

Functional Ecology

Low-quality carbon and lack of nutrients result in a stronger fungal than bacterial home-field advantage during the decomposition of leaf litter

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Acknowledgements

Authors would like to show their gratitude to the Municipality of Donostia/San Sebastián and to the Sociedad de Diviseros del Monte de La Isla (Peñacerrada/Urizaharra) for providing permission to use and access to the studied sites. This work was supported by networking support by WG2 of the COST Action FP1305 “BioLink” and grants from the Swedish research council (grant no 2015-04942 and no 2020-04083), the Swedish research council Formas (grant no 2018-01315), the Knut and Alice Wallenberg Foundation (grant no KAW 2017.0171) and INIA predoctoral grant FPI2014-040. Additionally, funding for this study was provided by the Spanish Ministry of Economy and Competitiveness through CHARFOR (RTA2012-00048-00-00) and the Department of Economic Development and Infrastructure of the Basque Government.

Authors' contributions

NGB and AAG established the field sites, field experiments, and litter traps. GBC and JR designed the study, and GCB performed experiments, analyses, data analyses and interpretations supported by JR. GCB

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as doi: [10.1111/1365-2435.13822](https://doi.org/10.1111/1365-2435.13822)

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and JR wrote the MS, and all co-authors contributed to revising the MS and gave approval for its final submission.

Data accessibility

Data upon which this study is based are available through the Dryad Digital Repository <https://doi.org/10.5061/dryad.prr4xgxm4> (Benito-Carnero et al. 2021).

Abstract

1. Decomposition of litter is a key biochemical process that regulates the rate and magnitude of CO₂ fluxes from biosphere to atmosphere and determines soil nutrient availability. Although several studies have shown that plant litter decomposition accelerated in their native compared to a foreign environment, i.e. a home field advantage (HFA) for litter degradation, to date HFA has only been considered in term of respiration or litter mass loss.
2. The competitive success of the decomposer microorganism will be determined by its ability to transform used OM into population growth. Therefore we hypothesized that HFA for microbial growth would be more pronounced than that for decomposition. We also expected that HFA effect for decomposition and microbial growth would increase with lower quality litter, which the fungal role in litter decomposition would be more dominant than that of bacteria, and that HFA effects would strengthen with more pronounced differences between 'home' and 'away' environments.
3. We designed a 2-month microcosm reciprocal transplant experiment with litter from two sites with contrasting climates (Atlantic and Sub-Mediterranean climates) and including 3 tree species (*Quercus robur*, *Pinus sylvestris* and *Fagus sylvatica*).
4. We found a stronger HFA for microbial growth than for decomposition, that the nutrient content and C-quality of litter influenced the microbial HFA, and that interactions between bacterial and fungal communities during litter decomposition modulated the HFA for litter degradation.
5. Low litter nutrient content, strong nutrient limitations and low C-qualities all favoured fungal over bacterial decomposers, and our results suggest a dominant functional role of the fungal community and gave rise to HFA effect for fungal growth but that this translated to only marginal implications for overall decomposition of litter.

Keywords

Bacteria, fungi, home field advantage, leaf, litter decomposition, microbial growth, microcosm, reciprocal transplant experiment

Introduction

The decomposition of litter is a key ecosystem process critical to both carbon (C) and nutrient cycling, determining both the flux of CO₂ from the soil to the atmosphere and soil nutrient availability (Coûteaux et al., 1995; Prescott, 2010). It is well-known that climate and litter quality are the primary determinants of the rate at which leaf litter is decomposed (Berg et al., 1993; Aerts, 1997), which together explain around 70% of the variation in global decomposition rates (Gholz et al., 2000; Cornwell et al. 2008). However, recent studies have also revealed an important role for the decomposer community independent of climate and litter quality. Thus, there is now growing evidence that decomposer community composition can influence litter decomposition rates over and above climate and litter quality controls (Wall et al., 2008; Keiser & Bradford, 2017). Increasing evidence suggests that specific interactions between microbial decomposers and plant litter, named home-field advantage (HFA), influence litter breakdown (Gholz et al., 2000; Ayres et al., 2006; Veen et al., 2019). Although HFA effects for litter decomposition has been demonstrated in several studies, there has been considerable variation in the reported strength and direction of these effects (Veen et al., 2015a), yielding situations where native litter can decompose faster, slower, or similarly in a foreign compared to native environments (Giesselmann et al. 2011; St John et al. 2011; Perez et al., 2013). Despite growing evidence for the widespread occurrence of HFA effects, their causes remain difficult to define and to generalise (Veen et al., 2015a; Veen et al., 2018).

If there are feedbacks between the litter and the soil that will generate HFA effects for litter use, the decomposer trait that needs to be selected for is a better ability to exploit resources to generate growth (Kirchman, 2016; Rousk, 2016). That is, the ecological and evolutionary success of decomposer microorganisms will be determined, not by their ability to mineralise OM, but by their ability to transform used OM into population growth. To date, studies on HFA effects for litter decomposition have considered the decomposer ability by resolving the contribution to litter mass loss (St John et al., 2011; Veen et al., 2015a, 2018; Lin et al., 2019) or respiration (Ayres et al., 2009). However, no studies to date have investigated HFA effect for metrics of microbial growth.

It is known that plant species diversity and traits represent important drivers of decomposer communities (Freschet et al., 2012; Wardle et al., 2006; Perez et al., 2013). Leaf litters derived from different plants have a large variation in chemical and physical characteristics. Because plant litter is an important source of nutrients and energy for soil biota, and because the ability to extract such resources from different types of litter differs between soil organisms, an ecological and

evolutionary incentive is created where the soil decomposer community is specialized for the litter it receives (Morriën et al., 2017). That is, the quality of the litter may act as environmental filter and prevent the establishment of certain microorganisms (Kraft et al., 2015) resulting in litter-type-specific microbial communities (Veen et al., 2019). In line with this, previous studies observed that litter quality will influence the strength of the soil-litter interaction, with observations suggesting that HFA was greater for recalcitrant litter types than for labile litter (Ayres et al., 2009; Freschet et al., 2012; Veen et al., 2015a). To explain this, Zhang et al., (2008) suggested that the compounds found in labile litter can probably be degraded by a wide variety of organisms, whereas the complex compounds found in recalcitrant litter likely require specialized enzymes, restricted to only a few taxa, in order to be decomposed (Ayres et al., 2009; Wallenstein et al., 2013; Veen et al., 2015a). Since it is the microbial community present in the soil that to a large extent determines which species are available to colonize the litter during decomposition, this implies that HFA effects may grow stronger in situations where ‘home’ and ‘away’ environments, along with their resident soil communities, are more distinct (Lin et al., 2019).

Although it is known that the microbial community could be important determining the HFA effect, reports of the relative contributions of soil microorganism to HFA are scarce in the literature (Fanin et al., 2016). In the few reports available, the contribution of different microbial groups to litter decomposition seem distinct. In general, fungi are thought to have a greater ability to (i) decompose recalcitrant plant material (ii) and tolerate very low nutrient conditions whereas bacteria are thought to be more efficient at rapidly exploiting labile C compounds (de Boer et al. 2005; Deveau et al., 2018). From these observations, it would follow that the fungal community is important for determining the direction and magnitude of HFA effect for recalcitrant litter decomposition, while the bacterial community may be dominant for labile litter decomposition (Lin et al., 2019; Veen et al., 2019).

In our study, we targeted responses of HFA effects not only for decomposition (respiration or mass loss), but also for the colonizing microbial community (bacterial and fungal growth) in 3 different forest types growing in two contrasting environments in addition to explore home and away microbial community functioning and composition. We hypothesized that: 1) HFA effects would be stronger for microbial growth than for decomposition (respiration); 2) HFA effects for decomposition and growth would increase (a) with lower quality litter (i.e. higher lignin content and total fibre content and lower nutrient concentrations) and (b) with stronger microbial nutrient limitation (higher ratios of enzymes for nutrient acquisition to those for C acquisition); 3) The HFA effects for

fungal growth would increase more than those for bacteria with lower quality litter (i.e. higher lignin content and lower nutrient concentrations). 4) The HFA effect would increase with the difference between the quality of litter exchanged between 'home' and 'away' sites.

2. Material and methods

2.1 Experimental design and sample collection

We designed a 2-month microcosm reciprocal transplant study with litter and soil from 2 sites with contrasting climatic conditions (M-site was in a Sub-Mediterranean climate and A-site was in an Atlantic climate) and including 3 different tree species (*Quercus robur* L., *Pinus sylvestris* L. and *Fagus sylvatica* L.) at each of the two sites. The laboratory microcosm study was carried out at controlled temperature and moisture to standardise any direct influence by climate on the litter decomposition processes. We measured respiration, bacterial growth and fungal growth throughout the experiment to determine the responses of the microbial community to different treatments and estimated the HFA effect for each process. To elucidate putative factors that could drive HFA effect, we determined the litter quality of all studied litters, and the physiochemistry of the studied soils. In addition, to link HFA to the perceived nutrient quality of the studied litters, enzymatic activities related with C, N and P cycles were also measured. Finally, PLFA analysis were performed to screen for differences between microbial community structures resulting from different combinations of soils with litter.

The O layer (approx. 5-10 cm deep) and leaves for the microcosm experiment were collected within the forest at Artikutza (43°13'N 1°48'W, Goizueta) and Montoria (42°38'N, 2°44'W, Urizaharra), located in the northern and southern Basque County, respectively, in north Spain. Both study sites have acid soils, with pH-H₂O 3.9 in Artikutza ("A-site") and 4.4 in Montoria ("M-site"). Soil sampled from the A site has a sandy clay loam soil texture with 13% of organic matter and that sampled from the M site also had a sandy loam soil texture, with 9.4% of organic matter. Both soils were classified to be Umbisols (WRB, 2015). These two study sites differ in climatic conditions. While the northern site (Artikutza, or A-site), is located in an Atlantic climate with annual mean precipitations about 2500 mm, 16°C annual mean temperature and annual 31 frost days, the southern M-site is in a sub-Mediterranean climate in which mean annual precipitation is approximately 650 mm, i.e. 4 times lower than in A site, and the mean annual temperature is 10.5°C, i.e. about 5.5 °C cooler than the A-site, and the frost days are approximately double than in A-site (58 days). We selected three

different tree species, similar in age, density and structure, which were present in the two areas: common oak (*Quercus robur* L.), scots pine (*Pinus sylvestris* L.) and European beech (*Fagus sylvatica* L.).

Naturally senesced leaves were collected with litter traps (composited from 4 litter traps per forest type) between late October 2017 and early January 2018 from oak, pine and beech stands within each study site. They were oven dried (40°C) until dry (>48 h) and ground. O layer samples (top 5-10 cm) were collected in early January 2018 during frost free conditions in both sites. Composite samples were collected in each stand around each litter trap. O layer samples were sieved (4 mm) and kept at 4°C for a week until microcosm experiment started.

Different microcosms were prepared for a reciprocal transplant experiment of litter from different study sites for each of the three tree species (Figure 1). Litter from the A-site was combined with its native soil ('home'), but also with soil from the other site ('away'), and *vice versa*, resulting in 4 different treatments per tree species, and thus a total of 12 treatments. The treatments were replicated in three (n=3), resulting in 36 microcosms that were monitored over time. As we wanted to investigate the soil environment's role in affecting the decomposition of litter by microbes, capturing this necessitates a sufficiently large proportion of soil to litter. Without it, the environments in the microcosm environments would be dominated by the litter only. Therefore, in each microcosm, 60 g of soil (O-layer) were adjusted to optimal moisture, at 50% of water holding capacity, after which 12 g of dried and ground litter were added. This combination of O layer with litter was used to simulate incoming litter into the top of a forest floor. Unsterilized litter will bring with it a small amount of microbes that could contribute to seed the soil environment below. However, the dried litter samples should have carried only a negligible microbial community compared to that already active in the O-horizon sample used, which we argue natural conditions of litter fall on a forest floor well. The microcosms were incubated at 16°C, thus falling within a range of summer soil temperatures normal for both sites, and constant moisture (adjusted as needed) for 2 months, and respiration, bacterial growth and fungal growth were monitored (10 sampling times: 1, 3, 5, 8, 12, 21, 28, 35, 42, and 55 days). In addition, biomarkers for the microbial community size and composition (PLFA) were determined 3 times (1 day, 28 days and 55 days) over the experiment. Basic soil chemical properties, pH and electrical conductivity, and enzymatic activities were measured in the first and in the last sampling times to capture the differences in microbial nutrient availability.

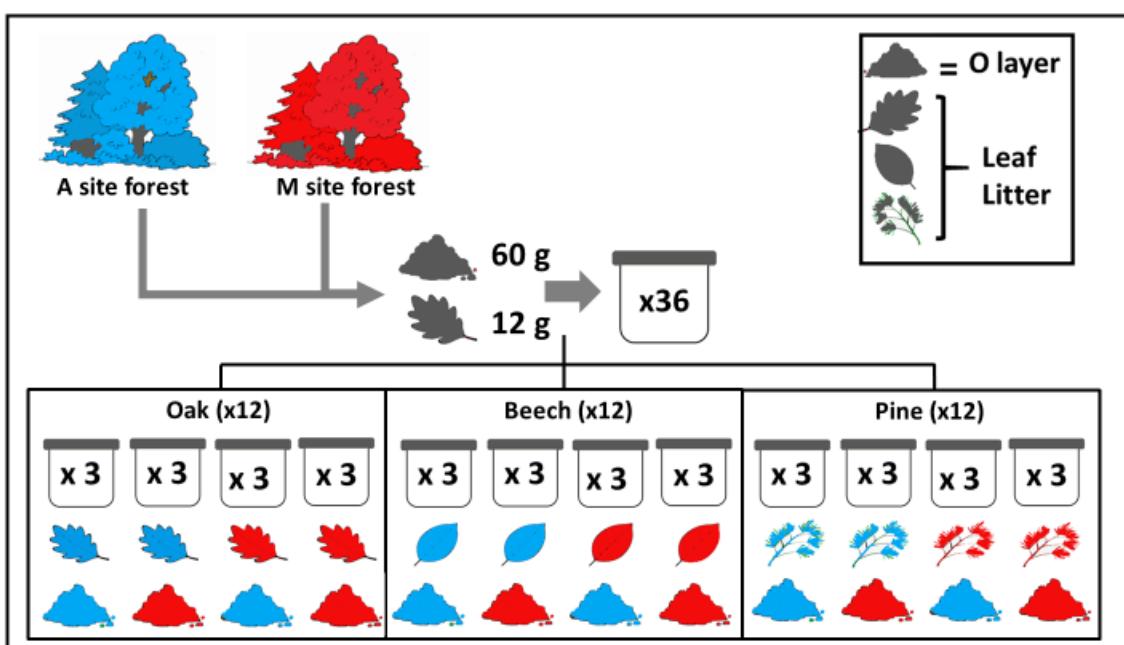


Figure 1. Experimental design used in the microcosm study where the effect of a reciprocal litter transplant between different climates was tested in oak, beech and pine forests. In each microcosm a mix of 60 g fresh O layer and 12 g dried leaf litter was added to simulate incoming litter into the top layer of the forest floor. Blue symbols indicated samples taken in A-site while red symbols indicated samples taken in M-site. A total of 36 microcosms were used, 3 replicates per treatment.

2.2 Microcosm microbial parameters

Bacterial growth was measured by the incorporation of ^{3}H -Leucine (Leu) into bacteria (Bååth et al., 2001). At each time point, 1 g of soil was mixed with 20 ml demineralized water by vortexing for 3 min. The supernatant with a bacterial suspension was sampled after low speed centrifugation (1000 g for 10 min) and the incorporation of Leu was measured in 1.5 ml aliquots of the bacterial suspension. A combination of nonradioactive and tritiated Leu (^{3}H -Leu, 37 MBq ml⁻¹, 5.74 TBq mmol⁻¹, Perkin Elmer, USA) were added to yield a final concentration of 275 nM in the bacterial suspension. The extracted bacteria were incubated for 1 h at 16°C. The samples were washed (Bååth et al., 2001) and the radioactivity of the incorporated Leu was measured on a liquid scintilator. Bacterial growth was expressed as the amount of Leu that was incorporated in the extracted bacteria per g dry soil and per h (Rousk and Bååth, 2011).

Fungal growth was assessed using the acetate-into-ergosterol-incorporation (Bååth, 2001; Rousk et al., 2009). A radioactively labelled acetate solution (20 µl 1-[^{14}C] acetic acid (sodium salt), 37

MBq/ml, 2.10 GBq/mmol, Perkin Elmer), was combined with 30 µl unlabelled 16 mM acetate and 1.95 ml water was added to 0.5 g of soil, yielding a final concentration in the soil mixture of 0.2 mM, and incubated for approximately 4 hours at 16°C. Growth was terminated by adding 500 µl 10% formalin, and ergosterol was extracted and separated using high performance liquid chromatography and a fraction collector (Rousk and Bååth 2007). The incorporated ¹⁴C-acetate into ergosterol was measured using liquid scintillation and used as a proxy for fungal growth (Rousk & Bååth, 2011).

Soil respiration was measured using a gas chromatograph equipped with a methanizer and a flame ionization detector. For each sampling time, 0.5 g of soil was put into a 20 ml glass vial, which were purged with air, sealed and incubated at 16°C (microcosm incubation temperature) for 2-6 hours (Silva-Sánchez et al., 2019). The concentration in the air used for purging was subtracted from the headspace concentrations, which was then divided by the incubation time to estimate the respiration rate. The respiration rates were expressed as µg CO₂ per g dry soil and per h.

2.3. PLFA analysis

The microbial phospholipids were extracted in a one-phase mixture of chloroform, methanol, and citrate buffer (Frostegård et al., 1991) from frozen subsamples (see above). Then, phospholipids were fractionated and methanolyzed into free fatty acid methyl esters (FAMEs) with FAME 19:0 added as an internal standard (Frostegård et al., 1993; Cruz Paredes et al., 2017). The resulting phospholipid fatty acids (PLFAs) were separated and quantified on a GC with a flame ionization detector (Frostegård et al., 1993). A total of 18 PLFAs were selected and used to indicate the microbial community composition, and to estimate total, bacterial and fungal biomass concentrations. Fungal biomass was quantified based on 18:2ω6,9 content (PLFAfun), and bacterial biomass (PLFAbact) was quantified as the sum of i15:0, a15:0, i16:0, 16:1ω7c, i17:0 cy17:0, 18:1ω7, 18Me18:0 and cy19:0 (Frostegård and Bååth, 1996; Ruess and Chamberlain, 2010), and the total biomass (PLFAtot) was calculated as the sum of the 18 PLFAs.

2.4. Enzymatic activities

Microbial biomass C:N:P ratios are relatively invariant across ecosystems (Cleveland and Liptzin, 2007). However, it is suggested that differences in the microbial effort directed towards different resources could be used as indicators to infer limiting factors for growth. A high enzymatic activity

targeting one resource in relation to that targeting another should then indicate the relative strength of limitation by those two resources (Sinsabaugh et al., 2008). As such, and ratio of enzymes targeting C compared to those targeting N is lower than 1, N is more limiting than is C, and *vice versa*. Following the comparison of enzymes targeting N compared to those targeting P, followed by a comparison of enzymes targeting C compared to the more limiting nutrient will provide an assessment for the nutrient most limiting microbial growth in the studies system (Sinsabaugh et al. 2008).

Hydrolytic enzyme activities were determined as described by ISO/TS 22939. Briefly, 1.5 g of frozen sample was homogenized in 150 ml distilled water using ultrasonic probe at 40% intensity (50 watts) for 1 min in an ice bath. The homogenate was used as a sample. The activities of β -glucosidase (B-glu), quitinase (Qui), β -xylosidase (B-Xy), acid phosphatase (Phos), Leucine-aminopeptidase (Leu-am) and Alanin-aminopeptidase (Ala-am) were then measured using 4-methylumbelliferyl (MUF) or 7-amido 4-methylcoumarine (AMC) based substrates. The substrates at final concentration of 500 μ M were combined with 4 technical replicates of 100 μ l of extract in a 96-microwell plate. The pH used in the assay were used to optimize fluorescence, as established by ISO/TS 22939 (6.1 for activities whose substrates produce MUF and 7.8 for activities whose substrates produce AMC). For the background fluorescence measurement, a standard curve of MUF and AMC for each soil sample was added. The multi-well plates were incubated at 30°C and the fluorescence was recorded at different times (0, 20, 40, 60 minutes) using a microplate reader at an excitation wavelength of 360 ± 40 nm and an emission wavelength of 460 ± 40 nm. The quantitative enzymatic activity was calculated based on a standard curve of MUF and AMC and expressed as $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$. The enzyme ratios presented represent molar ratios.

2.5. Home-field advantage (HFA) of litter decomposition and microbial colonization

The home field advantage index was estimated for each tree species paired plot as described by Ayres et al., (2009). The index is a measure of the change in decomposition of both litter types at home based on the relative decomposition within each habitat (Ayres et al., 2009; St John et al., 2011). To estimate decomposition, we used the cumulative CO₂ produced. If we let "A" and "M" represent the litters of the three species under consideration and "a" and "m" their respective habitats, then their relative CO₂ released ('R' for respiration) within each habitat are calculated as:

$$A_{Ra} = Aa / (Aa + Ma)$$

$$\text{HFA index} = (A_{Ra} + M_{Rm}) / (A_{Rm} + M_{Ra})$$

We also calculated the HFA index for fungal and bacterial growth resolved over the course of our experiments.

2.6. Data analysis

Values of bacterial growth, fungal growth and respiration rates as well as on the enzymatic activities' ratio, and bacterial, fungal and total biomass over time were compared with repeated measures 3-way ANOVAs, considering the factors "soil environment" ("A-site" soil or "M-site" soil), "litter origin" (litter from "A-site" or "M-site") and "litter species" (oak, pine or beech), and in all factorial combinations, using the Restricted Maximum Likelihood (REML) method in JMP (SAS Inst., USA). In addition, cumulative bacterial growth, fungal growth and respiration were estimated as the integral of rates overtime and compared with 3-way ANOVAs with the same fixed factors as above. The HFA effects for bacterial growth, fungal growth and respiration were determined using t-tests comparing the HFA results obtained at each sampling point with 0 (i.e., distinguishable from no-effect).

The relative difference between qualities of reciprocally transplanted litters were calculated as Veen et al., (2015b), using the equation:

$$\frac{|\text{Quality(A site)} - \text{Quality (M site)}|}{\text{Quality(A site)} + \text{Quality (M site)}}$$

In order to study the correlation between litter quality values and litter quality dissimilarities with different HFA index, in addition to study the correlation between bacterial HFA index with fungal HFA, Pearson correlations were carried out. Pearson correlations were also used to explore links between HFA effects on bacterial and fungal growth and for respiration.

Finally, PCA analysis was performed using PLFA data of each treatment and at each sampling point to an overview of the differences in microbial community between soil environments, litter origin and litter tree species and the development over the 2 months of the microcosm study.

3. Results

3.1. Chemical analysis

Litter quality for each tree species and study site combination were illustrated in Table 1. The A-site litter had higher nutrient contents and lower C:N ratio, indicating a better litter quality, but also a

higher lignin content, suggesting persistence. Pine litter has a lower amount of N and lignin, and a higher ratio of C:N and cellulose content the greatest differences in chemistry in comparison with beech and oak.

Table 1. Litter content of C (%), N (%), the C:N-mass ratio and lignin, cellulose and hemicellulose contents (%). Shown are mean values (n=4) and standard errors (SE) for different tree species at each incubation site. “A” and “M” letters represent the factors “soil environment” (“A-site” soil or “M-site” soil) and “litter origin” (litter from “A-site” or “M-site”).

Sp.	Soil enviroment	Litter origin	Soil		Litter		N:P	C:N	Lignin (%)	Cell (%)	Hemi (%)
			C %	N %							
Oak	A	A	50.2 (0.4)	1.45 (0.12)	3.85 (0.31)	34.8 (3.2)	35.8 (0.9)	5.00 (1.16)	5.93 (0.8)		
	M	A	48.7 (0.9)	1.38 (0.09)	3.54 (0.24)	35.5 (3.0)	36.4 (3.3)	4.38 (0.91)	6.87 (1.42)		
	M	M	48.8 (0.3)	1.06 (0.04)	1.70 (0.31)	46.1 (1.8)	30.9 (1.6)	4.99 (1.48)	9.24 (0.71)		
	A	M	49.1 (0.2)	1.06 (0.05)	1.69 (0.24)	46.2 (2.2)	28.5 (28.5)	4.47 (1.32)	8.00 (1.56)		
Pine	A	A	51.0 (0.4)	0.64 (0.08)	2.91 (0.22)	81.1(8.9)	28.7 (2.2)	17.00 (1.25)	9.00 (0.31)		
	M	A	50.8 (0.3)	0.72 (0.1)	2.64 (0.38)	71.7(10.8)	27.6 (0.9)	17.36 (1.15)	7.55 (0.83)		
	M	M	51.6 (0.3)	0.75 (0.11)	4.40 (0.84)	64.3(4.7)	33.9 (1.0)	11.98 (1.21)	8.76 (0.22)		
	A	M	53.2 (2.8)	0.65 (0.11)	4.54 (0.42)	83.1(13.3)	32.3 (1.6)	12.74 (0.69)	8.35 (0.64)		
beech	A	A	48.6 (2.2)	1.34 (0.14)	3.40 (0.67)	36.6(4.3)	38.9 (2.9)	8.32 (2.02)	6.66 (0.90)		
	M	A	49.9 (0.9)	1.27 (0.04)	4.36 (0.43)	39.4 (1.5)	43.7 (0.9)	9.60 (1.26)	4.78 (0.64)		
	M	M	43.9 (2.9)	0.96 (0.09)	2.51 (0.18)	48.8 (3.1)	31.2 (5.2)	11.96 (1.07)	8.82 (1.59)		
	A	M	45.1 (2.3)	0.99 (0.03)	2.47 (0.41)	45.8(3.0)	30.5 (2.7)	11.63 (2.66)	6.99 (1.87)		

Abbreviations: cellulose = “Cell” and hemicellulose = “Hemi”)

The soil pHs in freshly sampled soil were between 4.7 ± 0.3 and 5.1 ± 0.1 in the litter combinations with the A-soil environment, and in all three species were significantly higher ($p < 0.001$; $F = 20.3$; $df = 1$; Table 2), between 5.2 ± 0.1 and 6.7 ± 0.2 , with the litter combinations with the M-soil environment. Higher values were also found in the soil-litter combinations of beech compared to oak or pine ($p < 0.0001$; $F = 133$; $df = 2$; Table 2). The soil pH values changed after 2 months of incubation, and a significant interaction between tree species and litter origin ($p < 0.0001$; $F = 34.8$; $df = 2$) indicated that the initial patterns between litter and soil environments had reversed (Table 2). Estimated electrical conductivities were higher in the M than the A environment for oak and beech while values for pine were similar in the two soil environments (Table 2). Overall, a decrease in these values were observed after 2 months of incubation (Table 2).

Table 2. pH and electrical conductivity ($\mu\text{S cm}^{-1}$) of each microcosm at the beginning and end of the

Tree species	Litter origin	Soil environment	pH		Electrical conductivity ($\mu\text{S cm}^{-1}$)	
			Initial pH	2 months	Initial EC	2 months
Oak	A	A	4.8 (0.1)	4.9 (0.1)	140 (25)	112 (32)
	M	M	6.0 (0.3)	5.9 (0.2)	376 (55)	146 (10)
	A	M	6.0 (0.3)	5.4 (0.2)	376 (55)	141 (17)
	M	A	4.8 (0.1)	5.6 (0.2)	140 (25)	108 (5)
Pine	A	A	4.7 (0.3)	5.3 (0.1)	173 (33)	66 (7)
	M	M	5.2 (0.1)	6.0 (0.1)	156 (18)	94 (3)
	A	M	5.2 (0.1)	5.6 (0.1)	156 (18)	75 (5)
	M	A	4.7 (0.3)	5.6 (0.1)	173 (33)	85 (4)
Beech	A	A	5.1 (0.1)	5.6 (0.1)	121 (13)	68 (7)
	M	M	6.7 (0.2)	7.0 (0.2)	244 (84)	120 (95)
	A	M	6.7 (0.2)	6.1 (0.1)	244 (84)	102 (7)
	M	A	5.1 (0.1)	6.6 (0.1)	121 (13)	135 (9)

incubation. Values are means with standard errors (SE).

3.2 Soil microbial growth and respiration

Bacterial growth was similarly stimulated in all treatments and the three tree species, with highest bacterial growth rates in the first 5-8 days of incubation, and then decreased continually over time (Table S1; Fig. 2. a-c). Overall bacterial growth rates over time were distinguishable between both litter origin ($p<0.001$; $F=26.9$; $df=1$) and tree species ($p<0.001$; $F=8.26$; $df=2$), being higher in microcosms with M litter in oak and beech and lower in pine, but not between soil environments. No clear differences between home and away treatments were found (interaction term litter origin*soil environment $p=0.6$; $F=0.28$; $df=1$). These dynamics translated into cumulative bacterial growth as indicated by [^3H]Leu incorporation with a similar pattern home and away (interaction term litter origin*soil environment $p=0.42$; $F=0.67$; $df=1$) in both litter types and in the three tree species (Fig. 2.d). The M site litter resulted in the highest cumulative bacterial growth in the two broadleaved trees oak and beech. On average, the cumulative bacterial growth showed values between 30-50% higher in comparison to bacterial growth in A site litter ($p <0.001$; $F=31.6$; $df=1$). In contrast, in the case of pine litter, cumulative bacterial growth values were similar for all microcosm treatments with

final values between 1.2 and 1.5 $\mu\text{mol g}^{-1}$, with an interaction between tree species and litter origin factors ($p<0.001$; $F=16.9$; $df=2$).

The variability between fungal growth rates in different treatments was higher in the first 5-8 days of incubations, but after that the fungal growth rate remained stable throughout the entire experiment ($p<0.001$; $F=8.4$; $df=9$). Differences in rates differed by tree species ($p<0.05$; $F=3.42$; $df=2$), soil environment ($p<0.05$; $F=5.9$; $df=1$) but not by litter origin (Table S1; Fig. 2. e-g), and effects were dynamics over time, leading to interactions between fixed factors and time (Table S1). These dynamics resulted in a cumulative fungal growth (Fig. 2. h), that increased linearly overtime. Overall fungal growth was indistinguishable between the different microcosm conditions, with marginally lower rates occurring in pine litter than in the two broadleaved species ($p<0.001$; $F=3.42$; $df=2$). Cumulative fungal growth was between 13-20% higher for oak and beech than in pine samples. No differences were found between soil environments ($p=0.14$ $F=2.3$; $df=1$) or between litter origins ($p=0.88$; $F=0.03$; $df=1$) (Fig. 2. h).

Respiration rates peaked immediately, and then decreased over time in all treatment and in the three tree species. Although initial respiration rates were higher in pine samples, reaching levels approximately 40% higher than initial respiration rates in broadleaved species, the final respiration rates were similar for all tree species, with overall mean values between 25 and 89 $\mu\text{g CO}_2\text{g}^{-1}\text{h}^{-1}$ (Table S1; Fig. 2. i-k). Overall, respiration rates could be distinguished between tree species ($p<0.001$; $F=116$; $df=2$), litter origin ($p<0.001$; $F=29.9$; $df=1$), and soil environments ($p<0.001$; $F=32.6$; $df=1$), and effects varied over time creating interactions with time (Table S1). These subtle but consistent differences in rates over time accumulated into levels that could be distinguished for each tree species in cumulative respiration (Fig. 2. l). Overall higher cumulative values were observed in pine samples, followed by oak and beech ($P<0.0001$ $F=109$; $df=2$). Similarly to bacterial growth, there was an interaction between tree species and litter origin ($p<0.001$ $F=14.6$; $df=2$) with cumulative respiration values higher in microcosms with M litter ($p<0.0001$ $F=30.3$; $df=1$) in the case of broadleaves species. In contrast, no differences were found in the case of pine litter between soil environments or between litter origins, with final respiration values between 1.4 and 1.5 $\mu\text{g CO}_2\text{g}^{-1}$.

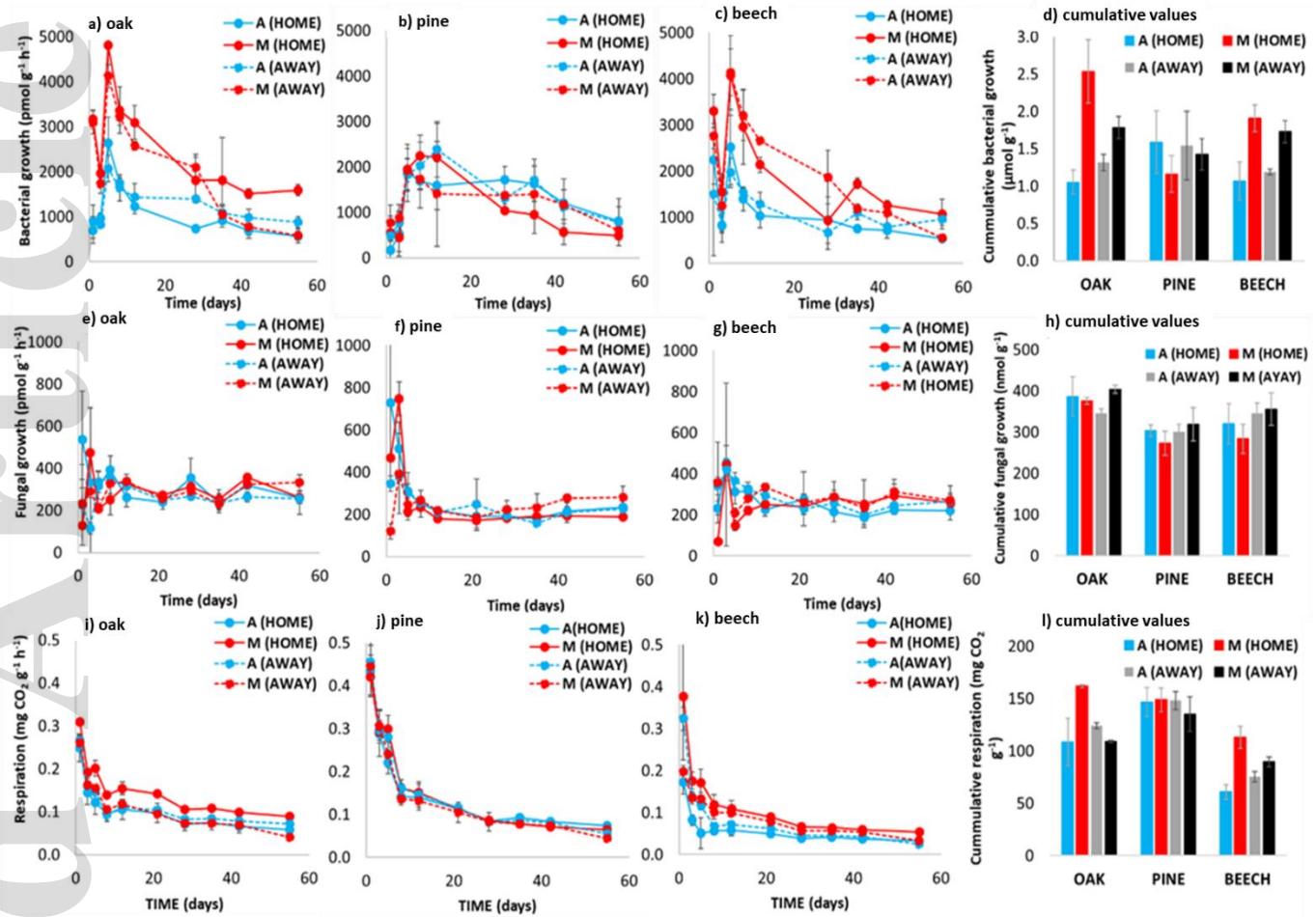


Figure 2. Bacterial growth rates ($\text{pmol g}^{-1}\text{h}^{-1}$) (a-c), Fungal growth rates ($\text{pmol g}^{-1}\text{h}^{-1}$) (e-g) and Respiration rates ($\text{mg CO}_2\text{ g}^{-1}\text{h}^{-1}$) (i-k) for oak samples, pine samples and beech samples, and final cumulative bacterial growth ($\mu\text{mol g}^{-1}$) (d), cumulative fungal growth (nmol g^{-1}) (h) and cumulative respiration ($\text{mg CO}_2\text{ g}^{-1}$) for all treatments and tree species. Data points and bars show mean values $\pm 1 \text{ SE}$; for some data points the error bars are smaller than the symbol itself.

3.3 Home-field advantage indices for respiration, bacterial growth and fungal growth

HFA effect for respiration were of limited size and mostly constant over the 2 months of the incubation (Fig. 3. a). No HFA for decomposition was found for oak or pine, while small and transient reductions of HFA effect were found for beech litter. Different patterns were observed for HFA indices for bacterial and fungal growth. For bacterial growth, there were initial positive HFA effects for the broadleaves oak and beech, while the HFA effects for pine initially were negative (Fig. 3. b). The HFA effect was still detectable for oak during most of the initial three weeks, while the negative HFA effects for pine were discernible during the first week. For beech, this initial HFA effect could

only be discerned the first few days. For fungal growth there were initially positive HFA effects for oak and pine, while there were negative HFA effects for beech (Fig. 3. c). There were significant and negative correlations between bacterial and fungal HFA effects for pine ($p<0.001$) and beech ($p<0.001$; Fig. 3. d), while no links were found for oak ($p=0.7$).

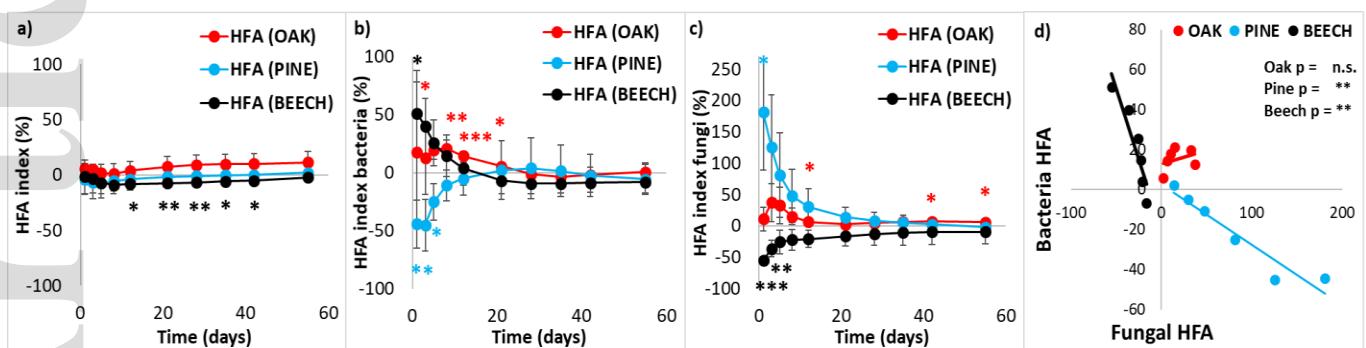


Figure 3. HFA index values (%) for different tree species (oak, pine and beech) over incubation time, and the correlation between fungal and bacterial HFA (%). a) HFA index for respirations data (panel a); HFA index for bacterial growth (panel b) and HFA index for fungal growth (panel c). Correlation between bacterial and fungal HFA index (%) (panel d). Data points show mean values ± 1 SE; * $p<0.05$; ** $p<0.01$; *** $p<0.001$ based on t-test comparisons with zero effect for individual time-points.

3.4. Enzymatic activities

In all cases values below 1 were found for N/P ratio, with the lowest values for pine litter, followed by oak and finally beech ($p<0.0001$; $F=379$; $df=2$). There were interactions between the different factors ($sp*soil$ $p<0.0001$; $F=29.9$; $df=2$; $sp*litter$ $p<0.0001$; $F=130$; $df=2$; $soil*litter$ $p<0.001$; $F=18.8$; $df=1$). Higher values were found in M-site litter for oak (mean value for M litter 0.43 and mean value for A litter 0.22) and beech (mean value for M litter 1.0 and mean value for A litter 0.34) in the first sampling point and the differences were maintained throughout the incubation. However, no differences were found for pine in the enzymatic N/P ratio (Fig. 4. d-f).

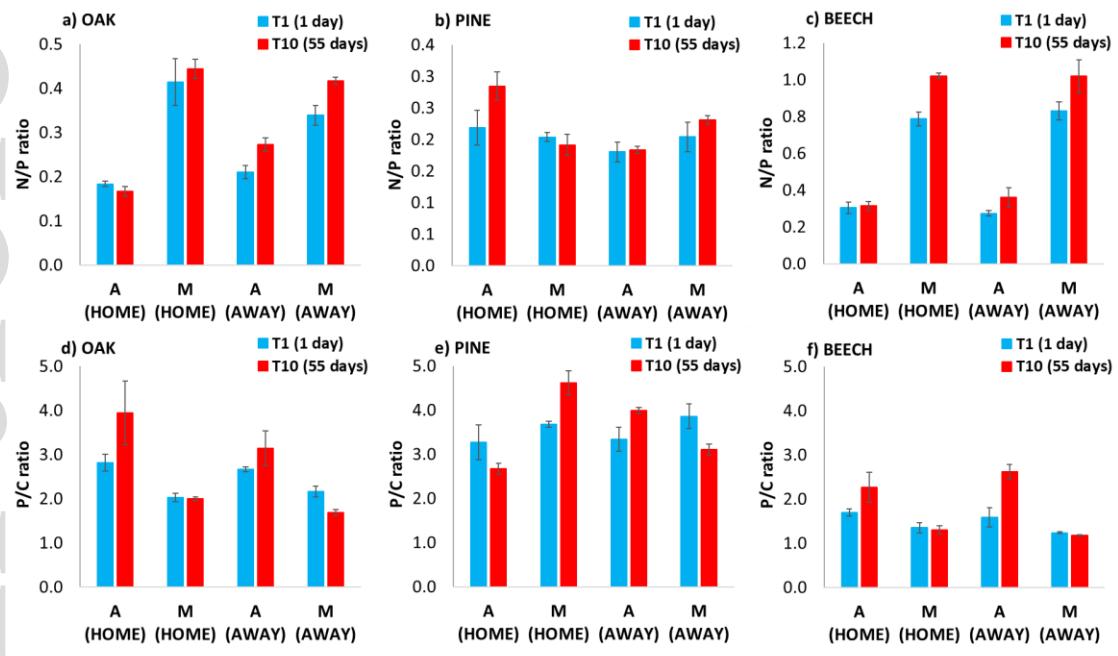


Figure 4. The ratios of extracellular enzyme activities for enzymes targeting N compared to those targeting P (Panel a-c), or those targeting P compared to those targeting C (panels d-f). High values indicate that microorganisms are dedicating more effort towards acquiring the enzyme in the numerator compared to that in the denominator, and vice versa, while an enzymatic ratio of 1 indicates a balanced need. Thus, values of enzymatic N/P lower than 1 and values of enzymatic P/C exceeding 1 indicate a microbial lack of P. Bars show mean values \pm 1 SE.

The values for enzymatic P/C ratios were overall higher than 1, and different for each tree species ($p<0.0001$; $F=155$; $df=2$). Values between 1-2 were found for beech, between 2-3 for oak and highest values were observed for pine, with values between 3-4, indicating increasing strengths of P limitation with higher values. There was an interaction between tree species and litter origin ($p<0.0001$; $F=31.8$; $df=2$), where higher values, suggesting stronger P limitation, were obtained for A-site litter in broadleaved species, and no differences were found between litter origins. While no relationship could be discerned between nutrient limitations inferred from enzyme ratios and respiratory or bacterial HFA. However, stronger limitation of P in litters with strong fungal HFAs were found (Table S2).

3.5. Biomass and community composition

Bacterial biomass measured using PLFA indicators revealed no marked differences between 'home' and 'away' combinations (p -value litter origin*soil environment=0.09; $F=3.37$; $df=1$) (Table S3).

Overall, an increase was observed in all treatments for microcosms with A-site litter ($p<0.01$; $F=11.4$; $df=1$) and also in microcosms with M-site litter when it was incubated in the A-site soil. Even so, bacterial PLFA concentrations decreased in samples with M-site litter at 'home'. Contrary to bacterial biomass, fungal biomass measured using PLFA indicators did not change markedly over time and no differences were observed between 'home' and 'away' treatments (Table S2). Higher ergosterol concentrations were found for pine and oak samples, and lower concentrations for beech. Moreover, microcosms with M-site litter had higher ergosterol concentrations. Total biomass measured using PLFA indicators were stable over time (Table S3), and no differences were observed between 'home' and 'away', between litter type or soil environment.

A principal component analysis of the PLFA composition from different microcosm revealed significant differences in PC scores between treatments, tree species and between sampling times. A large fraction of the variance, 26.6%, was explained by PC1, which aligned with differences between sampling times (Fig. 5.a) and tree species (Fig. 5.b). The PC2, which explained the 15.8% of the variability, aligned with differences between treatments, with litter types in their home environments in the extremes of the axis and litters in their away environments at intermediate positions (Fig. 5.a). Effect of different treatments at 'home' on the PLFA pattern was linked to higher concentration of the PLFAs 16:1 ω 7c and 18:2 ω 6,9 in the first day of the incubation with M-site with M-site litter while 16:0 and 18:1 were higher in A-site with A-site litter (Fig. 5.c). After 1 and 2 months of incubation, higher relative abundances of PLFAs markers PLFAs 15:0, cy17:0, 16:1 ω 5, i15:0, i16:0 and a15:0 were found in M-microcosms, and of PLFAs 18:0, 20:0, i17:0 and cy19:0 in the A-site microcosms. Intermediated values of these markers were found for 'away' treatments (A-site soil with M-site litter and M- site soil with A-site litter).

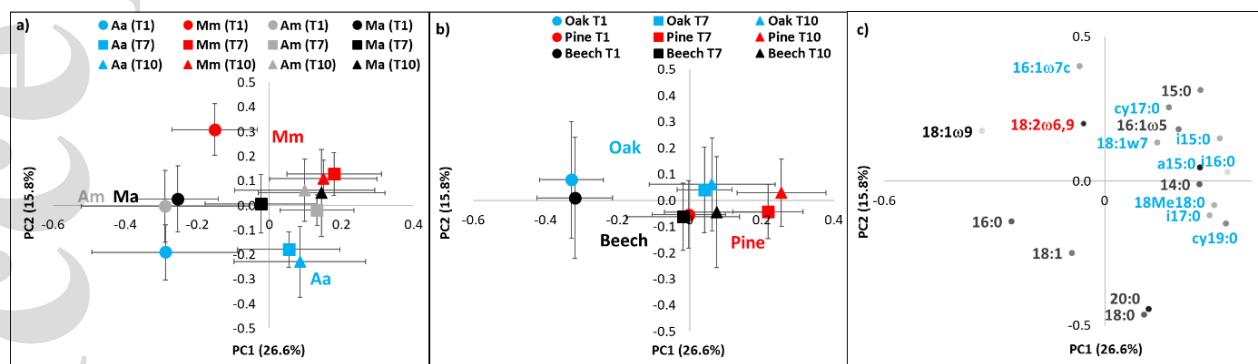


Figure 5. Principal component analysis of the PLFA composition. PC1 explains the 26.6% of the variability and PC2 explains the 15.8% of the variability. The principal component scores (PCs) were

coded either by treatments (panel a), where the capital letter (A or M) indicated the litter origin and lowercase letter (a or m) indicate the soil environment of each treatment, or coded by tree species (panel b; see legend). The variable loadings were also shown (panel c) showing which PLFA markers drove the sample separation. In blue there are the phospholipids used to determined bacterial biomass, in red the phospholipid to determined fungal biomass and in black the rest of the phospholipids. T1, T7 and T10 refer to the sampling times that corresponds to 1, 28 and 55 days. Values are means \pm 1 SE.

3.6. Linking HFA effects to litter quality, limiting factors and community structure

3.6.1. Litter quality

The HFA effects for respiration were not correlated to any litter quality parameter (Table 3). We found a negative link between bacterial HFA effects and the A-site litter C:N-ratio and C-content. In contrast, we found a positive link between fungal HFA effects and litter C-content, N-content and C:N-ratio for both sites. In addition, litter N:P, litter lignin content and litter lignin/N ratio all influenced fungal HFA effects (Table 3; Fig. S1).

Table 3. Results of the Pearson correlation matrix between bacterial growth (HFA bact.), fungal growth (HFA fung.) and respiration HFAs (HFA res.) and litter quality parameters. Correlation between litter quality dissimilarities (pairs of reciprocally transplanted litters calculate as Veen et al., (2015b).) with HFA indexes were also calculate.

	Dissimilarities between A								
	A site litter			M site litter			and M site litters		
	HFA res.	HFA bact.	HFA fung.	HFA res.	HFA bact.	HFA fung.	HFA res.	HFA bact.	HFA fung.
P(mg/kg)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
C (%)	n.s.	-0.84**	0.83**	n.s.	n.s.	0.81**	n.s.	n.s.	0.65**
N (%)	n.s.	n.s.	-0.76*	n.s.	n.s.	-0.80**	n.s.	n.s.	n.s.
N:P	n.s.	0.73*	-0.74*	n.s.	n.s.	n.s.	n.s.	n.s.	0.19*
C:N	n.s.	-0.68*	0.78*	n.s.	n.s.	0.91**	n.s.	n.s.	n.s.
lignin(%)	n.s.	n.s.	-0.91**	n.s.	n.s.	n.s.	n.s.	n.s.	-0.45**
lignin/N	n.s.	n.s.	n.s.	n.s.	n.s.	0.49**	n.s.	n.s.	0.69*

n.s.= not significant ($p>0.05$). * denote significant correlations (* $p<0.05$; ** $p>0.01$; *** $p>0.001$).

3.6.2. HFA correlation with dissimilarities in litter quality between sites and microbial community structure

Dissimilarities between chemical characteristics of both litter types did not explain variation in respiration or bacterial HFA effects. However, a negative relationship was found between C%, N:P, lignin and lignin/N differences and fungal HFA effects and a positive contribution of C:N dissimilarities to fungal HFA effects (Table 3; Fig. S2). The HFA effects of respiration and fungal growth were not correlated to PC1 of the PCA on the PLFA composition, while HFA effects for bacterial growth were negatively associated ($R=-0.43$; $p<0.001$; Table S4), and were thus linked to higher relative abundances of PLFA markers oriented towards negative PC1 variable loadings, and *vice versa* (Fig. 7c). For the second component, PC2, only HFA effects for respiration were weakly and positively linked to PC2 ($R=0.22$, $p=0.032$; Table S4).

3.6.3. Correlations between respiration and bacterial and fungal growth

We found a positive link between bacterial HFA effects and those of respiration. In contrast, a negative link between fungal HFA effects and those of respiration found (Table S5).

4. Discussion

4.1. HFA for respiration, bacterial growth and fungal growth

We found stronger and more variable HFA effects for bacterial and fungal growth than for decomposition. However, effects varied between litter species and between fungal and bacterial decomposers. As such, we find support for our first hypothesis with a stronger HFA effect for microbial growth than that for decomposition. This makes ecological sense. The ecological and evolutionary success of decomposer microorganisms will be determined, not by their ability to mineralise OM, but by their ability to transform used OM into population growth (Rousk, 2016). However, the variation in responses between the three studied litters also raises new questions. The negative HFA effects for decomposition observed for beech litter coincided with strong but transient HFA effects for bacterial growth concomitant with an inhibited fungal growth, while the inhibited bacterial growth and stimulation of fungal growth in home-field conditions left decomposition unaffected for pine litter. It has been suggested that rapid colonization of labile resources is a life history trait which has a trade-off to effective decomposition of persistent fractions of organic

matter (Allison, 2005; Kaiser et al., 2015; Malik et al., 2020). Such a trade-off could lead to a situation where early and rapid use of labile resources by bacteria inhibited the growth and OM decomposition of the fungal community which possessed a greater ability to degrade complex OM (de Boer et al., 2005; Deveau et al., 2018). In line with this, we observed a positive relationship between the HFA effect of bacteria and respiration and a negative relationship between the HFA effects of fungi and respiration, which supports our reasoning. In contrast, the simultaneous stimulation of bacterial and fungal growth in home-field conditions were linked to a small but systematic tendency for a positive HFA effect for decomposition. It is possible that complementary use of resources derived from the oak litter could simultaneously benefit both groups and thus stimulate overall decomposer functioning (Fester et al., 2014; Deveau et al., 2018). These potential links between the HFA effects for decomposition and the colonizing microbes would also need to align with the nutrient qualities of the litters, the dissimilarities between litter qualities in home vs away conditions, the nutrient limitation of the microbial communities, as well as the microbial community structure associated with the litter in home or away conditions.

4.2. Relationship between HFA effect, litter quality and nutrient limitation

It is known that litter quality is different not only between tree species but also within species (LeRoy et al., 2007). These differences in the chemistry will influence the decomposition process, both in the mineralization of OM and in the structure and traits of the colonizing microbial community (Bani et al., 2018), thus influencing HFA effects. Previous research found a greater HFA effect in the most recalcitrant leaf litter (Ayres et al., 2009; Freschet et al., 2012; Veen et al., 2015a) and we expected to find this pattern not only for respiration, but also for the HFAs of bacterial and fungal growth, as the breakdown of persistent litters may require the action of specialized decomposers (Ayres et al., 2009). However, our results do not support this hypothesis. HFA effect for respiration showed no significant relationship with different chemical parameters (Table 3). Fungal and bacterial HFA effects, in turn, did show significant relationships with some of the chemical parameters. The bacterial HFA effects were negatively correlated to C/N and C%, while the relationship between litter chemistry and the fungal HFA effects were more complex. The fungal HFA effects correlated negatively with N, N/P and lignin, and positively with C and the C/N ratio, thus directly opposing some patterns found for bacterial HFA effects (Table 3). We could not confirm that recalcitrant litter promoted greater HFA effects (hypothesis 2a), but it is clear that litter chemistry explained a large proportion of the variation in fungal HFA effects and a smaller but significant proportion of bacterial HFA effects (hypothesis 3). Moreover, the opposing patterns for fungi and

bacteria suggested that the differences in litter quality related with these parameters could have generated resource exploitation interactions between the two microbial groups during the decomposition of beech and pine litter (Fig. 4d).

Independently of the litter quality, nutrient availability was also a powerful candidate controller of microbial processes. We found enzyme-ratios exceeding 1 for the P/C enzymatic ratio and ratios below 1 for the N/P enzymatic ratio in most cases (Fig. 5). This suggests that microbial communities were limited by P. These results match previous studies reported from forest ecosystems (Gartzia-Bengoetxea et al., 2009; Fanin et al., 2015; Rosinger et al., 2019). We did *not* observe a relationship between nutrient limitations inferred from enzyme ratios and respiratory or bacterial HFA effects. However, again we found a strong relationship for fungal HFA effects, where a stronger limitation of P in litter coincided with pronounced fungal HFA effects. These patterns were consistent with findings by Güsewell & Gessner (2009) that suggested that fungi were relatively more important for decomposition of submerged plant litter with higher N/P ratios, when decomposition was P limited. It should be noted that inferring limiting nutrients for the microbial community from enzyme ratios recently has been challenged (Rosinger et al. 2019; Mori, 2020), calling for confirmation of the obtained results using methods that directly target the limiting factors for growth. On the other hand, several studies found that litter quality had weak or no impact on HFA effects and that HFA effects became stronger when the quality of 'home' and 'away' litters became more dissimilar (Ayres et al., 2006; Wallenstein et al., 2013; Veen et al., 2015a). In our study, litter dissimilarities did not affect HFA effects of respiration bacteria, but they did strongly influence fungal HFA effects (Table 3). As such, fungal HFA effects matched the prediction of hypothesis 4, with dissimilarities in litter C, N:P, lignin and lignin:N affecting fungal HFA effects negatively for site A and positively for site M.

Taken together, our results suggest a strong link between the fungal community's contribution to litter decomposition and litter quality. There are observations associating the fungal community with decomposition of more recalcitrant litter (de Boer et al., 2005), however, there are also several recent studies linking fungi with the decomposition of plant leaf litter in general (Rousk and Frey, 2015; Lin et al., 2019). It is possible that this is an outcome driven by the stronger nutrient rather than C limitation of the decomposer community induced in a litter compared to the soil matrix (Güsewell and Gessner, 2009; Silva-Sánchez et al., 2019), which is predominantly C-limited (Demoling et al. 2007; Rosinger et al., 2019), and a greater ability of fungi to cope with nutrient deficiency (Güsewell and Gessner, 2009; Sterner and Elser, 2002). This may be the reason why fungal HFA was more related to the quality of leaf litter than that of bacteria.

4.3. HFA and microbial composition

It is often expected that the HFA relies on the specialization of the microbial community for a given OM and the conditions of its environment. Lin et al., (2019) showed that fungal communities on broadleaved litter were most dissimilar between litters incubated in ‘home’ environments. In line with this, we expected that differences in microbial community structures would be largest between the two “home” treatments while community structures in the “away” treatments would be intermediary, effectively being a mixture of the two ‘home’ environments (hypothesis 5). There are studies which suggest that the different results in HFA could be due to the ability of the microbial community to shift its composition to match the quality of a particular substrate on a short temporal scale (Giesselman et al., 2011). Our results are consistent with these expectations, since the PCA of the microbial PLFA composition indicated that the microbial community changed to adapt to the new situation in the away treatments, gradually aligning their composition to the microbial compositions in treatments away than from the home environments they were sourced from. In addition, we can see that the beech and the oak had very similar microbial communities despite different responses in the HFA indices. Although recent works have demonstrated that structural differences in microbial community can influence ecosystem functioning (Strickland et al., 2009a, 2009b), our findings suggest that although the communities were aligning, this had relatively small functional implications in this study system (Fig. 7, Table S5).

6. CONCLUSIONS

Overall, our results support that HFA effect will be stronger and more responsive over time for microbial growth than for decomposition during the first weeks of decomposition. Moreover, we observed that the different patterns for bacterial and fungal communities were associated with differences in the HFA effects for decomposition, suggesting causal links that will deserve targeted attention. We found that litter chemistry plays an important role by determining the magnitude and direction of bacterial and fungal HFA effects, and that the outcome of the interaction between fungi and bacteria during the decomposition of litter, resulting in competition or facilitation, seems to drive the HFA for litter degradation. The fungal community was influenced not only by litter chemistry, but also by the chemical differences between litter from ‘home’ and ‘away’ environments

and to the nutrients that limited microbial growth, suggesting a dominant functional role of the fungal community that determined the decomposition of litter in the studied forest floor systems.

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