary productivity (Tilman et al. 2001, Hooper et al. 2005) and organic matter decomposition (Gartner and Cardon 2004, Hättenschwiler et al. 2005). Biodiversity effects on decomposition have been shown empirically through resource manipulations of plant leaf litter (Gartner and Cardon 2004, Swan and Palmer 2004, Hättenschwiler et al. 2005, Kominoski et al. 2007), as well as consumer-level manipulations of invertebrates (Jonsson and Malmqvist 2000, Dangles and Malmqvist 2004, Hättenschwiler and Gasser 2005) and microorganisms (Bärlocher and Corkum 2003, Dang et al. 2005, Lecerf et al. 2005, Tiunov and Scheu 2005, Duarte et al. 2006).

Forested headwater streams are ideal environments to test effects of litter diversity on invertebrate detritivores and microbial decomposers and subsequent litter decomposition. Terrestrially derived litter is the dominant organic matter resource in headwater stream ecosystems (Wallace et al.

1997, 1999, Webster et al. 1997, Hall et al. 2001), and consumers in these donor-controlled ecosystems do not directly affect the diversity of allochthonous organic matter resources. In addition, litter species diversity is especially relevant to stream ecosystems because global-scale processes such as climate change, species invasions, and the spread of pathogens are nonrandomly altering plant species composition within riparian communities (Ellison et al. 2005). Shifts in riparian tree species composition will alter litter inputs to streams, which may affect the structure of microbial communities and overall organic matter processing in these ecosystems.

Forested watersheds in the eastern United States provide an excellent case study of these phenomena. Current declines in *Tsuga canadensis*, eastern hemlock, resulting from infestation by an invasive invertebrate pest, *Adelges tsugae*, woolly adelgid, are altering forest communities along the Appalachian Mountains (Orwig et al. 2002). In the southern Appalachians, declines in eastern hemlock could increase the dominance of *Rhododendron maximum*, rhododendron, which is known to inhibit canopy tree seedling establishment (Nilsen et al. 1999, Walker et al. 1999).

Since *R. maximum* is an abundant, low-quality litter species (Webster and Benfield 1986), potential increases in *R. maximum* litter could have structural and functional implications for forested, headwater streams.

Although composition and diversity of riparian tree species influences species diversity of aquatic fungi (Bärlocher and Graça 2002, Laitung and Chauvet 2005, Lecerf et al. 2005), mechanistic explanations remain largely unexplored. Several recent studies have used molecular techniques to characterize microbial communities associated with single-species litter (Das et al. 2007, Nikolcheva et al. 2003, 2005, Mille-Lindblom et al. 2006a). However, no study has tested effects of litter species diversity on microbial community diversity associated with individual litter species, which would enhance our understanding of synergistic and antagonistic effects of litter species diversity on microbial communities. Our previous research showed that bacterial and fungal biomass was lower in mixtures containing *R. maximum* (Kominoski et al. 2007). In contrast, we recorded stimulatory effects of *Liriodendron tulipifera*, tulip poplar, on bacterial and fungal biomass when it was present in mixtures (Kominoski et al. 2007). However, since we measured microbial biomass on composite samples from litter mixtures, we do not know how litter diversity affected microbial communities on individual litter species within a mixture.

Our primary objective was to test the effect of mixing low- and higher-quality litters on microbial community diversity and litter processing among individual litter species. We used molecular techniques to assess bacterial and fungal community diversity. We hypothesized that breakdown dynamics of individual, low- and higher-quality litter species would differ when incubated individually versus in mixtures. Specifically, we predicted that mixing higher- and low-quality litter species would: 1) decrease rates of litter mass loss and microbial respiration of higher-quality species and increase rates of litter mass loss and microbial respiration of low-quality species as compared to individual species incubated alone; and 2) increase microbial community diversity on higher-quality species due to increased resource competition (Tilman 1982) and decrease microbial diversity on low-quality species due to decreased resource competition as compared to individual species incubated alone.

Methods

Study site

Research was conducted in a second-order reach of Ball Creek at Coweeta Hydrologic Laboratory Long-term Ecological Research facility (Coweeta) located in Macon County, North Carolina, USA. Coweeta is a 2185 ha forested basin in the Blue Ridge physiographic province of the southern Appalachian Mountains (Swank and Crossley 1988). Vegetation is mixed hardwood with a dense understory of *R. maximum* that provides year-round shading of streams. Mean monthly air temperature ranges from 3 to 22°C, and mean annual precipitation ranges from 180 cm at low elevations to 250 cm at high elevations (Swift et al. 1988). Mean stream temperature during the study was 9.7°C (range: 4.7 – 16.4°C).

Sampling

We collected litter from four dominant riparian tree species that differ in litter quality (*Acer rubrum* (A), red maple; *L. tulipifera* (L), tulip poplar; *Quercus prinus* (Q), chestnut oak; *R. maximum* (R), rhododendron). After initial dry mass was measured, approximately five grams total of single- (A, L, Q, R) and mixed-species (A+R, L+R, Q+R) litter were placed in plastic mesh litterbags (19.1 × 38.1 cm, 5 × 5 mm mesh) in equal mass proportions. On 17 September 2006, corresponding with peak litterfall, we randomly deployed litterbags (n=35; 7 single- and mixed-species litter treatments × 5 replicates) in blocks along a 75 m reach of Ball Creek. Additional litterbags were used to estimate handling loss and measure initial litter chemistry. We retrieved litterbags after 50 d, and transported them to the laboratory on ice for processing, which was completed within 12 h. Litter was rinsed over sieves to remove sediment and debris. Invertebrates were removed from litterbags and preserved for later identification. These data are not included in this manuscript. Individual litter species within mixtures were removed and analyzed separately, enabling us to analyze effects of litter mixing on breakdown dynamics of individual litter species. Leaf disks (17 mm ø) were removed for microbial respiration and microbial community analyses. Remaining litter was oven-dried at 60°C for 48 h and ball-milled prior to litter chemistry analyses and ash-free dry mass (AFDM) determination.

Litter chemistry and mass loss

Litter carbon and nitrogen ratios (C:N) were measured with a CHN analyzer. Oven-dried litter was weighed and combusted at 500°C for 4 h to estimate AFDM remaining. Litter AFDM was measured by subtracting ash weight from dry weight.

Microbial respiration

We measured microbial respiration in the laboratory as oxygen uptake of incubated litter. Ten leaf disks (17 mm ø) were taken from single-species litter, and 20 leaf disks (10 per species) were excised from litter mixtures. Leaf disks were placed in darkened respiration chambers (30 ml) containing unfiltered stream water, and oxygen concentrations were measured every five min for 30 min (Gulis and Suberkropp 2003). Controls contained only unfiltered stream water. Oxygen consumption was determined as the slope of the regression of oxygen concentration over time, adjusted for unfiltered stream water controls and temperature, and expressed per gram litter AFDM per hour (Gulis and Suberkropp 2003).

Microbial community diversity

Deoxyribonucleic acid (DNA) of bacteria and fungi was isolated from frozen (−80°C) leaf disks (surface area = 453.7 mm²) of individual litter species using an Ultraclean Soil DNA extraction kit. Five replicates of extracted DNA from single- (A, L, Q, R) and mixed-species

(A + R, L + R, Q + R) litter and DNA of bacterial (*Pseudomonas aeruginosa*) and fungal (*Cortinarius multiformis*) reference cultures were amplified with polymerase chain reaction (PCR). Universal primers were used to amplify a fragment of 16S rRNA gene of bacteria and the intergenic transcribed spacer (ITS) region of fungi. Bacterial primer pairs 341F + GC and 534R were used to amplify a 200 bp fragment of the 16S rRNA gene (Muyzer et al. 1993). The ITS region of fungi was amplified with primer pairs ITS3GC (May et al. 2001, Nikolcheva et al. 2005) and ITS4 (White et al. 1990, Nikolcheva and Bärlocher 2005, Nikolcheva et al. 2005). Both forward primers contained GC-clamps at the 5'-end, which ensured separation during denaturing gradient gel electrophoresis (DGGE; Muyzer et al. 1993). Each 50 µl PCR reaction contained 0.4 µM forward primer, 0.4 µM reverse primer, 2.5 mM MgCl₂, 0.2 mg ml⁻¹ bovine serum albumin, 160 µM dNTP, 1 × GoTaq buffer, 3 units GoTaq DNA polymerase, and 3 µl of sample DNA. A 1:1000 and 1:1 dilution of DNA extract was optimal for amplification of bacterial and fungal DNA, respectively. All PCR reactions were performed using a thermocycler and included an initial denaturing step at 94°C for 2 min, followed by 40 cycles of denaturing at 94°C for 1 min, annealing at 56.4°C for 1 min, and extension at 72°C for 1 min. A final extension cycle was run at 72°C for 5 min. Additional extension times have been suggested to eliminate artifactual double bands in DGGE (Janse et al. 2004).

Microbial community diversity from one replicate of extracted DNA from bacteria and fungi was assessed with denaturing gradient gel electrophoresis (DGGE; Muyzer and Smalla 1998). PCR products were loaded onto 6% (fungi) or 8% (bacteria) polyacrylamide gels with 20 to 60% urea-formamide denaturing gradients and electrophoresed at 60 V for 14 h. After electrophoresis, gels were removed and stained with GelStar nucleic acid stain (1:10 000 dilution), and imaged with a digital gel imaging system. Bacterial and fungal ribotypes, corresponding to distinct DNA base sequences, were represented as bands on the polyacrylamide gel (Nikolcheva et al. 2003, Das et al. 2007). Bands were identified across lanes and band intensity values for each band were recorded using Gel Doc imaging software. The number of distinct bands (i.e. DNA ribotypes) was used as a surrogate for bacterial and fungal community diversity.

Statistical analyses

Initial C:N was compared among individual litter species using one-way analysis of variance (ANOVA) and post hoc comparisons (Tukey's HSD). Linear regressions were used to assess relationships among litter mass loss, litter C:N, and microbial respiration after 50 d incubation. We used two-way ANOVA and Tukey's HSD to compare litter C:N, mass loss, and microbial respiration rates of individual, higher-quality litter species (*A. rubrum*, *L. tulipifera*, *Q. prinus*), incubated alone and in mixtures with *R. maximum*. We used ANOVA and Tukey's HSD to compare the effects of mixing higher-quality litter species (*A. rubrum*, *L. tulipifera*, *Q. prinus*) on litter C:N, mass loss, and microbial respiration rates of *R. maximum*. Analyses were conducted with SAS

ver. 9.1 with an alpha (type I error rate) of 0.05. Assumptions of normality of residuals were met for all analyses (Shapiro–Wilkes test).

Results

Litter species incubated individually

Litter C:N was different among individual species both initially ($F_{3,4} = 37.6$, $p = 0.002$; Table 1) and after 50 days of incubation ($F_{3,16} = 62.7$, $p < 0.001$; Table 1). Among all four tree species, leaf litter C:N ratios decreased after 50 days of individual incubation in Ball Creek (Table 1), but C:N of *R. maximum* litter remained higher than all other litter species ($p < 0.05$; Table 1). For leaf species incubated individually, there was a negative relationship between day 50 litter C:N and percent AFDM lost ($r^2 = 0.34$, $p < 0.01$). However, there were no relationships between microbial respiration and day 50 litter C:N ($r^2 = 0.05$, $p > 0.10$) or percent AFDM lost ($r^2 = 0.15$, $p > 0.10$).

Effects of mixing low- and higher-quality litter

Litter mass loss

Mass loss among higher-quality litter from single-species litterbags was different ($F_{2,20} = 25.6$, $p < 0.001$) but was not dependent on mixing with *R. maximum* ($F_{2,20} = 0.63$, $p = 0.54$). Specifically, *A. rubrum* and *L. tulipifera* lost more mass than *Q. prinus* (Tukey's $p < 0.05$) from single-species and mixtures with *R. maximum* (Fig. 1A). Despite differences among higher-quality litter being independent of the presence of *R. maximum*, mass loss of *Q. prinus* mixed with *R. maximum* was greater than *Q. prinus* incubated alone (Fig. 1A).

There was no effect of mixing higher-quality litter on mass loss of low-quality *R. maximum* ($F_{3,16} = 0.45$, $p = 0.72$; Fig. 1B).

Litter C:N

Differences in litter C:N among higher-quality species was dependent on the presence/absence of *R. maximum* ($F_{2,20} = 3.64$, $p = 0.045$; Fig. 2A). In single-species litterbags, C:N was lowest for *L. tulipifera*, but *Q. prinus* had the lowest C:N of species mixed with *R. maximum* (Tukey's HSD, $p < 0.05$; Fig. 2A).

Litter C:N of *R. maximum* was higher when incubated in mixtures with some higher-quality litter species ($F_{3,16} = 3.77$, $p = 0.03$; Fig. 2B). Specifically, a mixture of *R.*

Table 1. Carbon to nitrogen ratio (C:N) per litter ash-free dry mass for individual litter species at day 0 and after 50 days of incubation. Values are means (± 1 SE). Differences in lower case letters denote significant ($p < 0.05$) differences among species on the same day species using ANOVA. Ratios calculated as mass.

Species	C:N	
	Day 0	Day 50
<i>Acer rubrum</i>	69.0 (2.9) ^a	43.2 (1.6) ^a
<i>Liriodendron tulipifera</i>	45.8 (2.9) ^a	35.4 (1.0) ^a
<i>Quercus prinus</i>	47.0 (8.3) ^a	40.7 (3.5) ^a
<i>Rhododendron maximum</i>	110.1 (3.1) ^b	80.3 (3.3) ^b

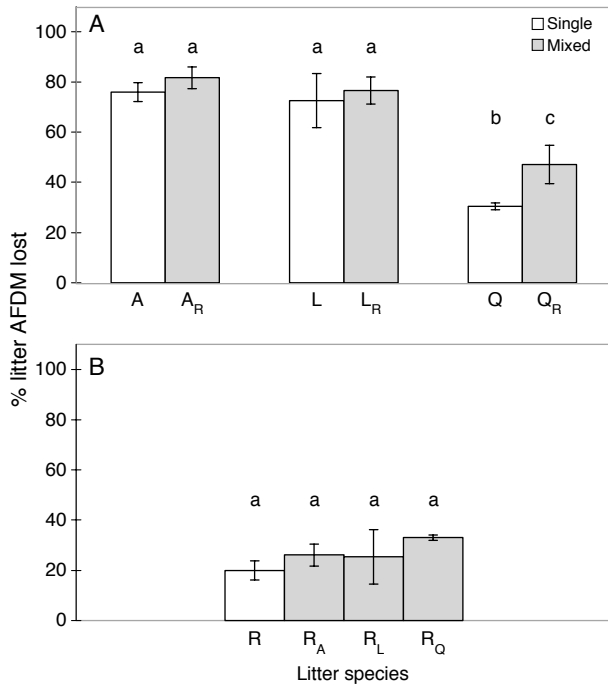


Figure 1. Percent litter ash-free dry mass (AFDM) lost from individual (A) higher-quality litter species (A = *Acer rubrum*; L = *Liriodendron tulipifera*; Q = *Quercus prinus*) and (B) low-quality *Rhododendron maximum* (R) after 50 days incubation as single species and mixtures. For mixtures, the subscript letter corresponds to the litter species that was mixed with the individual litter species of interest. Values are means (± 1 SE). Different lowercase letters denote significant differences with ANOVA and Tukey's HSD.

maximum with *L. tulipifera* had higher C:N than *R. maximum* alone ($p < 0.05$; Fig. 2B).

Litter-associated microbial respiration

Differences in rates of microbial respiration among higher-quality litter species were dependent on the presence of *R. maximum* ($F_{2,20} = 4.18$, $p = 0.038$, Fig. 3A). When *R. maximum* was present, rates of microbial respiration were greater for *A. rubrum* and *Q. prinus* but not for *L. tulipifera* (Tukey's HSD, $p < 0.05$; Fig. 3A).

Mixing higher-quality litter with *R. maximum* had no effect on its associated microbial respiration ($F_{3,11} = 2.42$, $p = 0.12$; Fig. 3B).

Bacterial and fungal communities

DGGE analyses detected differences in bacterial and fungal communities on individual litter species from single- and mixed-species treatments (Supplementary material Appendix 1). The highest number of bacterial DNA ribotypes (e.g. DNA bands) was observed on *L. tulipifera* and *R. maximum* when both were in mixed together (Fig. 4A); however, *L. tulipifera* and *R. maximum* from single-species litterbags contained the lowest bacterial diversity (Fig. 4A). Bacterial diversity was greater for *R. maximum* when it was incubated with higher-quality litter (*A. rubrum*, *L. tulipifera*, *Q. prinus*; Fig. 4A). When *R. maximum* was present, bacterial diversity associated with *A. rubrum* was lower, but higher for

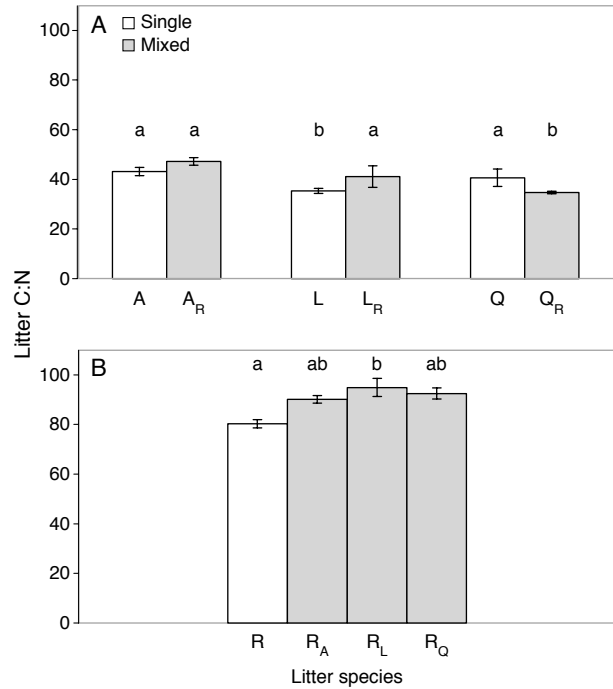


Figure 2. Litter C:N (based on mass) of individual (A) higher-quality litter species (A = *Acer rubrum*; L = *Liriodendron tulipifera*; Q = *Quercus prinus*) and (B) low-quality *Rhododendron maximum* (R) after 50 days incubation as single species and mixtures. For mixtures, the subscript letter corresponds to the litter species that was mixed with the individual litter species of interest. Values are means (± 1 SE). Different lowercase letters denote significant differences with ANOVA and Tukey's HSD.

L. tulipifera and *Q. prinus* (Fig. 4A). Among single species, fungal DNA bands were greatest on *R. maximum* (Fig. 4B), and fungal diversity associated with *R. maximum* was greater when it was incubated with *L. tulipifera* (Fig. 4B). Fungal diversities associated with *A. rubrum* and *Q. prinus* litter were not affected by mixing with *R. maximum*, and *L. tulipifera* litter had lower fungal diversity in the presence of *R. maximum* (Fig. 4B). Fungal diversity associated with *R. maximum* appeared slightly inhibited by the presence of *A. rubrum* and *Q. prinus* in litter mixtures (Fig. 4B).

Discussion

Enhanced resource heterogeneity, achieved by mixing litter of different litter qualities, resulted in variable effects on microbial communities associated with individual litter species. For leaf litter incubated individually, there was a clear relationship between litter quality, defined here as C:N ratio, and litter mass loss. Higher-quality litter (lower C:N ratio) showed higher rates of mass loss (Fig. 1A–B). Presence of *R. maximum* had variable effects on litter quality, mass loss, and microbial respiration of higher-quality species compared to single-species incubations. Similarly, *R. maximum* quality, mass loss, and associated microbial respiration were affected by higher-quality litter species present in mixtures. In general, presence of higher-quality litter species decreased *R. maximum* litter quality but had no effects on its mass loss or associated microbial

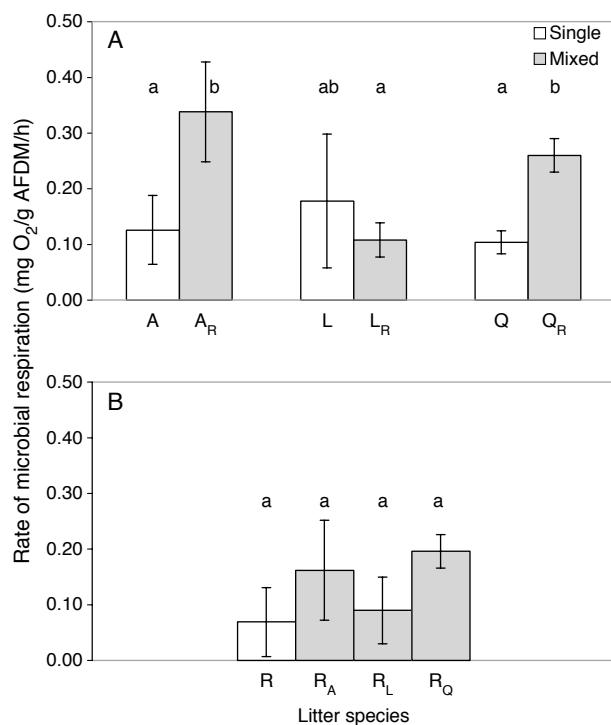


Figure 3. Rates of microbial respiration associated with individual (A) higher-quality litter species (A = *Acer rubrum*; L = *Liriodendron tulipifera*; Q = *Quercus prinus*) and (B) low-quality *Rhododendron maximum* (R) after 50 days incubation as single species and mixtures. For mixtures, the subscript letter corresponds to the litter species that was mixed with the individual litter species of interest. Values are means (± 1 SE). Different lowercase letters denote significant differences with ANOVA and Tukey's HSD.

respiration. Despite the lack of a strong effect of litter mixing on mass loss of individual species, our results partially support our hypotheses of changes in microbial respiration and apparent shifts in microbial community diversity on higher-quality litter species from mixtures with low-quality *R. maximum*. Overall, litter mixing had stronger effects on microbial respiration and community diversity than litter C:N and mass loss.

Mixing *R. maximum* with higher-quality litter species also resulted in patterns contrary to our predictions. We hypothesized that mixing *R. maximum* with higher-quality litter would decrease rates of litter mass loss and microbial respiration associated with the higher-quality species relative to either species incubated alone. We based this hypothesis on results of our previous study that found apparent microbial inhibition by *R. maximum* litter (Kominoski et al. 2007). However, when we examined individual species litter within mixtures, we found that *R. maximum* presence increased microbial respiration on two of the three higher-quality litter species (Fig. 3A) and increased the rate of litter mass loss for one litter species (Fig. 1A). These results indicate that within a mixed-species leaf pack, the presence of a rigid, recalcitrant litter species such as *R. maximum* may have important structural contributions that affect the resource utilization of individual litter species by microorganisms. For example, *R. maximum* may increase dissolved oxygen availability within leaf packs by permitting greater interstitial space between individual leaves. Higher

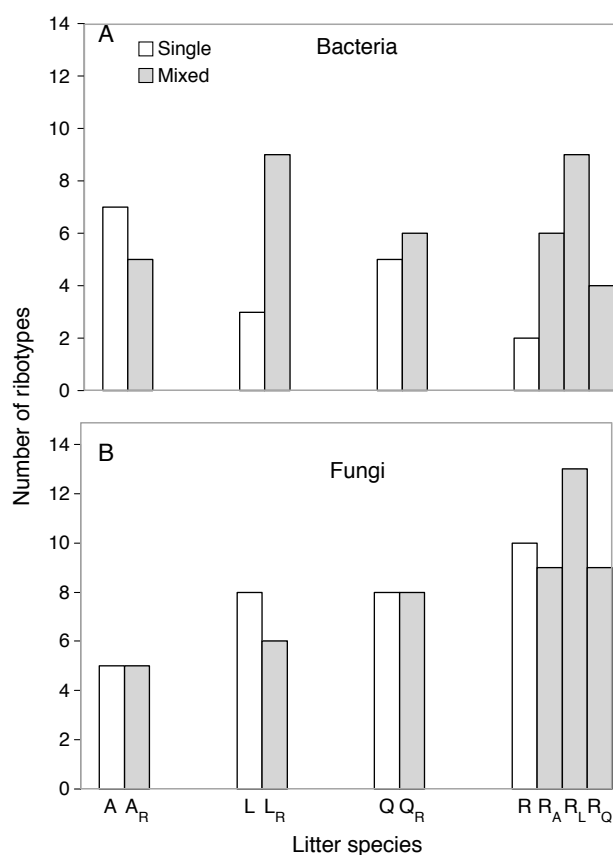


Figure 4. Bacterial (A) and fungal (B) DNA ribotype richness (n) from denaturing gradient gel electrophoresis (DGGE) analyses of individual species litter (A = *Acer rubrum*; L = *Liriodendron tulipifera*; Q = *Quercus prinus*; R = *Rhododendron maximum*) from single-species and mixtures. For mixtures, the subscript letter corresponds to the litter species that was mixed with the individual litter species of interest.

oxygen availability, as determined by the architecture of leaf packs, may explain the higher microbial respiration rates we observed for mixed-species leaf packs containing *R. maximum*.

Detritus increases food web stability and supports consumer biodiversity because it can be a persistent resource (Moore et al. 2004). Enhanced detrital heterogeneity, which we experimentally achieved through mixing *R. maximum* with higher-quality litter species, resulted in apparent shifts in microbial community diversity on individual litter species. Bacterial and fungal community diversity was higher on *R. maximum* compared to higher-quality litter species, and microbial diversity on individual litter species in mixtures with *R. maximum* responded variably (Fig. 4A–B). Stream microbial communities appear to respond to litter mixing more than litter quality. These results represent a unique contribution to the literature, because most studies of stream microbial communities associated with litter in headwater streams have been done with single-species litter (Nikolcheva et al. 2003, 2005, Nikolcheva and Bärlocher 2005, Das et al. 2007). These studies have emphasized the importance of individual litter species characteristics on microbial communities and litter breakdown. However, our results suggest that litter mixing could have bottom-up effects on microbial communities and their influence on ecosystem processes.

Further, as in situ leaf packs are commonly comprised of mixed-species assemblages (Swan and Palmer 2004), understanding the ecological importance of mixed-species litter is crucial for placing the results of litter breakdown experiments in the context of real-world ecological relevance.

Our study shows mixing low- and higher-quality litter can stimulate microbial respiration. It is unclear whether this is due to a change in total activity of similar microbial communities across experimental combinations of leaf litter, or due to differences in microbial community structure. Although changes in bacterial and fungal biomass related with litter chemistry (Webster and Benfield 1986, Gulis and Suberkropp 2003, Kominoski et al. 2007) and litter diversity (Kominoski et al. 2007) have been reported in the literature, biomass is a static measurement and may not fully integrate microbial response to resource quality during breakdown. In contrast, respiration is a measure of overall heterotrophic microbial activity and is a functional metric of microbial communities. In this study, respiration appeared to be linked with litter quality and litter mixing, as microbial respiration increased on higher-quality litter when *R. maximum* was present. Because respiration rates quantify the activity of a broad range of biofilm constituents (i.e. all aerobic organisms), future understanding of the ecological importance of litter mixing may benefit by measuring ecosystem processes that display greater variability among biofilm organisms, such as extracellular enzyme activity, nutrient transformation rates, or microbial production estimates.

Potential mechanisms for differential litter-mixing effects on microbial community diversity and processing of individual litter species include niche partitioning, resource competition (Tilman 1982), and competitive interactions between bacteria and fungi (Mille-Lindblom et al. 2006b, Romani et al. 2006). Close proximity of bacteria and fungi on substrates can result in synergistic and antagonistic interactions. For example, bacterial growth on decomposing litter is enhanced by the presence of fungi, but bacteria can also inhibit fungal growth (Romani et al. 2006). Antagonistic interactions between bacteria and fungi have been linked to litter substrate competition (Mille-Lindblom et al. 2006b). However, in our study, the number of fungal ribotypes was higher than bacteria on all litter, except *L. tulipifera* mixed with *R. maximum*. We observed apparent synergistic effects of litter mixing on microbial diversity associated with *R. maximum* and only slight, apparent antagonistic effects on higher-quality litter mixed with *R. maximum*. Litter mixing appears to enhance microbial community diversity on low-quality *R. maximum*. Therefore, it is possible that bacteria and fungi on *R. maximum* from mixtures are competing for resources with bacteria and fungi on higher-quality litter (Tilman 1982).

Our findings expand on previous studies that used molecular techniques to estimate stream microbial communities colonizing litter during breakdown (Nikolcheva and Bärlocher 2005, Nikolcheva et al. 2005, Mille-Lindblom et al. 2006a, Das et al. 2007). Das et al. (2007) did not observe differences in bacterial and fungal communities colonizing individual litter species with different chemical qualities. The microbial communities in their study system were dominated by generalist taxa, and they concluded that interspecific differences in litter breakdown were explained by leaf toughness and microbial

activity (Das et al. 2007). We observed apparent differences in microbial community diversity (Fig. 4A–B) among individual litter species from single-species and mixtures. Although microbial communities on individual litter species were likely influenced by litter quality and, as our data suggest, litter mixing, we observed no significant functional effects (e.g. litter mass loss or microbial activity) attributed to changes in microbial community diversity. Our results represent qualitative analyses of bacterial and fungal communities associated with single- and mixed-species litter, and it should be noted that finer resolution techniques, such as cloning and DNA sequencing could have allowed us to further identify taxa associated with DNA ribotypes (Supplementary material Appendix 1). In addition, we recognize the methodological limitations of DGGE, such as lack of detectability of low abundance taxa and difficulties with gel replication. These are realistic limitations to the current methodology that will likely be improved upon with future technological advances. Despite these limitations, our results suggest that litter mixing effects stream microbial community diversity and activity associated some individual litter species.

Environmental changes within riparian ecosystems are altering species diversity on a global scale, the structural and functional implications of which need to be further tested. As species losses and composition shifts are likely to be nonrandom (Huston et al. 2000, Tilman and Lehman 2001), we have the ability to test effects of predicted species composition shifts on ecosystem function. For example, loss of eastern hemlock *Tsuga canadensis* in eastern US forests will alter riparian tree community composition (Orwig et al. 2002), and it is probable that *R. maximum*, where present, will increase (Ellison et al. 2005). Our results suggest that changes in riparian tree species composition resulting in increased dominance of *R. maximum* may alter stream microbial community structure and function through changes in litter species composition and subsequent species interactions.

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