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Microbial Decomposer Dynamics: Diversity and Functionality Investigated through a Transplantation Experiment in Boreal Forests

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Abstract

Litter decomposition is the main source of mineral nitrogen (N) in terrestrial ecosystem and a key step in carbon (C) cycle. Microbial community is the main decomposer, and its specialization on specific litter is considered at the basis of higher decomposition rate in its natural environment than in other forests. However, there are contrasting evidences on how the microbial community responds to a new litter input and if the mass loss is higher in natural environment. We selected leaf litter from three different plant species across three sites of different altitudinal ranges: oak (*Quercus petraea* (Matt.) Liebl., 530 m a.s.l), beech (*Fagus sylvatica* L., 1000 m a.s.l.), rhododendron (*Rhododendron ferrugineum* L., 1530 m a.s.l.). A complete transplantation experiment was set up within the native site and the other two altitudinal sites. Microbial community structure was analyzed via amplified ribosomal intergenic spacer analysis (ARISA) fingerprinting. Functionality was investigated by potential enzyme activities. Chemical composition of litter was recorded. Mass loss showed no faster decomposition rate on native site. Similarly, no influence of site was found on microbial structure, while there was a strong temporal variation. Potential enzymatic activities were not affected by the same temporal pattern with a general increase of activities during autumn. Our results suggested that no specialization in microbial community is present due to the lack of influence of the site in structure and in the mass loss dynamics. Finally, different temporal patterns in microbial community and potential enzymatic activities suggest the presence of functional redundancy within decomposers.

Keywords Bacterial community · Fungal community · Potential hydrolytic activities · Alpine region · Functional redundancy

Introduction

Litter fall is one of the most important components of net forest primary production, playing a key role in nutrient cycling and forest health [1]. Litter provides also habitats for microfauna in forest ecosystems and is able to buffer fluctuation in temperature, soil water moisture, and leaching [1]. Litter is the major source of organic matter entering the decomposition cycle [2]. Its decomposition is a key step in the carbon C flux [3], releasing more C than fossil combustion [4]. It is also an important source for nutrients, including nitrogen (N) for which litter decomposition contributes up to 70% of the annual input [2].

Fungi have been considered the main agents of litter decomposition in forest systems [5, 6] as they have functionally different enzymes on all major degradable components of litter. They have the ability to secrete a range of degrading enzymes targeting specifically plant tissue such as cellulase, xylosidase, and β-glucosidase able to attack cellulose and hemicellulose [7]. They are also able to decompose recalcitrant compounds such as lignin with a set of extracellular enzymes [5]. Differently, the role of bacteria is still unclear and under debate despite their functional potential in the process. Some authors suggest a more active role [8–10] considering that enzymes such as laccase and peroxidase, involved in the degradation of lignin, have been found in several bacterial strains [11]. It is reported that 24% of the bacterial genomes sequenced have genes encoding for cellulose-degrading enzymes [12]. Bacteria may also have a role as

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helper for fungal communities: Purahong et al. [8] and Tlaskal et al. [9] have found in beech and oak litter the presence of N fixing bacteria suggesting a beneficial relationship within fungi that require external N for their metabolic activities.

Temporal variation in microbial fungal and bacterial community composition across the seasons has been identified to be associated with a shift in litter chemical composition [8, 9, 13–15]. The phyllosphere community inhabiting the leaf surface is replaced by taxa able to decompose the senescent leaf enriched with cellulose, hemicellulose, and lignin [8, 14, 15]. For instance, there is an increasing fraction of *Basidiomycota* over time able to degrade lignin and humic acids [8, 15]. Additionally, there is an increase of abundance of bacterial cellulolytic taxa peaking in early phases of the decomposition (usually the first year), which persists until the late phase of decomposition [8, 9]. The same temporal patterns had been observed also for the enzymatic activities [13, 16]. However, the correlation between functionality and microbial community structure has been recently questioned by different researches where the difference between temporal dynamics of the community and the enzymatic dynamics has been found [17, 18].

Due to the importance of microbial communities, researchers have used transplantation experiment not only to untangle the importance of quality of the litter and site of decomposition [19, 20] but also to test possible scenario following global change modification of plant species composition or change in land use [20, 21]. Based on the results of transplantation experiment, authors have found that litter usually decomposes faster in its natural environment than away (Home Field Advantage, HFA), and one of the possible explanations proposed was the presence of a specialized microbial community [22, 23]. Different studies have found that microbial community (bacteria and fungi) has specific preference based on the dominant vegetation [24–26], but it is not clear how it will respond to the presence of alien species and under which condition the increase rate of decomposition is maximized by the presence of specialized decomposers [27]. More pronounced effects are expected when litter species differ in chemistry composition and when sites are differentiated by temperature, because it is expected that in a cold environment, there would be present a more specialized microbial community [27]. We selected three sites and their dominant species in order to maximize the differences: a dominant oak forest (530 m a.s.l.), a beech forest (1000 m a.s.l.), and a rhododendron bush area (1530 m a.s.l.). Our hypotheses were that mass loss should be higher in the native site (H1) and that the microbial community and their potential enzymatic activities should be strongly influenced by time and site of decomposition (H2). To answer these questions, a complete transplantation experiment was set up to follow the litter mass loss and the microbial community structure as well as chemical composition of litter (total C and total N) and monitoring the temperature of litter layer.

Materials and Methods

Study Area

In South Tyrol territory (Italy), we selected litter of three different plant species and altitudes: oak forest (*Quercus petraea* (Matt.) Liebl.) at 530 m a.s.l. in the Monticolo forest area (M), beech forest (*Fagus sylvatica* L.) at 1000 m a.s.l. in San Genesio Atesino (SG), and rhododendron bush (*Rhododendron ferrugineum* L.) at 1530 m a.s.l at Renon (RN). The experimental sites were chosen to represent the three natural environments of each selected species. Detailed information are provided in Table 1. Temperature was constantly measured in every site at the litter horizon over the entire experiment with HOBO data logger (Onset Computer Corporation, Bourne, USA). Soil pH was determined in soil: distilled water extracts (1:10, *w/v*) using a pH-Meter BASIC 20+ (Crison, Modena, Italy). Other data about soil characteristics have been taken elsewhere [28, 29].

Experimental Design and Sampling

In each sampling site, litterbags containing leaves from their own sites and from the other two sites were located and left for 519 days. Fresh fallen leaves were collected in the autumn of 2014 and left in an incubator at 0 °C until May 2015. Leaves were maintained at 0 °C to guarantee surveillance of endophytic and phyllosphere microbial community. Leaves were air-dried before being placed in litterbags (20 × 20 cm, 40 μm nylon mesh size, Manifattura Fontana, Macherio, Italy) [19] for deployment and to be brought back to the study sites. Oak and beech litterbags contained each 3 g of air-dried samples while rhododendron litterbags contained 1 g of leaf litter. In total, 162 litterbags were placed on the upper layer of litter horizon of the three experimental sites during May 2015. In each experimental area, 54 litterbags were present: three replicates for each sampling time ($n = 6$) for every plant species ($n = 3$). During this time, litterbags were collected on the following six dates: May 22, 2015 (10 days), August 7, 2015 (87 days), November 17, 2015 (189 days), March 17, 2016 (310 days), July 7, 2016 (422 days), and October 12, 2016 (519 days) (see Fig. S1).

In each sampling event and site, three replicates for each plant species were randomly collected. Replicates were placed into separate sterile plastic bags to reduce the loss of small fragments and cross contamination. Samples were immediately transported on ice to the laboratory. Within 24 h, each litterbag was divided into subsamples for mass loss determination, biomolecular analysis, chemical analysis, and enzymatic assay.

Table 1 Main characteristics of the selected study sites

Site characteristic	Monticolo	San Genesio Atesino	Renon
Elevation	530 m a.s.l.	1000 m a.s.l.	1530 m a.s.l.
Lythology	Quartz porphyritic	Quartz porphyritic	Quartz porphyritic
Soil type	Acid brown soil	Podzol	Podzol
Soil texture	Sandy loam	Sandy loam	Sandy loam
pH soil	5.50	6.13	4.83
Mean annual precipitation	800 mm	735 mm	970 mm
Mean annual temperature	11.8 °C (max. 21.3, min. 3.6)	10.5 °C (max. 17, min. 3)	6.8 °C (max. 14.3, min. − 0.7)
Dominant vegetation	Oak (*Quercus petraea*)	European beech (*Fagus sylvatica*)	Rhododendron (alpine rose) (*Rhododendron ferrugineum*)

Mass Loss and Chemical Analysis

Mass loss was measured for each sample after being oven-dried at 105 °C until constant weight. Mass loss was expressed as percent of remaining mass compared to the initial dried mass. Subsamples from each replicate were analyzed to detect total C and N. Each subsample was oven-dried for 72 h at 60 °C and then grounded to fine powder [30] with a Mixer Mill MM 400 (Retsch GmbH, Haan; Germany). Total C and total N concentrations were determined by an elemental analyzer (Elemental Analyzer Flash 2000, Thermo Scientific, USA) using 1 mg of dried sample.

DNA Extraction for Automated Ribosomal Intergenic Spacer Analysis (ARISA) Analysis

Litter samples, previously stored at − 20 °C, were grounded to fine powder with liquid N in sterile condition. DNA extraction was performed with 0.25 g of sample using the PowerSoil DNA isolation kit (MoBio, Arcore, Italy) according to the user manual. The concentration of the eluted genomic DNA was determined by spectrophotometry with the Qubit (Invitrogen, Milan, Italy) and adjusted to 10 ng/μl for all samples.

Automated Ribosomal Intergenic Spacer Analysis was performed on both bacterial (B-ARISA) and fungal (F-ARISA) community. The bacterial community was investigated using primers ITSF and ITSReub labeled with 6-FAM according to the protocol of Cardinale et al. [31]. The primer pair ITS4 and ITS1F labeled with 6-FAM was used to study the fungal community following the protocol reported in Carson et al. [32]. PCR products were sent to STAB Vida Lda. (Caparica, Portugal) for capillary electrophoresis.

Raw data were analyzed using AB Peak Scanner Software 1.0 (Applied Biosystems, Monza, Italy). The output matrix was then analyzed as described by Borruso et al. [33].

Potential Enzyme Activities and Microbial Biomass

Selected activities involved in the principal nutrient cycles were determined according to Bardelli et al. [34]. Briefly, enzymes were extracted from soil samples by a heteromolecular exchange procedure [35], using a 3% solution of lysozyme as desorbant and bead-beating to disrupt soil. An amount of 0.4 g of litter (fresh weight) was placed into a 2-mL Eppendorf tube, together with 1.4 mL of a solution containing 3% lysozyme, glass, and ceramic beads. Tubes were then subjected to bead-beating using a Retsch 400 beating mill at 30 strokes s^{-1} for 3 min, followed by centrifugation at 20,000*g* for 300 s. The supernatant containing desorbed enzymes was dispensed into 384-well white microplates with the appropriate buffer to fluorometrically quantify the enzymatic activities using fluorogenic, 4-methyl-umbelliferyl- (MUF) and 4-amido-7-methyl-coumarine (AMC) substrates. A Synergy HT (BIO-TEK) microplate reader was used for quantifying fluorescence. Cellulase, xylosidase, β-glucosidase, and acid phosphomonoesterase assays were performed in 100 mM, pH 5.8 MES buffer, and phosphodiesterase was assayed in 100 mM pH 7.5 THAM buffer. All assays were performed at 24 °C, and all measurements were done in duplicate. Enzyme activities were expressed as nanomoles of MUF g^{-1} dry litter $hour^{-1}$. Microbial biomass was estimated by quantifying dsDNA extracted from soil [36]. Briefly, 0.4 g of litter (fresh weight) were placed into 2-mL Eppendorf tubes, together with 1.2 mL of 0.12 M, pH 7.8 sodium phosphate buffer together with glass and ceramic beads. Tubes were then subjected to bead-beating using a Retsch 400 beating mill at 30 strokes s^{-1} for 2 min, followed by centrifugation at 20,000*g* for 30 s. An aliquot of supernatant was diluted in THAM-EDTA buffer before fluorometric quantification of dsDNA by using Pico-Green reagent (Life Technologies) in black 384-well microplate and a Synergy HT (BIO-TEK) microplate reader.

Table 2 Initial chemical litter characteristics

	Beech litter	Oak litter	Rhododendron litter
Total C%	47.6 ± 1.6 b	46.0 ± 2.2 b	51.2 ± 0.7 a
Total N%	0.82 ± 0.1 b	0.66 ± 0.1 c	1.8 ± 0.1 a
C/N	59 ± 6 b	68 ± 8 a	28 ± 2 c

Values are mean values ± standard deviation ($n = 3$). Different letters indicate significant difference between the three plant species

Table 3 Decay rates (k) of different litter in all site of decomposition

	df	Beech litter	Oak litter	Rhododendron litter
M	2	0.66 ± 0.16	0.61 ± 0.05	0.75 ± 0.14
RN	2	0.49 ± 0.07	0.57 ± 0.1	0.64 ± 0.14
SG	2	0.53 ± 0.05	0.65 ± 0.18	0.66 ± 0.13

Values are mean values ± standard deviation ($n = 3$). No significant differences were found between the k values of the different litter in the three site of decomposition

df degree of freedom, *M* Monticolo, *RN* Renon, *SG* San Genesio Atesino

Statistical Analysis

R software was used to perform statistical analysis using the R package "vegan" [37]. B-ARISA and F-ARISA fingerprint data were analyzed with nonmetric multidimensional scaling (NMDS), based on Bray Curtis distance measurements. Environmental variables were fitted to the NMDS ordination plot of the bacterial and fungal overall communities using the function envfit, with the p value calculated on 1999 permutations. To evaluate the differences between different groups, the ANOSIM function was applied. Decomposition rates (k) were calculated based on negative exponential decay for each litter type and each site of decomposition following the same equation as Purahong et al. [38]: $X/X_0 = e^{-kt}$. X was the final dried mass, X_0 the initial dried mass and t is the time of the experiment expressed in years (1.42, 519 days). Mass loss, k value, chemical composition, and potential enzymatic activities were tested for normality and equality of the variance with Shapiro-Wilk test and Levene test (car package [39]). ANOVA was used to test the effect of the site and the time, and when significant differences were found, a Tukey test (HSD) post-hoc analysis was applied. When the data failed the normality test, the Kruskal-Wallis was used to test the importance of the factor, and the applied post-hoc analysis was least significant difference (LSD) test. ANOVA residuals were then plotted using Shapiro-Wilk test to determine the normal probability. Mantel test was used to test the correlation between microbial community structure (ARISA matrix) and function (enzyme activity matrix).

Results

Mass Loss and Chemical Composition

The initial characterization of litter species is shown in Table 2. The oak litter had a higher C/N ratio, followed by beech and finally rhododendron. Over the entire experiment,

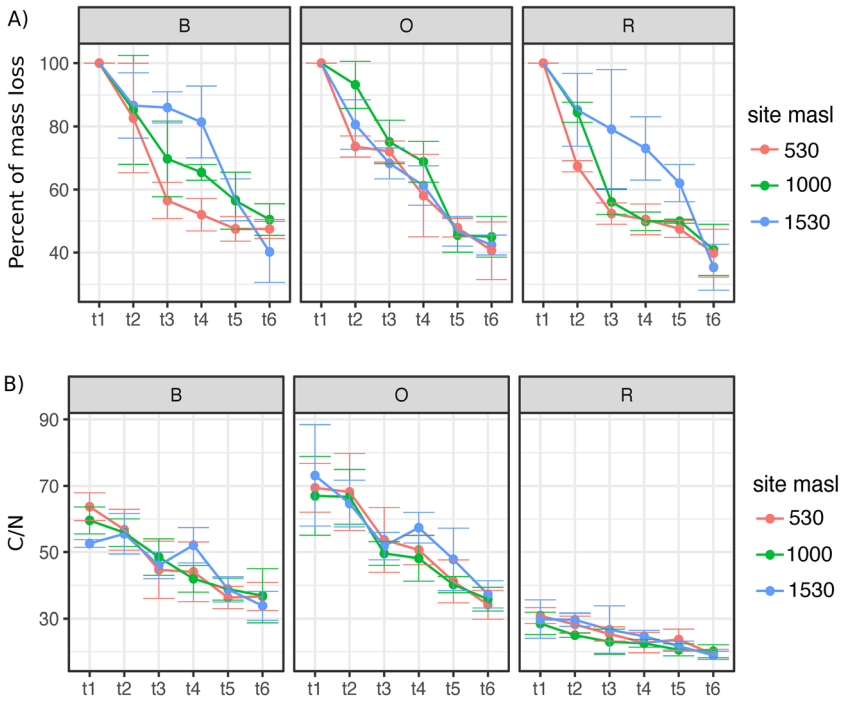

Fig. 1 Mass loss (**a**) and C/N (**b**) dynamics of different litter species. Values shown are mean values ± standard deviation ($n = 3$). Each panel represents a different litter: B, beech; O, oak; and R rhododendron. Different colors represent site of decomposition M, Monticolo; RN, Renon; and SG, San Genesio Atesino

the remaining mass in all litter species were similar in all the sites around 40% (Fig. 1a). The mass loss was influenced by time (p value < 0.05, Table S1) while no statistically significant differences were found based on site of decomposition (Table 3). K values were calculated for each decomposition pattern, and no significant differences were found within each litter between different sites of decomposition (Table 3, Table S1). C/N ratio over time decreased in all the species, with similar behavior in the different sites (Fig. 1b, Table 4).

Litter Microbial Community Structure

Bacterial richness, peaks from 150 to 1200 bp, in beech litter was on average 116 ± 47, for oak litter was 119 ± 39, and rhododendron bacterial richness was 93 ± 59. Bacterial richness increased over time in all the bacterial communities. Moreover, it was influenced by time but not by the decomposition site (Table 4 and Fig. S2A). Bacterial community structure, across all the litter types, showed clustering based on the sampling date, but not on the decomposition site (Fig. 2). The presence of six clusters based on time was confirmed by ANOSIM (p value < 0.001). The same analysis did not show a differentiation based on the location (Table S3). In particular, time 1 and time 2 were well separated to the remaining clusters in all the three species. The split was associated with the nutrient status of the leaf expressed as C/N ratio (Fig. 2). In general, bacterial communities were strongly related to litter quality indicators such as the C/N ratio and the total litter N (Table S2). Temperature strongly correlated only with the bacterial communities of oak litter (p value < 0.001) and to a less extent with beech (p value <0.05), with no significant effect on the rhododendron communities.

An average of 47 ± 20 of fungal peak richness, ranging from 150 to 1200 bp, in rhododendron litter was found, while in beech litter, the average was 42 ± 21 and finally for oak litter was 47 ± 19. Fungal richness was influenced by time but not by site of decomposition (Table 4 and Fig. S2B) in every litter studied. Fungal richness was always lower than bacteria richness. Fungal communities showed similar results to the bacterial community structure with a succession of different clusters over the experiment (Fig. 3). However, the cluster separation was less pronounced and showed some overlaps (ANOSIM R < 0.75) within the different groups (Table S3). Times 5 and 6 appeared to be split from the others across all litter considered and associated with the N composition of the substrate. Also for fungal communities, the importance of the environmental variables over the ordination plot was tested (Table S2). Similar to the bacterial results, the fungal communities had robust correlation with the total N and the C/N ratio. The temperature factor resulted to be significant (p value < 0.005) only in case of the fungal community associated with oak litter. The total C was significantly correlated with the fungal community of the beech (p value < 0.05) and the oak litter (p value < 0.05), but not with the rhododendron.

Litter Microbial Community Functionality

The patterns observed in the NMDS plot (Fig. 4) for potential enzymatic activities were very different from the one observed for the microbial community structure. No clear clustering was observed based on time or on site of decomposition. However, ANOSIM results showed that time was significant (p value < 0.05), but the R value indicated clusters barely separated (0.50 < R < 0.25) [14]. The total content of N and the C/N ratio were strongly related to the potential enzymatic activities of all litter types (p value < 0.05) while temperature was significant only for beech litter (p value < 0.05) (Table S2).

The singular potential enzymatic activities are shown in Fig. 5. Over the 17 activities analyzed, only six are shown to present the general trend observed in the functionality (for the remaining enzymes see Table S4). All the activities were influenced by the time (p value < 0.05, Table 4), but not by the site (Table 4). The β glucosidase and the cellobiohydrolase activities showed similar behavior with a maximum of activity during the autumn seasons t3 and t6, respectively. Similar to the previous two enzymes, alkaline phosphomonoesterase had maximum activity in the two autumn seasons. Differently, the acid phosphomonoesterase increased constantly over time and reached the maximum at the end of the experiment. In beech litter, acid phosphomonoesterase activity was present, but not the alkaline phosphomonoesterase. Leucine aminopeptidase was almost stable over time across all types of litter. Arylsulfatase had an early maximum in beech litter and then rapidly decreased, while in the other two litter types, it increased over time. Results of the Mantel test were not significant indicating that the microbial community structure was not reflected in the overall potential enzymatic activities (Mantel test Bray-Curtis distance matrix R < 0.203, p value > 0.05).

Total microbial biomass (Fig. 6) was higher in the oak litter, followed by the one associated with beech and lower in rhododendron. There was an increase in microbial biomass over time in all the three types of litter and in every site of decomposition, with the highest peak in the autumn/winter season. Differently to the results of the microbial community, apart from the sampling date, also the site of the decomposition influenced the total microbial biomass (p value < 0.05 for time and site, Table 4).

Discussion

Mass Loss Dynamics

After 17 months (519 days), the mass remaining was 40% in the same percentage range of mass loss of other studies both

Table 4 Factorial ANOVA or Kruskal-Wallis for chemical and microbiological parameters

	Beech litter						Oak litter						Rhododendron litter					
	Time			Site			Time			Site			Time			Site		
Variable	df	F	*P*	df	F	*P*	df	F	*P*	df	F	*P*	df	F	*P*	df	F	*P*
Mass	5	**24.04**	***	2	1.19	ns	5	**71.95**	***	2	0.37	ns	5	**33.24**	***	2	1.57	ns
Total C	5	**2.42**	*	2	3.02	ns	5	1.79	ns	2	1.11	ns	5	**2.70**	*	2	0.09	ns
Total N	5	**24.91**	***	2	0.12	ns	5	**36.54**	***	2	1.25	ns	5	**19.52**	***	2	2.52	ns
C/N	5	**25.76**	***	2	0.25	ns	5	**31.88**	***	2	1.57	ns	5	**16.10**	***	2	3	ns
B rich	5	**11.74**	***	2	0.21	ns	5	**12.05**	***	2	0.21	ns	5	**74.89**	***	2	0.09	ns
F rich	5	3.05	ns	2	0.81	ns	5	**10.29**	***	2	0.14	ns	5	**5.69**	***	2	0.48	ns
dsDNA	5	**36.25**	***	2	**11.66**	***	5	**49.78**	***	2	**4.34**	*	5	**11.91**	***	2	**3.44**	*
gluc	5	**3.92**	**	2	1.11	ns	5	**4.24**†	**	2	**5.95**†	**	5	**33.96**†	***	2	0.84†	ns
cell	5	**15.23**†	***	2	2.17†	ns	5	**16.69**†	***	2	**7.44**†	*	5	**32.30**†	***	2	0.26†	ns
alkP	5	**15.61**	***	2	**7.64**	**	5	**28.24**†	***	2	2.96†	ns	5	**34.81**†	***	2	3.40†	ns
acP	5	**16.67**	***	2	2.68	ns	5	**16.23**	***	2	1.44	ns	5	**13.71**	***	2	1.13	ns
leu	5	**27.06**†	***	2	2.71†	ns	5	**35.13**†	***	2	3.90†	ns	5	**37.38**†	***	2	0.30†	ns
aryS	5	**25.80**†	***	2	1.06†	ns	5	**13.87**†	*	2	2.04†	ns	5	**18.50**	***	2	0.21	ns

Values in bold indicate significant difference according to ANOVA or Kruskal-Wallis. When Kruskal-Wallis was used instead of ANOVA the results are followed by (†)

df degree of freedom, *Mass* mass loss, *B rich* bacterial richness, *F rich* fungal richness, *dsDNA* soil microbial biomass index, *gluc* β glucosidase, *cell* cellobiohydrolase, *alkP* alkaline phosphomonoesterase, *acP* acid phosphomonoesterase, *leu* leucine-aminopeptidase, *aryS* arylsulphatase

on oak litter and beech litter decomposed in other European forests [15, 38]. Similar results were found for all plant species considered even if the chemical composition of litters were different as shown in Table 2. For example, rhododendron litter is covered in wax [40] that creates an external barrier to microbial attack. On the other hand, beech litter had a greater amount of lignin than oak litter [41]. Contrarily to our initial hypothesis (H1), mass loss was not fast in the natural environment (Fig. 1 and Table 4), and the decomposition rates were not influenced by site of decomposition (Table 3). These results were in accordance with recent studies on transplantation on similar altitudinal gradient but also on latitudinal gradient

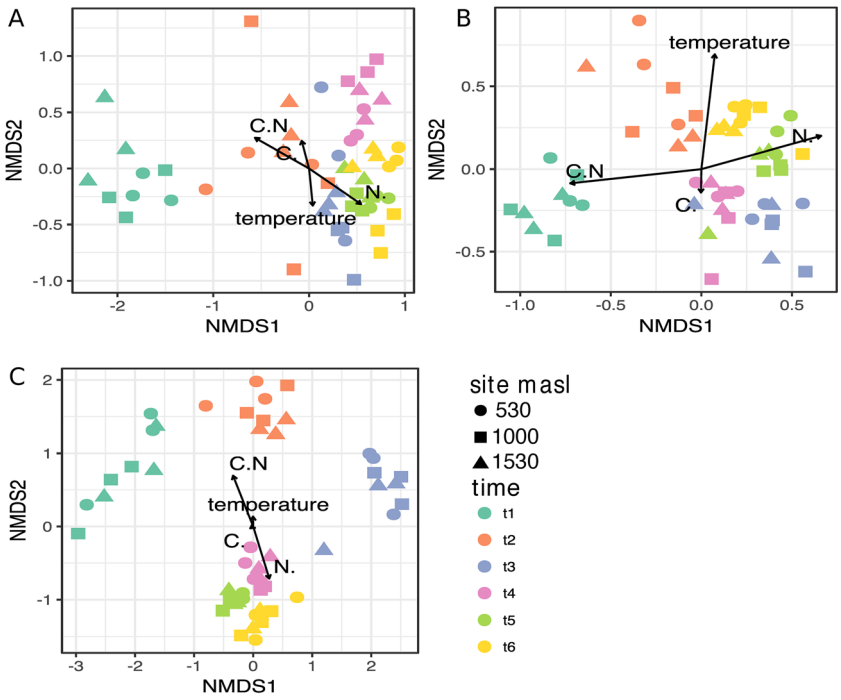

Fig. 2 NMDS ordination plots of the overall bacterial community across time associated with a specific plant litter. Environmental parameters were fitted to the plot. **a** Litter of beech, **b** oak, and **c** rhododendron. M, Monticolo site; RN, Renon site; and SG, San Genesio Atesino site

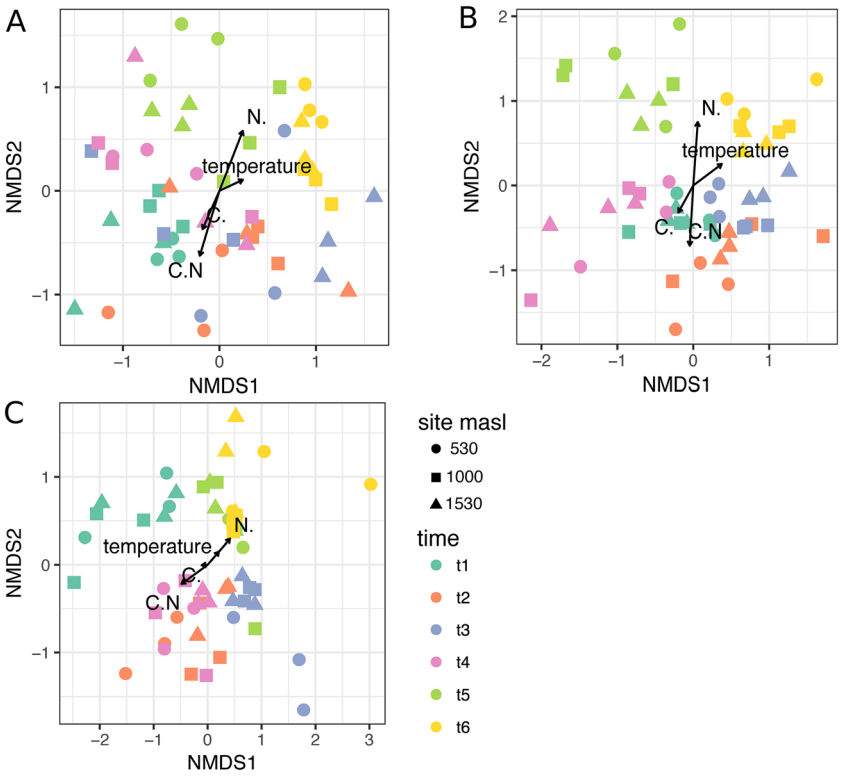

Fig. 3 NMDS ordination plots of the overall fungal community across time associated with a specific plant litter. Environmental parameters were fitted to the plot. **a** Litter of beech, **b** oak, and **c** rhododendron. M, Monticolo site; RN, Renon site; and SG, San Genesio Atesino site

across different biomes [20, 42]. In particular, Makkonen et al. [42] proposed that the plant species identity displays the strongest control on decomposition, having not found a preference in any biome in decomposition of the native vegetation. Additionally, she suggested that microbial community metabolism is very flexible and could easily use different types of litter even if not naturally present in the environment (see the "Influence of site of decomposition on microbial community"

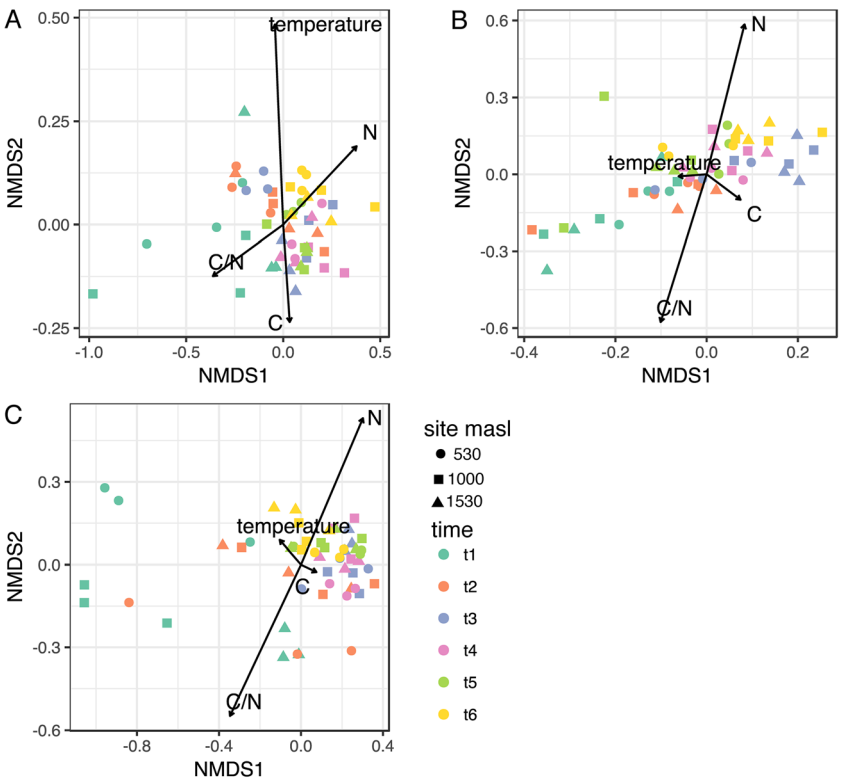

Fig. 4 NMDS ordination plots of the potential enzymatic activities across time. Environmental parameters were fitted on the plot. Different colors represent the different sampling time while the different shapes the different site. **a** Litter of beech, **b** oak, **c** rhododendron. M, Monticolo site; RN, Renon site; and SG, San Genesio Atesino site

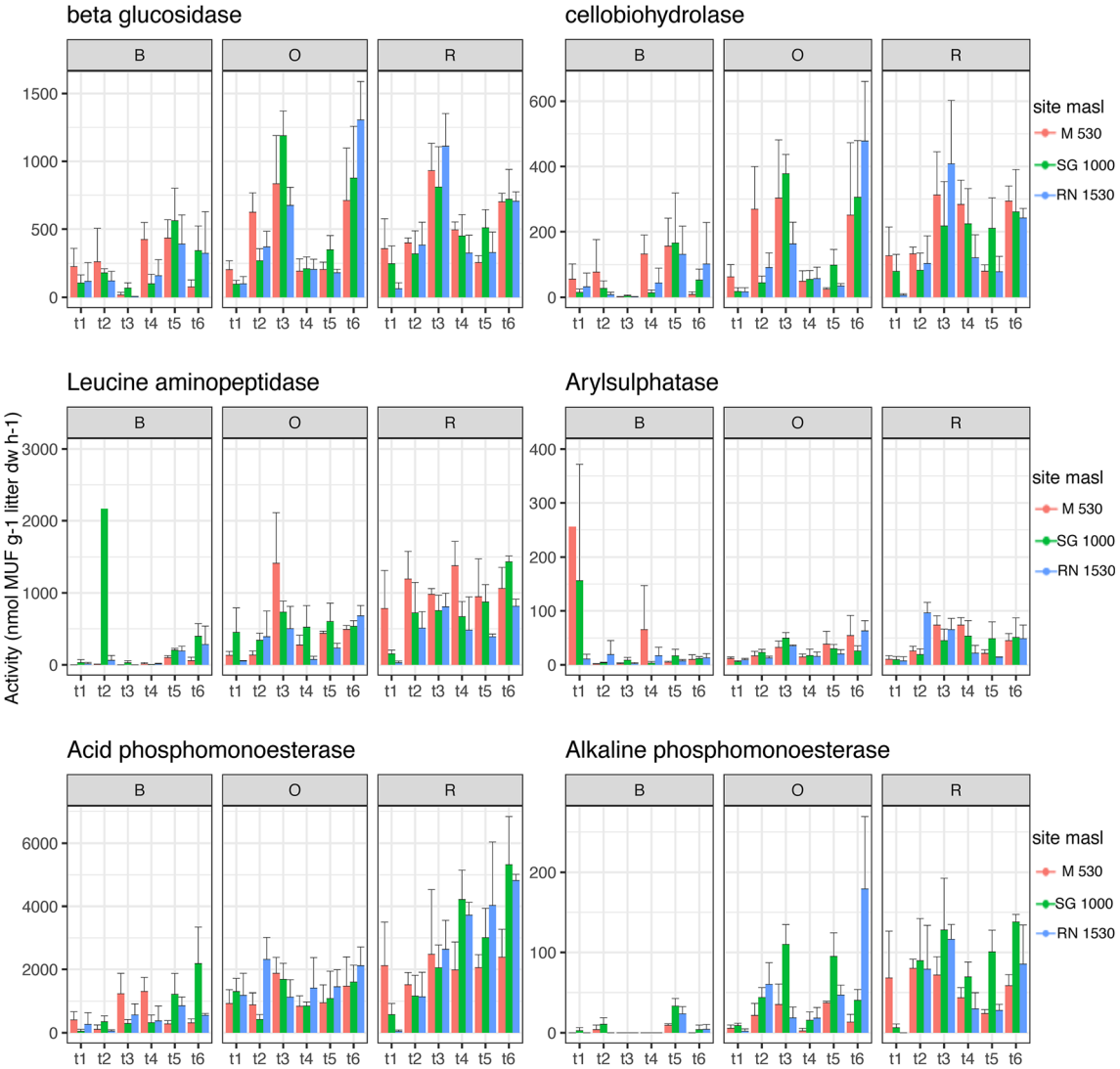

Fig. 5 Potential activities of the microbial community. Values shown are mean values ± standard deviation ($n = 3$). B, litter of beech; O, oak; R, rhododendron. M, Monticolo site; RN, Renon site; and SG, San Genesio Atesino site. β glucosidase, cellobiohydrolase, leucine-aminopeptidase, arylsulphatase, acid phosphomonoesterase, and alkaline phosphomonoesterase

section). Veen et al. [27] did not found a specialized microbial community, neither a quicker mass loss in the native site. However, they found that different litter species decomposed faster at lower altitude.

Microbial Structure Vs Functionality

The experiment aimed to shed light on the temporal patterns and the connection between microbial community structure and function within different types of litter decomposed in their natural environment and in transplanted areas.

ARISA techniques was used to have information on the structure of fungal and bacterial communities. This allowed having reliable results without the using of next generation sequencing [43]. The technique is well documented as reliable to correlate microbial structure and environmental parameters both for bacterial and fungal communities [43–45]. Microbial community structure associated with the different litter species showed a general trend: microbial communities were strongly influenced by the time according to our initial hypothesis (H2) (Figs. 1 and 2), sensitive to the stage of decomposition and to seasonal factors. Litter quality (C/N ratio) and total N (Table S2) also significantly influenced microbial community composition. Over time, litter becomes enriched in more complex molecules (such as lignin), leading to a succession of taxa able to decompose this complex matrix [14]. Several studies have confirmed these results based on different types of litters and forests [14, 19, 46]. Purahong et al. [46] found similar results with a succession of taxa, however considering only one type of litter in four different management forests.

Because results based on single potential enzymatic activity for each cycle could be misleading [34], there was a

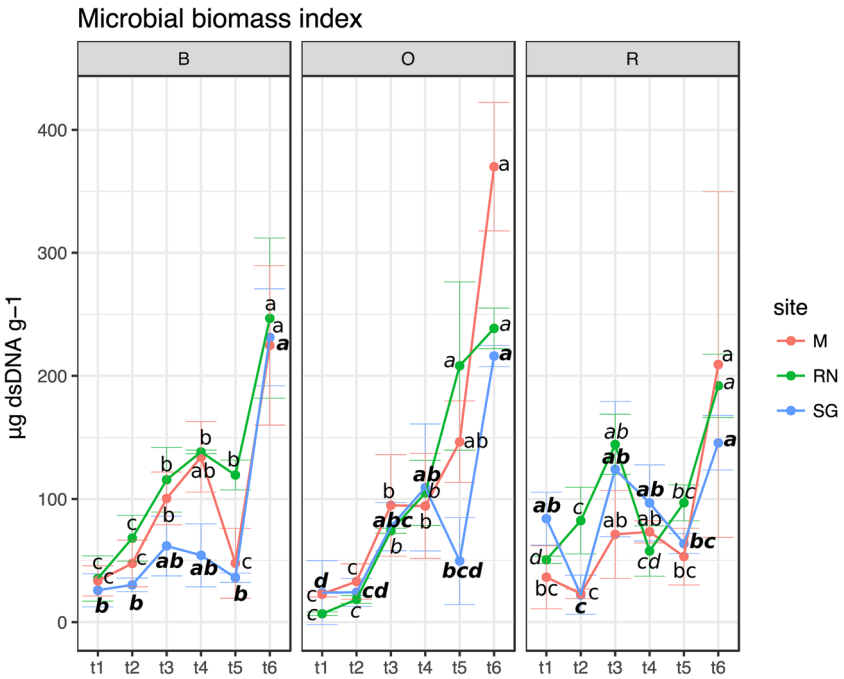

Fig. 6 Microbial biomass over time in the three site of decomposition. B, litter of beech; O, oak; R, rhododendron. M, Monticolo site; RN, Renon site; and SG, San Genesio Atesino site. Values shown are mean ± standard deviation ($n = 3$). Different letters indicate significant difference as function of time effect (p value < 0.05, ANOVA followed by HSD test) while different font of letters indicates significant difference between the different sites (p value < 0.05, ANOVA followed by HSD test)

selected range of different enzymes involved in different nutrient cycles including C cycle (cellobiohydrolase, β glucosidase), phosphate cycle (acid and alkaline phospomonoesterase), N cycle (leucine aminpeptidase), and sulfur cycle (arylsulfatase). The temporal succession that appeared in a clear pattern for the fungal and bacterial communities was not reflected by the potential enzymatic activities (Fig. 4). The results were similar to the finding of Frossard et al. [17] and Purahong et al. [18] that described a different dynamics for functionality and microbial community structure. Frossard et al. [17] found a decoupling of the bacterial structure and the function over time dynamics in nascent stream, where the structure was unaffected by temporal and spatial variability, but enzyme activity was influenced by time. The authors suggested a high functional redundancy in the bacterial community in which few generalist taxa could switch metabolic pathways excreting different types of enzymes [17]. Purahong et al. [18] found decoupled microbial structure and function over beech litter decomposition. The authors demonstrated that the microbial community was influenced by time and type of forest management, but not the functional enzymatic activities. Both studies suggested a functional redundancy in the microbial community. Functional redundancy is described as when different species of the same ecosystem perform the same function, even if a change in species composition happens, the overall ecosystem functionality is not affected [47]. Functional redundancy could be effective in the litter decomposer community due to the widespread presence of hydrolytic enzymes in bacterial genome. Consequently, more species could perform the same metabolic task. This could be the case of cellulases, found in at least 24% of bacterial sequenced genomes [12]. Functional redundancy could occur also when both fungi and bacteria perform the same function in the litter environment. Cellulases again could be replaced, not only within different species of bacteria or fungi but also by gram-positive-bacterial cellulases or from *Actinobacteria* [18]. Similarly, fungal xylanolytic enzymes could be replaced by bacterial xylanases present in *Streptomycetes* members [8], taxon found in the later phase of litter decomposition [48].

Influence of Site of Decomposition on Microbial Community

In contrast with our initial hypothesis, the influence of the site of decomposition has not been recorded. The mass loss in all litter species studied were similar, and no significant differences were present between the different sites within the same plant species (Fig. 1, Table 3). On the NMDS ordination plots (Figs. 2 and 3), it was not possible to cluster samples based on the decomposition site, whereas the time effect was clear. It was supported also by ANOSIM results, showing no significant clustering based on the decomposition site. Our results suggested that the type of litter had a stronger influence on the microbial community outperforming a potential site effect. Several studies confirmed that the type of litter is more important than the site. Cornwell et al. [49] showed, with a metadata study, that the magnitude of influence of the plant species on litter decomposition was greater than the climatic condition. In accordance, Makkonen et al. [42] found in a complete transplantation of 16 different plant species that the main drivers of the decomposition were the plant identity and not the climatic condition. The decomposer species pool quickly adapts to the

new litter, showing no preference for the native litter of the decomposition site. John et al. [50] obtained similar results when they tested in a transplantation experiment how the microbial community responded to a new input (grassland vs forest litter). Litter type was the strongest predictor of the bacterial and the fungal community, and only a weak influence of the habitat was recorded. All these results, including the one from our study, suggested that the microbial species pool of forest ecosystems is not specialized on a specific litter type but could quickly adapt to the new input [42].

Our results however were in contrast with those of Aneja et al. [19] and Dilly et al. [51] as both report a site effect in addition to the effect of the type of litter. Eventually, such results are related to the relatively short time of the experiment (only 8 weeks) compared to more than 500 days of our study and to the fact that Dilly et al. [51] used another type of litter, in detail agricultural litter across Germany.

In conclusion, our experiment found a decoupling of the structure of the microbial community and the potential enzymatic activities in three different decomposing types of litter in the alpine region. The decoupling could be explained by functional redundancy where different species, within fungi and bacteria or across the two domains, could replace the others in their functional role. Secondly, the site did not influence the microbial community being eventually outperformed by the importance of the litter type, leading to the hypothesis of a generalist microbial species pool in forest systems, quickly adaptable to new litter input.

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Author Contribution LBr conceived the study. AB, LB, SP and CW conducted the fieldwork. AB conducted the molecular and chemical analysis, AB and FF performed enzyme analysis. Statistical analysis was performed by AB and LB. AB drafted the manuscript. All authors read, edited and approved the manuscript.

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