Effects of riparian plant diversity loss on aquatic microbial decomposers become more pronounced at longer times

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Abstract

We examined the potential long-term impacts of riparian plant diversity loss on diversity and activity of aquatic microbial decomposers. Microbial assemblages were obtained in a mixed-forest stream by immersion of mesh bags containing three leaf species (alder, oak and eucalyptus), commonly found in riparian corridors of Iberian streams. Simulation of species loss was done in microcosms by including a set of all leaf species, retrieved from the stream, and non-colonized leaves of three, two or one leaf species. Leaves were renewed every month throughout 6 months, and microbial inoculum was ensured by a set of colonized leaves from the previous month. Microbial diversity, leaf mass loss and fungal biomass were assessed at the 2nd and 6th month after plant species loss. Molecular diversity of fungi and bacteria, as the total number of operational taxonomic units per leaf diversity treatment, decreased with leaf diversity loss. Fungal biomass tended to decrease linearly with leaf species loss on oak and eucalyptus, suggesting more pronounced effects of leaf diversity on lower quality leaves. Decomposition of alder and eucalyptus leaves was affected by leaf species identity, mainly after long time of diversity loss. Leaf decomposition of alder decreased when mixed with eucalyptus, while decomposition of eucalyptus decreased in mixtures with oak. Results suggest that effects of leaf diversity on microbial decomposers depended on leaf species number and also on which species were lost from the system, especially after longer times. This may have implications for the management of riparian forests to maintain stream ecosystem functioning.

Keywords: leaf decomposition, litter diversity, litter quality, microbial diversity, streams

Introduction

Human activities are affecting freshwater ecosystems worldwide leading to irreversible changes in biotic communities and the processes they support [1, 2]. A key ecological process in freshwaters is plant-litter decomposition, which is driven by microorganisms and invertebrate detritivores [3, 4]. Both aquatic fungi and bacteria play a key role in organic-matter decomposition by converting plant litter into a more nutritious food source for invertebrate detritivores [5]. Fungi have been recognized as the major microbial decomposers in streams accounting for more than 90% to the total biomass production on decomposing leaves [6]. However, the role of bacteria cannot be neglected because its contribution to plant-litter decomposition increases along time as smaller-size detritus are being produced [7, 8]. Protozoa (e.g., ciliates) can exert a top-down predation pressure on aquatic bacterial communities [9]; however, its role in plant-litter decomposition remains practically unexplored (but see [10, 11]).

Due to the importance of plant-litter decomposition and its tractability in field and microcosm experiments, the scientific community is increasingly using this process to better understand the relationships between biodiversity and ecosystem functioning in freshwaters [12, 13]. In fact, several studies have provided evidence of how leaf-litter decomposition is shaped by the diversity of resources [14] and of consumers (fungi [15-17]; invertebrates [18-20]).

Several studies indicate that composition and diversity of riparian plant species influence the diversity of aquatic fungi [14, 21]. The quality of plant-litter mixtures can also influence microbial biomass; for instance, the presence of high quality leaves of *Liriodendron tulipifera* in litter mixtures led to an increase of fungal and bacterial biomasses, while the presence of low quality leaves of *Rhododendron maximum* led to an opposite effect [22]. Most studies have focused on composite samples in litter

mixtures (but see [23]), but examining the effects of litter diversity loss within individual litter species might help to better understand the contribution of individual litter species for overall diversity effects on litter decomposition.

Microbes have faster growth rates than other organisms involved in plant-litter decomposition in streams, such as invertebrate detritivores. Generally, maximum doubling times of fungi on leaf litter decomposing in streams range from 5 to 50 days [24]. Therefore, microbes can have several generations throughout plant-litter decomposition, and may show strong responses to alterations in litter diversity at relatively short-time scales.

The aim of this study was to examine how aquatic microbial decomposers respond to riparian plant diversity loss. We used a microcosm approach to monitor the development of leaf-associated microbial assemblages during 6 months after inducing the loss of plant species from the system. Microbial assemblages were obtained in a mixed-forest stream by immersion of a pool of three leaf species (alder, oak and eucalyptus) commonly found in the riparian corridors of Iberian streams. After 2 and 6 months, we assessed leaf mass loss, fungal biomass, and diversity of fungi, bacteria and ciliates associated with individual leaf species. We expected that plant-litter mixtures, containing litter species with different chemical composition, would provide better resources to support higher microbial diversity and/or activity. We also expected that any constrain to microbial biomass development or leaf decomposition of lower-quality leaf species could be overcome by the presence of high quality leaf species in mixtures.

Methods

Microbial colonization of leaves in a stream

In October 2009, leaves from alder (*Alnus glutinosa* (L.) Gaertn.), oak (*Quercus robur* L.) and eucalyptus (*Eucalyptus globulus* Labill.) were collected from trees immediately before abscission and dried at room temperature. Leaves were soaked in deionised water, cut into disks, and sets of 12 leaf disks (four disks per plant species) were placed in fine-mesh bags (0.5-mm diameter pore size). On 28 October 2009, 28 bags containing leaf species mixtures were immersed in a mixed-forested stream in Portugal, the Estorãos stream (8.63800°W, 41.78194°N), to allow microbial colonization.

At the study site, the stream was about 0.5 m deep and 2.5 m wide, the stream bed was constituted by rocks, pebbles and gravel, and the riparian vegetation was dominated by *A. glutinosa*, *Q. robur* and *E. globulus*. During leaf colonization, stream water had on average (±SEM) a temperature of 14 ± 1.0 ºC, a pH of 5.9 ± 0.06, a conductivity of 31 µS cm^{-1} and a redox potential of 51 ± 1.5 mV (Multiline F/set 3 no. 400327; WTW, Weilheim, Germany). Nutrient concentrations in the stream water were 0.30 ± 0.04 mg L^{-1} of N-NO_3^-, 0.003 ± 0.000 mg L^{-1} of N-NO_2^-, <0.01 mg L^{-1} of N-NH_3 and <0.003 P-PO_4^{3-} (HACH kit, programs 351, 371, 385, and 490, respectively; HACH, Loveland, CO, USA).

Microcosm setup

After two weeks of stream immersion, mesh bags containing mixtures of alder, oak and eucalyptus leaves were brought to the laboratory. Simulation of leaf species loss was done in microcosms (500 mL Erlenmeyer flask with sterile stream water) containing non-colonized disks of three leaf species (30 disks per species, ome treatment), two leaf species (45 disks per species, three treatments) or one leaf species (90 disks, three treatments) enclosed in mesh bags, and 12 leaf disks (four per species) retrieved from the stream as microbial inoculum. After 30 days, 12 leaf disks of each microcosm with

treatments of three, two or one leaf species were kept as inoculum, and the remaining leaf disks were replaced by non-colonized leaf disks keeping leaf species treatments. This procedure was repeated every 30 days during 6 months. Four replicates were used per treatment. Microcosms were kept, aseptically, under aeration, with artificial light at 16 ºC, and stream water was renewed every 15 days. After 2 and 6 months, leaf disks were used to assess leaf mass loss, fungal biomass, and fungal, bacterial and ciliate diversity by denaturing gradient gel electrophoresis (DGGE), after PCR amplification of microbial DNA with specific primers targeting each microbial group.

Leaf mass loss

Leaf disks from all microcosms of each individual leaf species were freeze-dried for two days and weighed to the nearest 0.01 mg. Mass loss of each leaf species was expressed as percentage of the respective initial dry mass.

Fungal biomass

Fungal biomass was estimated from ergosterol concentration associated with decomposing leaf disks according to Gessner [25]. Briefly, lipids from each individual leaf species were extracted from sets of 6 leaf disks by heating (80 ºC for 30 minutes) in 8 g L^{-1} KOH-methanol. The lipid extract was purified by solid-phase extraction (Sep-Pak cartridges, Waters, Milford, MA) and ergosterol was quantified by high performance liquid chromatography (Beckmann Gold System, Brea, CA, USA), at 282 nm, using a LiChrospher RP18 column (250×4 mm, Merck). The system was run isocratically with methanol as mobile phase (1.4 mL min^{-1}, 33 ºC).

Microbial diversity

DNA was extracted from 4 freeze-dried disks of each leaf species with a soil DNA extraction kit (MoBio Laboratories, Solana Beach, California), according to the manufacturer instructions. The ITS2 region of fungal ribosomal DNA (rDNA) was amplified with the primer pair ITS3GC and ITS4 [26]; V3 region of 16S bacterial rDNA was amplified with the primer pair 338GC and 518 [26]; and 18S rDNA of ciliates was amplified with the primer pair 984GC and 1147 (adapted from [27]). For PCR of fungal, bacterial and ciliate DNA, 2x of GoTaq® Green Master Mix (Promega), 0.4 μM of the appropriate primers and 1 to 10 μL of DNA (1-10 ng μL^{-1}) were used in a final volume of 25 μL.

PCRs were carried out in a MyCycler Thermal Cycler (BioRad Laboratories, Hercules, CA, USA). The PCR program for bacteria and fungi was: initial denaturation at 95 ºC for 2 min; 36 cycles of denaturation at 95 ºC for 30 s; primer annealing at 55 ºC for 30 s and extension at 72 ºC for 1 min; and final extension at 72 ºC for 5 min [26]. The PCR program for ciliates was: initial denaturation at 94 ºC for 5 min; 30 cycles of denaturation at 94 ºC for 45 s; primer annealing at 55 ºC for 60 s and extension at 72 ºC for 90 s; and final extension at 72 ºC for 7 min [27].

DGGE analysis was performed using a DCode^{TM} Universal Mutation Detection System (BioRad Laboratories, Hercules, CA, USA). For fungi, 700 ng of the amplified DNA products with 380-400 bp were loaded on 8% (w/v) polyacrylamide gel in 1x Tris-acetate-EDTA (TAE 1x) with a denaturing gradient from 30 to 70% (100% denaturant corresponds to 40% formamide and 7 M urea). For bacteria, 700 ng of the amplified DNA products with ca. 200 bp were loaded on 8% (w/v) polyacrylamide gel in 1x TAE with a denaturing gradient from 40 to 75%. For ciliates, 700 ng of the amplified DNA products with 750-800 bp were loaded on 6% (w/v) polyacrilamide gel in 1x TAE with a denaturing gradient from 30 to 42.5%. Fungal and bacterial DNA was separated at 55

V and 56 ºC, while ciliate DNA was separated at 80 V and 60 ºC. All gels were run for 16 h. Gels were stained with 1x of GelStar (Lonza) for 10 min, and gel images captured under UV light in a gel documentation system (GenoSmart; VWR).

Nutrient content in leaves

To estimate initial carbon and nitrogen in leaves, samples of alder, oak and eucalyptus leaves were grounded with a ball mill and ca.100 mg of powdered leaf material was analyzed in a LECO-CNS 2000, using EDTA as a standard. Analyses were done in C.A.C.T.I. – Centro de Apoio Científico e Tecnolóxico á Investigación – University of Vigo, Spain.

Initial quality of leaves as C:N ratio differed between the three leaf types as follows: alder (13.29 ± 0.26) < oak (19.69 ± 0.71) < eucalyptus (30.51 ± 0.26).

Statistical analyses

DGGE gels of microbial DNA were aligned and normalized using Gelcompar II (Applied Maths, Sint-Martens-Latem, Belgium), and each DGGE band was considered an operational taxonomic unit (OTU).

Linear regressions were used to establish the relationships between leaf species number and total number of OTUs of each microbial group per leaf treatment. The distribution of fungi, bacteria and ciliates associated with leaf species identity and number at each time (2 and 6 months) was analysed by Correspondence Analysis (CA, [28]) downweighting the contribution of rare species. Data of fungal, bacterial and ciliates communities' structure were based on OTUs, as the relative intensity of each band in DGGE fingerprinting. Data was square root transformed.

For each leaf type, a three-way nested ANOVA was used to test if leaf species number, leaf species identity (nested within species number) and time after diversity loss significantly affected leaf mass loss and fungal biomass [29]. In punctual cases, in which diversity effects were marginally significant, a two-way nested ANOVA was done, testing for the effects of leaf species number and identity (nested with leaf species number) at each time separately. Because the experimental design was unbalanced, we applied Type III analyses of variance using the Variance Estimation and Precision (VEPAC) module in Statistica 8.0 (Statsoft, Tulsa, OK, USA). Differences between treatments were analysed using the Tukey-Krammer´s post-test, which is a modification of the Tukey´s post-test for unbalanced number of samples [29]. Linear regressions were used to establish the relationships between leaf species number and fungal biomass for each leaf species.

Linear regressions were done in Prism 4.0 for Windows (GraphPad software Inc., San Diego, CA), analyses of variance were done in Statistica 8.0 for Windows (Statsoft, Inc., Tulsa, OK) and multivariate analyses were done in CANOCO 4.5 for Windows (Microcomputer Power, Ithaca, NY).

Results

Effects of plant litter diversity on microbial diversity

Molecular diversity of microbial communities on decomposing leaves showed a total of 41, 64 and 29 operational taxonomic units (OTUs) for fungi, bacteria and ciliates, respectively (Fig. 1). In a general way, communities of fungi, bacteria and ciliates on each leaf species differed between single- and mixed-species treatments (Fig. 1 and Fig. 2). The number of fungal OTUs on individual leaf species was higher in mixed-species treatments than in single species treatments, particularly at the longer time, i.e. after 6

months of leaf species loss (Fig. 2). Conversely, bacterial diversity on individual leaf species was generally higher in single-leaf species treatments than in mixed-species treatments (e.g. 46 OTUs on oak alone versus 35-38 OTUs on mixtures, after long time of leaf diversity loss). The diversity of ciliates appeared to decrease with time because lower number of OTUs was found after long time in microcosms (except for oak leaves). Similarly to that found for bacterial diversity, the number of ciliate OTUs on individual leaf species was higher in single leaf species treatments than in mixed species treatments (Fig. 2).

However, when taking into account the total number of OTUs associated with all leaf species composing a given mixture, positive relationships were found between leaf species number and fungal or bacterial diversity (linear regression, P=0.0003 and P=0.024, respectively; Fig. 3). For ciliates, that relationship was not significant (linear regression, P=0.065). The dependence of microbial diversity on leaf species number strongly increased from ciliates to bacteria to fungi (slopes were 2.0, 4.2 and 6.0 OTUs/unit of leaf species diversity, respectively).

CA ordination of fungal assemblages according to leaf species number, leaf species identity and time after leaf diversity loss showed that the 1st factor explained 20.8% of the total variance in fungal assemblages, and separated assemblages established at short time from those established at long time (Fig. 4a). The 2nd factor explained 15.2% of the total variance and distinguished assemblages according to leaf species identity and leaf species number, mainly separating fungal assemblages on 3 leaf species from the others.

CA ordination of bacterial assemblages showed that the first two factors, explaining 24.5% of the total variance, separated assemblages on oak leaves from the others, and further discriminated assemblages according to leaf species number and the time after diversity loss (Fig. 4b). The 1st CA factor of ciliate assemblages explained 19.1% of the

total variance and separated assemblages by the time after diversity loss, while the 2nd factor explaining 15.1% of the total variance mainly distinguished assemblages according to leaf species number (Fig. 4c).

Effects of plant litter diversity on leaf decomposition

Leaf mass loss varied between 26% in microcosms containing eucalyptus mixed with oak after long time of leaf diversity loss and 43% in microcosms with oak in mixtures with alder and eucalyptus at short time (Fig. 5). Decomposition of alder leaves was not affected by leaf species number or time after leaf species loss, but effects of leaf identity were marginally significant (3-way nested ANOVA, Table 1). However, the effects of leaf identity on decomposition of alder leaves became stronger after long time of leaf diversity loss (2-way nested ANOVA, P=0.005, F=11.59). Decomposition of alder leaves was higher in mixtures with oak than in mixtures with eucalyptus or mixtures with oak and eucalyptus (Tukey-Kramer´s tests, P=0.024 and P=0.048, respectively). Decomposition of oak leaves was affected by leaf species number and interaction between species number and time after leaf species loss (3-way nested ANOVA, Table 1); leaf mass loss was higher in mixtures of 3 species than in treatments with oak alone (Tukey-Kramer´s test, P=0.040). Mass loss of eucalyptus leaves was affected by time after leaf diversity loss and marginally affected by leaf identity (3-way nested ANOVA, Table 1). Similarly to that found for alder leaves, the effects of leaf species identity on decomposition of eucalyptus leaves became stronger after long time of leaf diversity loss (2-way nested ANOVA, P=0.011, F=9.12). Mass loss of eucalyptus leaves was higher when eucalyptus was mixed with alder leaves than with oak leaves (Tukey-Kramer´s test, P=0.046).

Effects of plant litter diversity on fungal biomass

Leaf-associated fungal biomass varied between 55 µg ergosterol g^{-1} leaf dry mass, in alder leaves in mixtures with eucalyptus after long time of leaf diversity loss, and 468 µg ergosterol g^{-1} leaf dry mass in oak leaves in mixtures with alder at the shorter time (Fig. 5). Fungal biomass on alder leaves was affected by leaf species number (3-way nested ANOVA, Table 1), with higher values in mixtures with three leaf species than with two leaf species (Tukey-Kramer´s test, P=0.024). Fungal biomass on oak leaves was not affected by leaf species number, leaf identity or time after leaf diversity loss (3-way nested ANOVA, Table 1). However, when effects of leaf diversity were analysed at the longer time after diversity loss, leaf species number affected fungal biomass on oak leaves (2-way nested ANOVA, P=0.017, F=7.04). Moreover, fungal biomass on oak leaves decreased linearly with leaf species loss after long time (Linear regression, F=16.34, P=0.002, r^2=0.62, not shown). Fungal biomass associated with eucalyptus leaves was affected by leaf species number and time after leaf diversity loss (3-way nested ANOVA, Table 1), with overall higher biomass at the longer time (Tukey-Kramer´s test, P=0.006). The loss of leaf species led to a linear decrease in fungal biomass on eucalyptus leaves, with a stronger relationship at longer time (Linear regression, F=5.89, P=0.036, r^2=0.37, at short time; F=15.70, P=0.003, r^2=0.61, at long time; not shown).

Discussion

Our study suggests that changes in plant species diversity of riparian corridors affect diversity and activity of microbes on decomposing plant litter in streams. The leaf species (alder, oak and eucalyptus) used in our study provide resources with different quality due to differences in their leaf C:N ratio. Thus, each leaf species might harbour

different microbial assemblages that could provide inoculum to the different leaf species constituting the mixtures. Indeed, molecular diversity (as number of OTUs) of fungi on individual leaf species tended to be higher in leaf species mixtures. However, this trend was not observed on bacterial and ciliate diversity, which was higher in single species treatments. Fungi are reported to have antagonistic interactions with bacteria during leaf decomposition [30], but fungi have morphological and physiological adaptations that allow them to colonize plant litter earlier than bacteria [31], which might be outcompeted by fungi [32]. In addition, a reduction of bacterial diversity by the presence of fungi may decrease ciliate diversity because ciliates feed on bacteria and show preference for certain bacterial species [9]. This is consistent with the positive linear relationship between bacterial and ciliate diversity found in our study (not shown).

Although microbial assemblages on individual leaf species have shown different responses to leaf diversity loss, when microbial diversity as overall number of OTUs per leaf species treatment was considered, a positive relationship was found between leaf species diversity and the diversity of fungi and bacteria. A positive co-variation of fungal diversity with riparian plant species diversity was previously documented [14, 21]. Also, the replacement of native mixed forests by monocultures of eucalyptus in riparian corridors of streams in the Iberian Peninsula decreased the diversity of aquatic fungi with shifts in community composition [33]. In our study, the decrease in the number of OTUs per unit of leaf species lost was particularly high for fungi (6 OTUs/unit of leaf species diversity), pointing to a higher dependence of fungi than bacteria or ciliates on the diversity of plant litter resources. This agrees with the major role of fungi in early stages of plant-litter decomposition in streams [6, 7]; fungi have an efficient enzymatic machinery to degrade polysaccharides of plant cell walls, and their

hyphae have high ability to penetrate substrates [31]. The relationship between fungal diversity and litter diversity found in our study (6 OTUs decrease per unit of leaf species lost) was even stronger than reported by others (1.7 fungal species decrease per unit of leaf species lost [21]). This apparent discrepancy might be related to differences in the levels of leaf litter diversity investigated (1-3 leaf species in our study versus 7-17 leaf species in Laitung and Chauvet [21]). The positive relationship between the diversity of resources (litter) and the diversity of consumers (fungi) can be explained by mechanisms of niche differentiation [34]. We expected that more leaf species would provide a greater variety of resources that could allow the co-existence of more fungal species. However, it is conceivable that above a certain leaf diversity level, further increases in leaf diversity will not provide a proportional increase in nutrient supply or habitat structures for fungi. Therefore, the dependence of fungal diversity on plant litter diversity is expected to be stronger at lower leaf diversity levels.

The shifts in the structure of microbial assemblages on decomposing leaves in response to plant species loss were accompanied by changes in decomposition of oak leaves, but not of alder or eucalyptus leaves. However, the identity of litter mixture affected leaf decomposition of alder and eucalyptus leaves, mainly after long time of leaf diversity loss. Actually, the composition of litter mixtures appears to have a greater role in leaf decomposition in streams than diversity of litter species [35-37] with the differences in litter quality explaining the effects of leaf identity on leaf decomposition [38-40]. In our study, decomposition of eucalyptus leaves (higher C:N ratio, lower quality) tended to be faster when mixed with alder (lower C:N ratio, higher quality) than with oak leaves (intermediate C:N ratio), suggesting that microbial assemblages on eucalyptus leaves might have benefited from the presence of compounds released by high quality leaves to fulfil their metabolic needs [13]. Conversely, we found a deceleration of decomposition

of alder leaves by the presence of eucalyptus at the longest time after leaf species loss. Also, fungal biomass on alder leaves was consistently lower when mixed with eucalyptus, especially at the longest time. Eucalyptus leaves contain oils and tannic acids that inhibit the growth of aquatic fungi [41]. Thus, if inhibitory compounds were leached from eucalyptus leaves to the surrounding environment [13], microbial activity on other leaf species composing the mixture might also be inhibited.

In our study, the effects of leaf diversity were stronger on fungal biomass and diversity than on microbially-driven leaf decomposition. Moreover, fungal biomass and diversity tended to decrease as litter species were lost from the system, especially for oak and eucalyptus. This may have implications for freshwater invertebrates that preferentially feed on leaves colonized by microbes [42, 43]. Moreover, fungal diversity correlates positively with leaf consumption rates by invertebrate shredders [14]. Thus, the effects of leaf diversity loss on fungal diversity and biomass observed in our study might have indirect impacts on plant-litter decomposition in streams.

Overall, leaf litter diversity and quality changed the structure of microbial assemblages and affected leaf decomposition and fungal biomass on individual litter species. Fungal biomass tended to decrease with leaf species loss, especially for lower quality leaf species (oak and eucalyptus) after long time of diversity loss. Leaf decomposition was mainly affected by leaf species identity at the longer time. Microorganisms growing on low quality leaves appeared to benefit from the presence of other leaf species, as shown by higher fungal biomasses found in leaf mixtures. Conversely, the presence of eucalyptus lowered the decomposition of alder leaves at the longer time after leaf diversity loss. The eucalyptus species used in our study was introduced in the Iberian Peninsula almost two centuries ago, and nowadays vast areas are covered by monocultures of this exotic tree [44]. Alterations in diversity and quality of riparian

vegetation can jeopardize litter inputs into streams with possible bottom-up effects to the functioning of detritus food-webs [45]. Thus, protecting and/or restoring riparian vegetation is important to conserve microbial diversity and maintain the functioning of detritus food-webs in freshwaters.

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Figure captions

Fig. 1 DGGE patterns of DNA of fungal, bacterial and ciliate assemblages on individual leaf species (A, *Alnus glutinosa*; O, *Quercus robur*; E, *Eucalyptus globulus*) from single- and mixed-leaf species treatments, after short (2 months) and long time (6 months) of leaf diversity loss. M, marker used to align different gels belonging to the same microbial group

Fig. 2 Number of OTUs from DGGE analyses of fungal, bacterial and ciliate assemblages associated with individual leaf species (A, *Alnus glutinosa*; O, *Quercus robur*; E, *Eucalyptus globulus*) from single- and mixed-leaf species treatments, after short (2 months) and long time (6 months) of leaf diversity loss

Fig. 3 Relationship between the number of OTUs of fungi (a), bacteria (b) and ciliates (c) and leaf species diversity. In mixtures of two and three leaf species, data represent total number of OTUs per leaf species treatment. Data were fitted to linear regressions. Fungi, $Y=5.98X+7.25$, $r^2=0.69$, $P=0.0003$; Bacteria, $Y=4.21X+39.50$, $r^2=0.36$, $P=0.024$; Ciliates, $Y=1.99X+6.25$, $r^2=0.28$, $P=0.065$

Fig. 4 CA diagrams for ordination of fungal (a), bacterial (b) and ciliate (c) OTUs according to exposure time (short and long), leaf species number (1sp, 2sp and 3sp) and identity (alder, oak and eucalyptus)

Fig. 5 Percentage of leaf mass loss and fungal biomass from individual leaf species alone and in mixtures, after short (2 months) and long time (6 months) of leaf diversity loss. Values are mean + SEM; n=3 for fungal biomass and n=4 for leaf mass loss

Table 1. Effects of leaf species number (Sp nº), leaf species identity (ID), nested within leaf species number, and time after leaf diversity loss (T) on leaf mass loss and fungal biomass. In leaf mixtures, data came from individual leaf species

Parameter	Leaf species	Factor	SS	DF	F	P
Leaf mass loss	Alder	Sp nº	26.7	2	0.47	0.630
		T	75.8	1	2.67	0.115
		Sp nº*T	20.5	2	0.36	0.701
		ID (Sp nº)	117.2	1	4.13	0.053
		ID (Sp nº)*T	52.1	1	1.84	0.188
		Error	680.7	24		
	Oak	Sp nº	115.0	2	3.77	0.038
		T	58.1	1	3.81	0.063
		Sp nº*T	122.7	2	4.02	0.031
		ID (Sp nº)	8.8	1	0.58	0.455
		ID (Sp nº)*T	14.0	1	0.91	0.349
		Error	366.4	24		
	Eucalyptus	Sp nº	26.4	2	0.63	0.539
		T	132.9	1	6.40	0.018
		Sp nº*T	90.1	2	2.17	0.136
		ID (Sp nº)	87.4	1	4.21	0.051
		ID (Sp nº)*T	33.2	1	1.60	0.218
		Error	498.9	24		
Fungal biomass	Alder	Sp nº	39497.4	2	6.75	0.007
		T	221.1	1	0.08	0.787
		Sp nº*T	11955.3	2	2.04	0.162
		ID (Sp nº)	4051.0	1	1.38	0.257
		ID (Sp nº)*T	3468.7	1	1.19	0.292
		Error	46814.2	16		
	Oak	Sp nº	73834.0	2	3.05	0.076
		T	94.0	1	0.01	0.931
		Sp nº*T	6609.0	2	0.27	0.765
		ID (Sp nº)	18124.0	1	1.50	0.239
		ID (Sp nº)*T	11212.0	1	0.93	0.350
		Error	193740.0	16		
	Eucalyptus	Sp nº	27038.0	2	10.90	0.001
		T	10665.5	1	8.60	0.010
		Sp nº*T	2211.0	2	0.89	0.430
		ID (Sp nº)	1000.1	1	0.81	0.383
		ID (Sp nº)*T	34.0	1	0.03	0.871
		Error	19851.3	16		