Soil Biology and Biochemistry 144 (2020) 107778

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: http://www.elsevier.com/locate/soilbio

Linking plant litter microbial diversity to microhabitat conditions, environmental gradients and litter mass loss: Insights from a European study using standard litter bags

Silvia Pioli [a], Judith Sarneel [b,c,d], Haydn J.D. Thomas [e], Xavier Domene [f,g], Pilar Andrés [f], Mariet Hefting [c], Thomas Reitz [h,i], Hjalmar Laudon [j], Taru Sandén [k], Veronika Piscová [l], Mika Aurela [m], Lorenzo Brusetti [a,*]

[a] Faculty of Science and Technology, Free University of Bozen/Bolzano, Piazza Università 5, 39100, Bolzano, Italy
[b] Landscape Ecology Group, Department of Ecology and Environmental Science, Umeå University, SE-901 87, Umeå, Sweden
[c] Ecology & Biodiversity, Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, the Netherlands
[d] Plant Ecophysiology, Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, the Netherlands
[e] School of Geosciences, University of Edinburgh, Scotland, UK
[f] CREAF, E08193 Bellaterra (Cerdanyola del Vallès), Catalonia, Spain
[g] Universitat Autònoma de Barcelona, E08193 Bellaterra (Cerdanyola del Vallès), Catalonia, Spain
[h] Helmholtz Centre for Environmental Research - UFZ, Department of Soil Ecology, Theodor-Lieser Str. 4, 06120, Halle/Saale, Germany
[i] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Deutscher Platz 5E, 04103, Leipzig, Germany
[j] Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, 901 83, Umeå, Sweden
[k] Department for Soil Health and Plant Nutrition, Austrian Agency for Health and Food Safety (AGES), Spargelfeldstraße 191, 1220, Vienna, Austria
[l] Institute of Landscape Ecology – Branch Nitra, Slovak Academy of Sciences, Akademicka 2, 949 10, Nitra, Slovakia
[m] Finnish Meteorological Institute, Helsinki, Finland

ARTICLE INFO

Keywords:
Abiotic drivers
Litter decomposition
Microbial communities' diversity
Microbial co-occurrences
Molecular fingerprinting
Pan-European study

ABSTRACT

Plant litter decomposition is a key process for carbon dynamics and nutrient cycling in terrestrial ecosystems. The interaction between litter properties, climatic conditions and soil attributes, influences the activity of microorganisms responsible for litter mineralization. So far, studies using standardized litters to investigate the response of bacterial and fungal communities under different environmental conditions are scarce, especially along wide geographic ranges.

We used a standardized protocol to investigate the diversity of bacteria and fungi in plant litter with the aim of: (i) comparing the microbial communities of native and exotic litters with the community of local soil along a European transect from northern Finland to southern Italy, (ii) defining whether and to what extent, litter types with different traits represent selective substrates for microbial communities, (iii) disentangling the abiotic drivers of microbial diversity, and (iv) correlating the microbial diversity and species co-occurrences patterns with litter mass loss.

We buried native litter and three exotic standardized litters (*Deschampsia cespitosa*, rooibos tea and green tea) at 12 European study sites. We determined litter mass loss after 94 days. We used an automated molecular DNA-based fingerprinting (ARISA) to profile the bacterial and fungal communities of each litter type and soil (180 samples in total).

Microbial communities in native and exotic litters differed from local soil assemblages. Green tea and *D. cespitosa* litter represented more selective substrates compared to native litter and rooibos. Soil moisture and soil temperature were the major drivers of microbial community structure at larger scales, though with varying patterns according to litter type. Soil attributes (i.e. moisture and C/N ratios) better explained the differences in microbial abundances than litter type. Green tea degraded faster than all other litter types and accounted for the largest number of positive co-occurrences among microbial taxa. Litter mass loss was positively correlated with fungal evenness and with the percentage of positive co-occurrences between fungi.

* Corresponding author.
E-mail address: lorenzo.brusetti@unibz.it (L. Brusetti).

https://doi.org/10.1016/j.soilbio.2020.107778
Received 21 November 2019; Received in revised form 5 March 2020; Accepted 7 March 2020
Available online 9 March 2020
0038-0717/© 2020 Elsevier Ltd. All rights reserved.

Our findings suggest that the microbial community at larger scales reflects the complex interplay between litter type and soil attributes, with the latter exerting a major influence. Mass loss patterns are in part determined by inter- and intra-kingdom interactions and fungal diversity.

1. Introduction

Litter decomposition in terrestrial ecosystems is controlled by the synergic combination of its biochemical composition, abiotic conditions and the activity of soil invertebrates and microorganisms (Hättenschwiler et al., 2005; Bani et al., 2018b). Microorganisms, such as fungi and bacteria, are responsible for the transformation and mineralization of organic matter, primarily contributing to soil respiration and nutrient cycling (Talbot and Treseder, 2011; Allison et al., 2013). Fungi are known to produce a set of oxidative enzymes that degrade the recalcitrant biopolymers of litter (Mathieu et al., 2013; Hoppe et al., 2015). In contrast, only few groups of bacteria degrade all lignocellulosic polymers, as they typically target simple soluble compounds (de Boer and van der Wal, 2008), therefore, the role of bacteria in the decomposition of more recalcitrant material is still debated (Wilhelm et al., 2019).

Microbial community structure is mainly determined by climate, land-use legacy and vegetation community (Fichtner et al., 2014), along with a wide range of microhabitat conditions including pedoclimate, soil pH and nutrients availability (Gartner and Cardon, 2004; Gray et al., 2011). Litter quality is also important as both bacteria and fungi respond to litter physicochemical changes during the decay process (Aneja et al., 2006; Purahong et al., 2016). Among litter biochemical traits, the carbon/nitrogen ratio and the fraction of acid-unhydrolyzable residue (AUR: formerly referred to as lignin) are considered good indicators of litter quality as they are related to nutrient availability and decomposition stage (Prescott, 2010; Talbot and Treseder, 2012). In this way, above-ground plant composition and plant traits, can affect microbial community structure and diversity by selecting decomposer communities that are specialized in breaking down litter of the local plant community (Bezemer et al., 2010; Freschet et al., 2012). Different litter types can thus, with their specific traits, select microbial taxa that are more specialized in degrading their components. However, still little is known on how microbial communities specialize on litter types with different physical and chemical traits (Freschet et al., 2012) especially in relation to other drivers such as climate and soil characteristic across large geographical scales.

At larger scales, environmental changes that alter the climatic conditions, especially temperature and moisture, are expected to impact on the microorganisms that regulate decomposition and other ecosystem processes (Allison et al., 2013; Glassman et al., 2018). Therefore, understanding the effect of climatic variation on decomposer diversity and decomposition may provide important indications for predicting carbon cycling under global climate change (Cavicchioli et al., 2019).

Besides the abiotic drivers of litter quality and climate, the diversity and functioning of microbial communities are affected by intra and inter-kingdom interactions. In natural communities, interactions between taxa of fungi or bacteria generally involve competition for space and resources (Boddy, 2000). Yet, between bacteria and fungi, positive interactions may take place influencing the rate of ecosystem processes. For example, it has been suggested that bacteria can facilitate the activity of decaying fungi by providing important nutrients such as nitrogen (N) and phosphorous (P) (Purahong et al., 2016). It is therefore likely that, decomposition dynamics depend on microbial community diversity and on the facilitative/competitive interactions among different species of the same group (Hoppe et al., 2015) and between bacteria and fungi (Purahong et al., 2016). However, the importance of species interactions in decomposition dynamics are not well understood and more studies under natural conditions are needed.

Elucidating the environmental drivers of microbial diversity across environmental gradients represents a key aspect in ecology. However, disentangling the effects of abiotic conditions on the microbial community structure and diversity remains a challenge. Comparisons across different ecosystem types are complicated by the trade-off between using single or few litter types and achieving maximum geographical extent. Recently, a cost-effective method has been developed to study litter decomposition using commercially available tea bags as standardized plant litters (Keuskamp et al., 2013). This method allows uniform data to be gathered across global scales, thus enhancing comparisons between ecosystems and soil types. Moreover, it discriminates between the effect of environmental attributes and litter traits on decomposition, providing further support to develop accurate decomposition models (Didion et al., 2016; Althuizen et al., 2018).

In this study, we used molecular fingerprinting (Automated Ribosomal Intergenic Spacer Analysis - ARISA) to compare the microbial community structure in one native and three exotic standardized litters (two tea types and a common garden litter) with the microbial community in local soil. We tested whether, and to what extent, litter types with different traits represent selective substrates for microbial community composition. We included environmental descriptors to disentangle the main drivers of microbial diversity in litter and soil across different European ecosystems. Finally, we related the microbial diversity and species co-occurrences patterns with litter mass loss. We hypothesized that i) native and exotic litters are colonized by different subsets of soil local microbiota, and thus that each litter select a specialized community; ii) bacterial and fungal communities in litter and soil are primarily determined by C/N soil ratios, soil pH and climatic conditions; iii) litter mass loss is positively related to litter microbial diversity, and iv) positive co-occurrences between microbial taxa facilitate litter decomposition. To the best of our knowledge, this is the first study investigating the microbial community structure and diversity of multiple, standardized litter types with varying traits across a wide latitudinal gradient.

2. Materials and methods

2.1. Study sites

The study was conducted along a European transect covering a latitudinal distance of more than 3000 km. Twelve study sites were chosen to represent different ecosystems (see Fig. 1 and Table 1 for details) including cropland (AUS), grassland (GER2), temperate forest (FRA, GER1, NET, SLO, UK), boreal forests (FIN, SWE) and Mediterranean forests (ITA, SPA2). The study sites represented a climatic gradient from warm, dry sites (ITA, SPA1, SPA2) to cold and wetter locations (FIN, SWE). They included organic soils (GER1, GER2, ITA, UK) to fine-grained soils (AUS, FIN, FRA, SLO, SWE). C/N ratios of native litter collected at those study sites ranged from 29.7 (ITA) to 94.8 (AUS). The native litters collected at those study sites varied in the amount of labile material, with hydrolysable fractions (H = 1-AUR) ranging from 0.40 (FRA) to 0.61 (GER1).

2.2. Experimental design

Native litter was collected in late autumn/early winter 2016 at each study site, preferably by shaking trees and collecting freshly senescent leaves or needles that had not touched the ground. Litter was air dried and sent to Umeå for processing (Umeå University, Department of Ecology and Environmental Sciences, Umeå, Sweden). Directly after snow melt, standing dead material of the graminoid *Deschampsia*

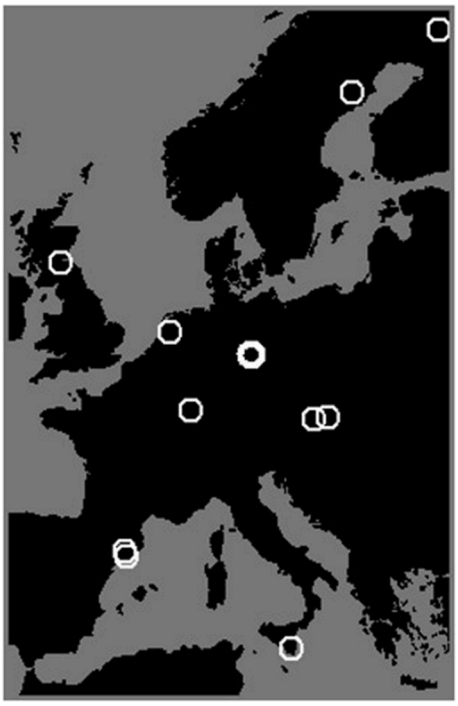

Fig. 1. Map of the study sites. Bold circles represent overlapping study sites.

cespitosa (hereafter referred as "common litter") was collected at the university campus in Umeå (63.819, 20.327). Using gloves to prevent microbial contamination, all litter types were cut in small pieces and passed through a sieve with 1 cm mesh. Nylon triangular mesh bags with mesh size 0.25 mm (Topzeven, Haarlem, the Netherlands) were made containing about 1.5 g litter. Bags were closed using a heat sealer. Each study site received seven bags containing its own native litter, seven bags with common litter and seven green tea and rooibos bags that had the same nylon mesh bags as the native and common litter (EAN 87 22700 05552 5, EAN 87 22700 18843 8, respectively. Lipton, Unilever). From the leftover material of each litter type, moisture content was determined as the mass loss after drying (48 h at 70 °C) using four replicates of approximately 1 g. We further determined the AUR of each native litter by acid fractionation using a Soxhlet extractor, following the method described in Keuskamp et al. (2013). The AUR represents the recalcitrant fraction of the material, which contains a high amount of lignin and other aromatic material. Native litter C/N ratio was determined by combustion on about 4 mg of finely ground and dried litter using a CHN-analyser at Utrecht University (EA NA 1110; Carlo Erba, Milan, Italy). C/N ratios and AUR for the exotic litter types were determined before the experimental period and reported in Table 2. Sequencing of the starting material showed a negligible microbial load in a parallel study (TeaTime4Schools consortium, 2018).

Upon receiving the bags, each study site re-weighed the bags to determine the loss of material by traveling. At each study site, litter bags were buried in a 2 × 2 m grid in June 2017, with one grid row containing the seven replicates of one litter type (Fig. 2). Bags were buried at 8 cm depth and retrieved after on average 94 days (ranging from 88 to 106 days, following Keuskamp et al., 2013). After retrieving, four replicate bags were cleaned of adhering soil, dried for at least 48 h at 60–70 °C, and weighed to determine mass loss. The three other replicates of each bag type were used to determine bacterial and fungal community composition and sent cooled and with express courier to Bolzano (Free University of Bolzano, Environmental Microbiology Lab, Bolzano, Italy), and stored at −20 °C until DNA extraction. To determine the bacterial and fungal composition of the soil community, three soil samples (ca 100 ml) were taken from 8 cm depth (Fig. 2) and sent to Bolzano along with the litter bags, where they were processed in the same way as litter samples. In addition, we measured soil temperature during the period the bags were buried by planting one i-button (Homechip, Milton Keynes, United Kingdom) next to the grid (Fig. 2), logging soil temperature every 3 h with a 0.5 °C precision.

2.3. Molecular analysis

We used Automated Ribosomal Intergenic Spacer Analysis fingerprinting to profile the community structure for both bacteria (B-ARISA) and fungi (F-ARISA), which gives a broad characterization of microbial community composition (Ramette, 2009).

The frozen content of each bag (12 per study site) was ground using liquid nitrogen under sterile conditions. DNA extractions were performed on 0.1- gram material using Power Soil isolation kit (MoBio Laboratories, Arcore, Italy) according to the manufacturer's instructions. The Internal Transcribed Spacer region (ITS) of bacteria, was amplified following the protocol described by Bani et al. (2018a) using primers ITSF (GTCGTAACAAGGTAGCCGTA) and ITSReub (GCCAAGGCATCCACC). The PCR amplification for fungi was carried out following Gleeson et al. (2005). The fungal ITS was amplified using primers ITS1-F (CTTGGTCATTTAGAGGAAGTAA; Gardes and Bruns, 1993) and ITS4 (TCCTCCGCTTATTGATATGC; White et al., 1990). The amplification failed for 9 replicates of bacteria and 21 replicates of fungi representing 5% and 12% of total samples, respectively. These samples were excluded from further analysis. The PCR products were shipped to STAB Vida Lda. (Caparica, Portugal) for fragment separation by capillary electrophoresis and the resulting profiles were analyzed using AB Peak Scanner Software 1.0 (Applied Biosystems, Monza, Italy) as described by Pioli et al. (2018).

2.4. Environmental parameters

Based on the GPS coordinates of each study site, we extracted the annual mean temperature and annual precipitation from WorldClim2 with 30 arc-seconds resolution, the elevation from topographic maps 7.5 arc-seconds resolution and soil pH (H_2O extractions at 5 cm depth) from www.soilgrids.org. In addition to these climatic and soil data that quantify general climatic settings, we calculated the mean soil temperature at each study site during the field study period from i-button readings. Soil moisture content was determined from soil samples, from which major roots and stones were removed, by measuring the mass loss of ca 15 g fresh soil after drying the soil for 48 h at 102 °C. After drying, the soil samples were ground by hand in a mortar and total soil carbon and nitrogen concentrations were determined by combustion of about 40 mg sample using a CHN-analyser at Utrecht University (EA NA 1110; Carlo Erba, Milan, Italy). In some soils with high percentages of carbonates (e.g. AUS), biologically available C may be lower than our estimates.

2.5. Statistical analyses

Operational Taxonomic Units (OTUs) richness (S), Shannon diversity (H′) and Pielou's Evenness (J) were calculated for bacteria and fungi on different substrate types per study site using the package 'vegan' (Oksanen et al., 2014. See Fig. S1 for indices formulas) in R version 3.4.1 (R Core Team, 2017). We tested the normality of data using Shapiro-Wilk test. For normally distributed data, we used one-way analysis of variance (ANOVA), followed by a Tukey's post hoc test ($P < 0.05$) to test the differences among substrate types for each diversity index. Where assumptions of normality were not met, we used the non-parametric Kruskal-Wallis test, followed by Bonferroni correction for multiple comparisons.

Multivariate analyses were performed on OTUs proportional abundances using the package 'vegan' (Oksanen et al., 2014). To reveal differences in bacterial and fungal community structure on litter and soil, we used nonmetric multidimensional scaling (NMDS) based on

Table 1
Details of the study sites. C/N soil ratios were determined as total (organic and inorganic) carbon to nitrogen ratio and expressed as mean values ± standard deviation (n = 3).

Locality	ID	Latitude	Longitude	Ecosystem type	Elevation (m a.s.l.)[a]	Mean annual precipitation (mm)[b]	Mean annual temperatures (°C)[b]	Dominant vegetation	Soil type	Soil C/N	Soil pH[c]	Native litter C/N[d]	Native litter H (1-AUR)[d]
Austria	AUS	48.163	16.705	Cropland	146	593	10.1	Maize (*Zea mays*)	Chernozem, sandy silt	29.66 ± 0.36	5.9	94.8	0.46
Finland	FIN	67.362	26.638	Boreal forest	186	520	−0.78	Scott pine (*Pinus sylvestris*)	Fluvial sandy podzol	25.23 ± 1.36	5.6	57.3	0.51
France	FRA	48.674	7.065	Temperate deciduous broadleaf forest	321	800	9.1	Beech (*Fagus sylvatica*)	Stagnic luvisol	12.36 ± 1.1	5.8	55.5	0.4
Germany	GER1	51.391	11.875	Unmanaged deciduous forest	113	514	9.3	*Acer pseudoplatanus, Acer platanoides, Fraxinus excelsior*	Haplic chernozem	12.42 ± 0.17	5.9	109.2	0.61
Germany	GER2	51.391	11.875	Extensively managed grassland	113	520	9.3	Bushgrass (*Calamagrostis epigejos), Poa angustifolia, Arrhenatherum elatius*	Haplic chernozem	12.8 ± 0.05	5.9	34.2	0.41
Italy	ITA	36.939	14.981	Mediterranean evergreen scrub	545	541	15.9	Kermes oak (*Quercus coccifera)*	Calcareous heavy claysoil (Luvisol)	13.55 ± 0.3	5.8	29.7	0.50
The Netherlands	NET	52.465	5.438	Temperate forest (plantation)	1.6	774	9.6	Norway spruce (*Picea abies*)	Calcareous heavy claysoil (Luvisol)	14.41 ± 0.09	5.8	30.2	0.56
Slovakia	SLO	48.303	17.889	Temperate deciduous mixed forest	191	574	9.7	European hornbeam (*Carpinus betulus*), field maple (*Acer campestre*), Norway maple (*Acer platanoides*), field elm (*Ulmus minor*)	Chernozem, parent material: loess	11.94 ± 0.36	5.9	35.9	0.56
Spain	SPA1	41.744	1.92	Mediterranean conifer forest	337	609	13.6	Aleppo pine (*Pinus halepensis*)	Calcareous Sandy Clay Loam. Parental material: sandstone	31.94 ± 8.31	6.5	64.8	0.52
Spain	SPA2	41.431	2.074	Mediterranean evergreen mixed forest	219	641	14.4	Holm oak (*Quercus ilex*), pubescent oak (*Quercus humilis*)	Sandy-loam. Parental material: shales and granite	15.18 ± 0.28	7.8	45.6	0.57
Sweden	SWE	64.256	19.775	Boreal forest	266	621	1.73	Scots pine *(Pinus Sylvestris*), Norway spruce (*Picea Abies*)	Primarily podzolised, unsorted glacial till	23.54 ± 5.57	5.8	53.2	0.53
United Kingdom	UK	55.924	−3.226	Temperate deciduous woodland	122	697	8.5	Sycamore (*Acer pseudoplatanus*), beech (*Fagus sylvatica*), elder (*Sambucus nigra*)	Brown forest loamy soil	17.31 ± 0.32	6.5	43.2	0.37

[a] Extracted from topographic maps.
[b] Extracted from WorldClim2.
[c] Extracted from www.soilgrids.org.
[d] Determined on material before burial period.

Table 2
Main chemical and physical characteristics of standard litter types used in the present study. C/N= Carbon to nitrogen ratios; H = 1- Acid-unhydrolyzable residue.

Standard litter types	C/N	H (1-AUR)
Green tea	12.23	0.842
Rooibos tea	42.87	0.552
Common litter (*Deschampsia cespitosa*)	61.02	0.398

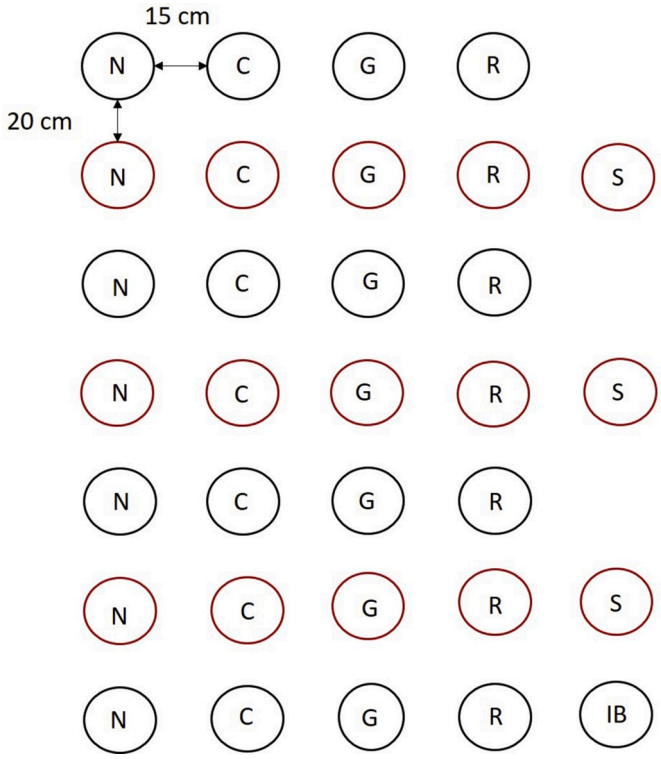

Fig. 2. Scheme of litter bags placement in the experimental plots. All the tea bags were buried at a depth of 8 cm. Black circles represent replicates used for mass loss determination. Three soil samples were taken for microbial and chemical analyses alongside the tea bags for microbial characterization (red circles). N = native litter; C = common litter; G = green tea; R = rooibos tea; S = soil IB = ibutton logger. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Bray-Curtis distances. To visualize how similar or different the community structure on the litters was compared to the soil, we calculated the absolute Euclidean distance between the centroid of the soil and the centroid of the different litter types of the same study site using the scores on the first two axes in NMDS space. Then, we calculated the average distances between the litters and soil communities for all the study sites. To determine statistical differences between community composition on soil and litters, we conducted two-way permutational multivariate analysis of variance (PERMANOVA) using the *adonis* function ('vegan' package, Oksanen et al., 2014) with microbial abundances as the dependent variable and litter/soil type as a fixed factor. We performed pairwise comparisons of the resulting PERMANOVAs with the package 'pairwiseAdonis' using the function *pairwise.adonis2* (Martinez, 2019).

We analyzed the importance of litter type compared to microclimatic conditions (independent variables) on bacterial and fungal community composition (dependent variables) in two ways. First, ten NMDS analyses were performed to identify the ecological drivers of community structure separately for bacteria and fungi on each of the four litter types and soil. We standardized the mean and standard deviations of the environmental variables (Table S1) and checked non-collinearities using the *vif* function of package 'usdm' (Naimi, 2015). This led to the exclusion of "Annual mean temperature" as it showed a strong collinearity with "Soil temperature" (VIF>10). We plotted significant environmental variables using *envfit* function of 'vegan' package with P values based on 999 permutations (Oksanen et al., 2013).

Second, we fitted multiple generalized linear models (GLMs) with negative binomial error distribution using package 'mvabund' (Wang et al., 2012) to detect the most important factor that influences microbial abundances. We used the bacterial and fungal community abundance matrix of the 100 most abundant OTUs as dependent variables and litter type, soil moisture, soil pH, soil temperature, soil C/N ratios, litter hydrolysable fraction and litter C/N as independent model input. We tested model terms for significance with a likelihood ratio test and a Monte Carlo resampling scheme with 999 bootstraps. We compared resulting models according to the Akaike's information criterion (AIC), with lowest AIC value representing the best fit model. We verified the model assumptions by plotting the residuals as described in Wang et al. (2012).

Finally, we calculated mass loss as percentage of litter mass loss during the study period including corrections for mass loss by handling and moisture content of the starting mass. Mass losses were correlated with diversity indices and species co-occurrences using Pearson's correlation. We calculated co-occurrences between bacterial and fungal OTUs with package 'cooccur' (Veech, 2013; Griffith et al., 2016) to reveal stable intra- and inter-kingdom interaction per litter type.

3. Results

3.1. Microbial community structure and diversity

A total of 1049 OTUs were detected for bacteria and 691 OTUs for fungi across all litter bags, soil samples (n = 171 for bacteria and 159 for fungi) and study sites (n = 12). The 100 most abundant OTUs accounted for 44% and 66% of the total OTU abundance observed for bacteria and fungi, respectively. On common litter, green tea, native litter and rooibos we found respectively 29, 31, 30 and 31% of bacterial OTUs that were also found in soils. On average, fungal soil communities shared 11, 15, 20 and 14% of total OTUs found on common litter, green tea, native litter and rooibos, respectively.

The diversity indices of the fungal communities did not differ between litter types and soil (Fig. S1). For bacteria, soils generally had highest Shannon and Evenness indices indicating a more diverse and balanced community compared to the community on the different litter types (Fig. S1). Maximum bacterial and fungal OTU richness was higher in native litter (up to 271 OTUs per sample for bacteria and up to 125 OTUs per sample for fungi) compared to other litter types and soil. However, across all sites the means were not significantly different (Fig. S1). Diversity indices of bacteria differed significantly between rooibos and green tea at one site (SPA1 - Shannon, $P < 0.05$; Evenness $P < 0.01$) and for fungi at three sites (SPA1 - Richness $P < 0.05$, Shannon $P < 0.001$; GER1 - Richness $P < 0.01$ Shannon $P < 0.001$; NET - Richness $P < 0.01$, Shannon $P < 0.01$, Table S2).

NMDS scores indicated that litter types were colonized by different subsets of taxa compared to the local soil assemblages in most cases, although with very specific patterns according to each study site (Fig. S2 and Fig. S3). The relative distances of different litter types from soil samples centroids in NMDS space, demonstrated that native and rooibos litter generally have the shortest distance from soil centroids in both bacterial and fungal communities (Fig. 3). Green tea samples are on average located furthest from the soil centroids, indicating the greatest difference in decomposer community. Overall, the PERMANOVA showed that microbial communities on all litter types were significantly different compared to the communities on soil, except for fungi in native litter (Table 3). Each litter type was also characterized by a significantly different community compared to other litter types for both bacteria and

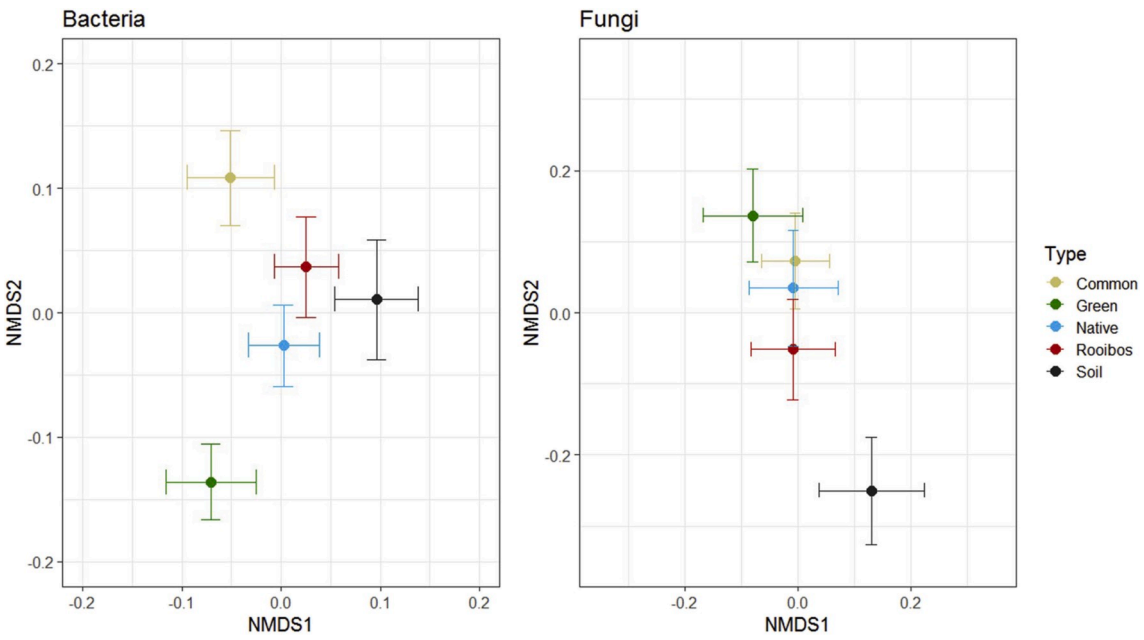

Fig. 3. NMDS ordinations of bacterial and fungal communities per litter type across the European transect. Different colors represent different litters/soil. Centroids and standard errors are displayed. Stress = stress value. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

fungi (Table 3).

3.2. Environmental drivers of microbial community structure

We found that several environmental variables related to bacterial and fungal community structure on the different litter types and soil (Figs. 4and5). Across Europe, both bacterial and fungal communities were frequently related to soil moisture and soil temperature. Spatial differences in bacterial communities were only related with major pedoclimatic variables (i.e. soil moisture and soil temperature) in native litter and rooibos tea. Soil pH variation across study sites significantly affected bacterial community structure in green tea, while soil pH was relevant for fungal community structure in common litter and soil. While environmental variables were the primary determinant of microbial community structure for most litter types, fungal community in rooibos was only related to native litter quality (C/N ratios, H).

At the European scale, soil moisture was the strongest driver of bacterial community abundances (Table 4; Model 1), whereas fungi were primarily affected by soil C/N ratios (Table 4; Model 4). Interactions between variables were never significant.

Table 3
Results of PERMANOVA and subsequent pairwise comparison between litter types using microbial community abundances as dependent variable, litter type as fixed factors and study site as strata. Based on 999 permutations. Asterisks denote significance levels (*P < 0.05, **P < 0.01, ***P < 0.001).

	Bacteria		Fungi	
	R2	Pr (>F)	R2	Pr (>F)
Litter type/soil	0.05233	0.001 ***	0.05179	0.001 ***
Common Vs Green	0.03516	0.001 ***	0.02003	0.001 ***
Common Vs Native	0.02474	0.004 **	0.02809	0.001 ***
Common Vs Rooibos	0.02371	0.007 **	0.02012	0.005 **
Common Vs Soil	0.03783	0.001 ***	0.0249	0.001 ***
Green Vs Native	0.02902	0.001 ***	0.03084	0.001 ***
Green Vs Rooibos	0.0269	0.001 ***	0.02392	0.001 ***
Green Vs Soil	0.0297	0.001 ***	0.02487	0.001 ***
Native Vs Rooibos	0.01886	0.018 *	0.02223	0.02 *
Native Vs Soil	0.02713	0.001 ***	0.02374	0.081
Rooibos Vs Soil	0.02818	0.001 ***	0.02293	0.005 **

3.3. Microbial diversity and litter mass loss

Litter type had a significant effect on mass loss (Table 5, chi-squared = 75.974, df = 3, P < 0.001, Kruskal-Wallis tested). At all study sites except one (SPA2), green tea had the significantly highest mass loss (P < 0.001, Table 5), whereas native litter showed the lowest mass loss at most study sites (Table 5). Mass loss was highest at the SPA2 site (for all litter types) and lowest at UK (for native litter), SPA1 (for common litter), ITA (for green tea) and SLO (for rooibos, Fig. S4). Across all litter types, mass loss showed no significant relationship with climatic factors, but was positively correlated with fungal evenness (P < 0.01, Table 6), species co-occurrences (see next section) and litter/soil chemical traits (Table S3).

3.4. Species co-occurrences

Intra- and inter-kingdom interactions were investigated through co-occurrences matrices for each litter type across sites (Fig. 6). In general, positive co-occurrences were more frequent compared to negative ones, as on average we found 19% of positive interactions and 1.2% of negative interactions among litter types. In all litter types, positive species interactions occurred mostly between fungal taxa, whereas negative interactions were most common between fungi and bacteria in common litter and green tea, and between fungi in native litter and rooibos. The percentage of positive co-occurrences between fungi was positively correlated with litter mass loss (Table 6; P < 0.001). Other intra- and inter-kingdom co-occurrences (both positive and negative) were negatively correlated with the mass loss. Negative co-occurrences between fungal OTUs were negatively correlated with Shannon diversity of fungi (r = −0.18; P < 0.05).

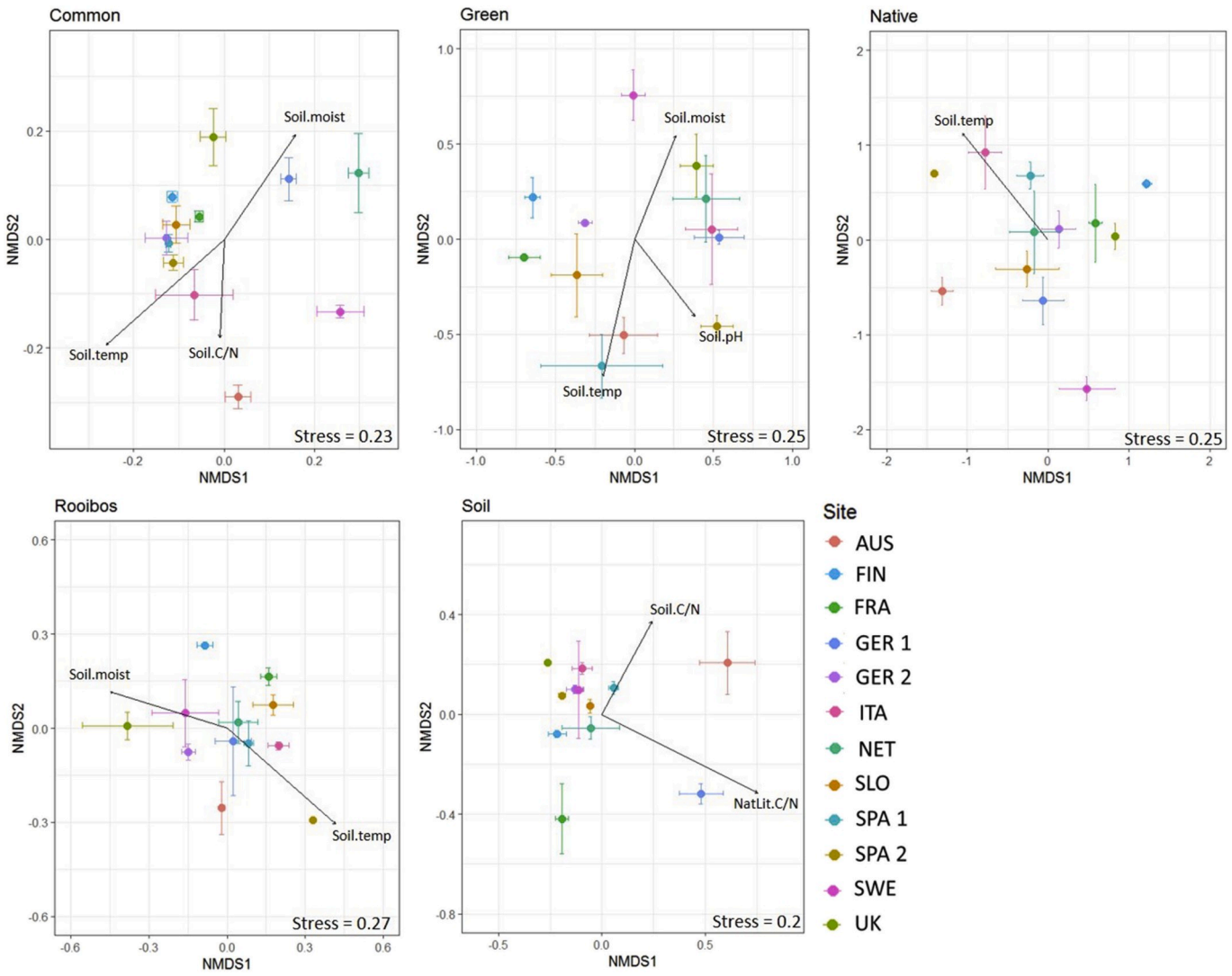

Fig. 4. NMDS ordinations of bacterial communities in different litters/soil. Colors indicate study sites. Centroids with standard errors (n = 3) are displayed. Arrows represent fitted environmental variables with $P < 0.05$. The significance was based on 999 permutations. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

4.1. Litter type as selective substrate for microbial diversity

We expected that the soil microbial community would represent the major source of potential microbial colonizers of litters, and thus that each litter type would be colonized by a subset of OTUs already present in the local soil pool. Unfortunately, the community composition of the litter bags before the experimental period was unknown, therefore, we were not able to prove whether the litter types acted as ecological filters by selecting or excluding species from the common soil pool. However, our first hypothesis was partly confirmed as we found that each substrate was characterized by a unique microbial community, suggesting a high specialization of fungi and bacteria in their resource use. Exotic litters likely represent new substrates, providing available niches that select for specific assemblages. Our findings therefore strongly support existing literature regarding the importance of plant species identity for the composition of microbial litter community (Prescott and Grayston, 2013; van der Wal et al., 2013). As expected, the microbial community of native litter was often the closest to that of soil community. However, the community structure of rooibos was also surprisingly similar to that of local soil. This may be partly explained by substrate characteristics, since both rooibos and many of the native litters consisted of recalcitrant material (needles, woody) whereas green tea had higher nitrogen content and other traits associated with more labile litters. It is therefore possible that the availability of labile compounds attracts a differently specialized community compared to more recalcitrant litter and soil organic matter (McGuire and Treseder, 2010).

4.2. Effects of environmental conditions on microbial diversity

The microbial communities colonizing different litter types and soils were strongly related to environmental (i.e. annual precipitation) and soil conditions (i.e. C/N ratios, pH, moisture, temperature). Notably, in agreement with our second hypothesis, soil conditions were always significantly related to the microbial structure, which is consistent with Lauber et al. (2008) that found soil pH and nutrient status to affect soil microbial community composition.

Both fungi and bacteria are known to respond to variation in litter and soil C/N ratios (Marschner, 2003; Blaško et al., 2013; Purahong et al., 2016). This was also confirmed in our study, as we observed that soil microbial community structure and fungal abundances are significantly related to soil C/N ratios. In general, fungi are able to utilize substrates of a higher C/N ratios than bacteria (Wallenstein et al., 2006),

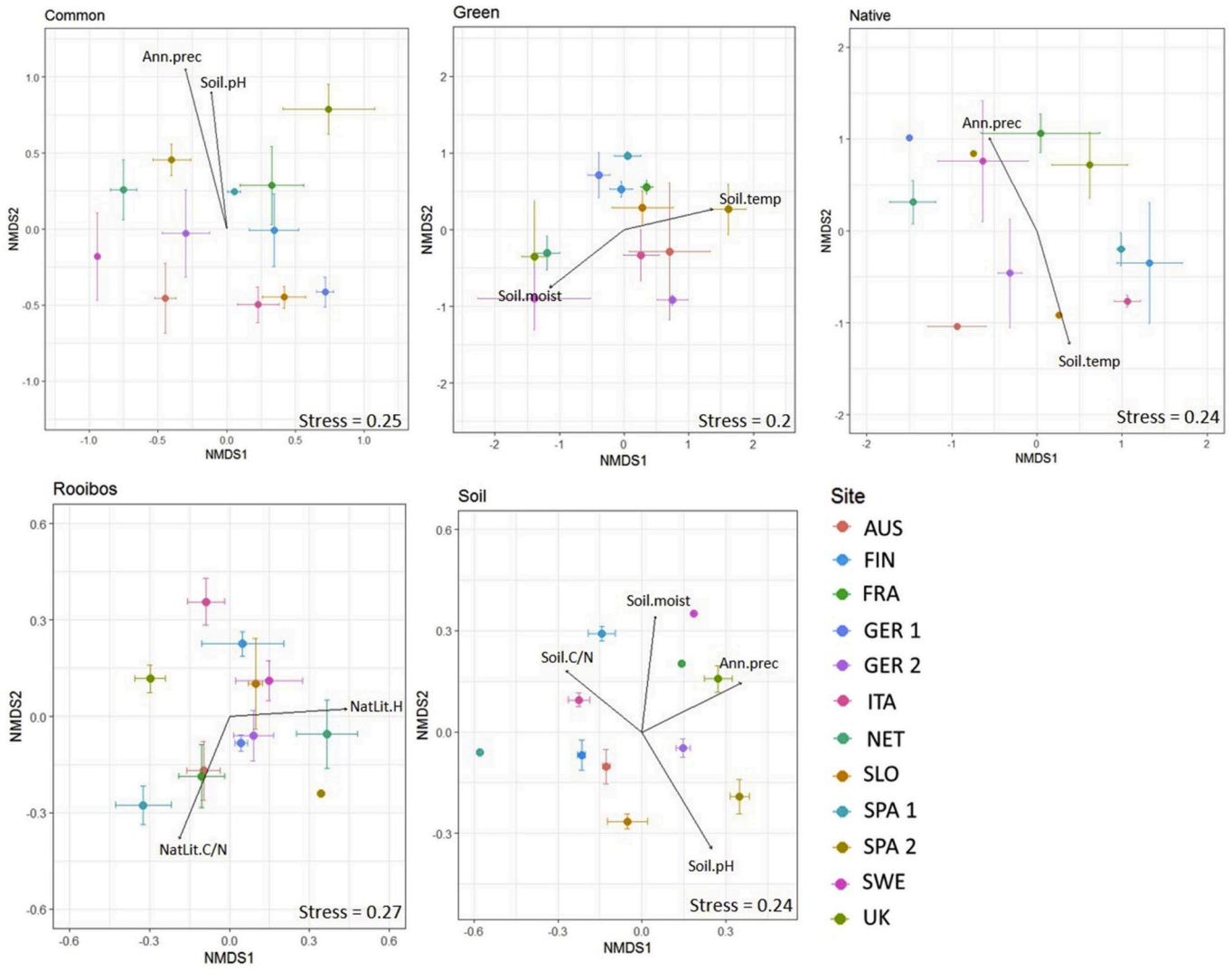

Fig. 5. NMDS ordinations of fungal communities in different litters/soil. Colors indicate study sites. Centroids with standard errors (n = 3) are displayed. Arrows represent fitted environmental variables with $P < 0.05$. The significance was based on 999 permutations. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

therefore soils characterized by recalcitrant materials are more likely to stimulate the fungal contribution to decomposition (Rousk and Bååth, 2007). However, in this study, litter community structure was less related to variation in soil chemistry, than to micro-climatic parameters (soil moisture and temperature).

Both temperature and soil moisture are known to affect bacterial and fungal community structure, their growth and functions (Feng and Simpson, 2009; Rousk and Bååth, 2011; Classen et al., 2015; Chen et al., 2018). Although studies on the effect of soil temperature on microbial communities in natural environments are still scarce, two recent works have demonstrated that the variation in mean annual temperature could affect continental-scale microbial diversity and distribution at the community level (Garcia-Pichel et al., 2013; Zhou et al., 2016). This suggests that annual mean temperature plays a major role in driving microbial community structure and composition in soils. These results are corroborated by our findings relating soil microbial community structure with soil temperature (which matched annual temperatures patterns). In addition, we provided evidences for a key role of soil temperature in shaping litter microbial communities as well. We also found that bacteria were more greatly influenced by soil temperature than fungi, irrespectively of the litter type.

Similarly to temperature, variations in soil moisture are commonly thought to affect microbial activity (Evans and Wallenstein, 2012; Averill et al., 2016), although the effect of different moisture conditions on the microbial community structure has been seldom assessed in litter (Brockett et al., 2012). We showed a clear differentiation of microbial communities on litter due to soil moisture and other climatic conditions, which is relevant in the context of global ecosystem processes under climate change. Fungi and bacteria respond differently to moisture fluctuations. For example, fungi are expected to be more tolerant to drought than bacteria (except for actinomycetes) as their extensive hyphal network allows to transfer water from more humid to dryer soil patches, whereas bacteria require water films for motility (Evans and Wallenstein, 2012). This is in agreement with our study, since soil moisture was a strong driver of microbial community structure. Further, for bacteria abundances soil moisture had a stronger impact than litter type and the other micro-habitat conditions. Other studies reported a shift in bacterial community composition under altered moisture regimes suggesting a differential sensitivity of bacterial taxa under certain moisture conditions (Evans et al., 2014). As an example, actinobacteria display a negative trend with soil moisture content and tend to dominate arid environments (Bouskill et al., 2013). Although we are not able to detect shifts in specific bacterial functional groups in this study, our findings are in agreement with recent literature on the role of edaphic

Table 4
GLM models of tested variables and their relative AIC scores for the 100 most abundant OTUs of bacteria (a) and fungi (b). Only significant models are reported. In bold the best model with lowest AIC score. Asterisks denote significance levels (*P < 0.05, **P < 0.01, ***P < 0.001).

a	Bacteria	AIC
Model 1	**Soil moisture****	**56719**
Model 2	Soil temp*	56727
Model 3	Soil C/N*	56727
Model 4	Soil moisture* + Soil temp	56801
Model 5	Soil moisture*** + Soil C/N	56817
Model 6	Soil temp *+ Soil C/N	56804
Model 7	Soil moisture **+ Soil temp + Soil C/N	56892
b	Fungi	AIC
Model 1	Soil moisture***	20006
Model 2	Soil temp***	20009
Model 3	Soil pH***	20001
Model 4	**Soil C/N*****	**19997**
Model 5	Soil moisture*** + Soil temp***	20032
Model 6	Soil moisture*** + Soil pH***	20010
Model 7	Soil moisture*** + Soil C/N**	20050
Model 8	Soil temp ***+ Soil pH***	20044
Model 9	Soil temp** + Soil C/N***	20039
Model 10	Soil pH*** + Soil C/N***	20027
Model 11	Soil moisture*** + Soil temp*** + Soil pH***	20038
Model 12	Soil moisture** + Soil temp*** + Soil C/N**	20068
Model 13	Soil temp** + Soil pH*** + Soil C/N***	20048
Model 14	Soil moisture*** + Soil pH*** + Soil C/N***	20028

Table 5
Mean values and standard deviation (n = 4) of mass loss fractions per litter types at all sites. Different letters indicate significant differences between litters (P < 0.05).

	Common	Green	Native	Rooibos
AUS	0.19 ± 0.01 b	0.69 ± 0.02 a	0.29 ± 0.01 b	0.26 ± 0.02 b
FIN	0.3 ± 0.06 b	0.61 ± 0.03 a	0.19 ± 0.02 c	0.24 ± 0.02 bc
FRA	0.25 ± 0.02 b	0.73 ± 0.07 a	0.17 ± 0.08 c	0.23 ± 0.06 b
GER1	0.22 ± 0.03 b	0.63 ± 0.03 a	0.25 ± 0.01 b	0.23 ± 0.05 b
GER2	0.35 ± 0.13 bc	0.71 ± 0.04 a	0.51 ± 0.08 b	0.27 ± 0.03 c
ITA	0.16 ± 0.02 b	0.53 ± 0.03 a	0.12 b	0.21 ± 0.04 b
NET	0.39 ± 0.13 b	0.68 ± 0.05 a	0.19 ± 0.05 c	0.26 ± 0.05 bc
SLO	−0.03 b	0.59 ± 0.01 a	0.07 ± 0.17 b	0.14 ± 0.02 b
SPA1	0.08 ± 0.02 c	0.61 ± 0.01 a	0.07 ± 0.01 c	0.17 ± 0.02 b
SPA2	0.82 ± 0.02 ab	0.82 ± 0.02 ab	0.77 ± 0.07 b	0.87 ± 0.02 a
SWE	0.16 ± 0.01 bc	0.58 ± 0.05 a	0.09 ± 0.01 c	0.21 ± 0.03 b
UK	0.23 ± 0.04 b	0.67 ± 0.03 a	0.07 ± 0.01 c	0.27 ± 0.02 b
All sites	0.28 ± 0.19 b	0.65 ± 0.09 a	0.25 ± 0.21 b	0.28 ± 0.19 b

conditions in driving the microbial diversity in soil (Fierer and Jackson, 2006; Lauber et al., 2008) and further provide evidences for a similar pattern in litter communities across large spatial scales.

4.3. The effect of microbial diversity on litter mass loss

Green tea had higher mass loss compared to the other litter types in our study, likely due to its higher fraction of labile components and lower C/N ratios (Keuskamp et al., 2013). However, a growing number of studies have shown the importance of microbial diversity for a variety of ecosystem processes including decomposition (Xiao et al., 2019), though the relationship between increased microbial diversity and decomposition efficiency has been often debated in the literature (Wohl et al., 2004; Tiunov and Scheu, 2005; Nielsen et al., 2011). Contrary to our third hypothesis, we did not observe a clear effect of bacterial or fungal diversity on litter decomposition as among the diversity indices, only fungal evenness was related to mass loss. In litter and soil where the high availability of resources can support species-rich communities, high levels of functional redundancy are expected. Under these conditions, multiple species are adapted to utilize the same substrate and thus, contribute with similar degrees to the decomposition process and nutrient cycling (Purahong et al., 2014; Bani et al., 2018b).

Among our study sites, the Mediterranean evergreen forest in Spain (SPA2) accounted for the highest decomposition for all the litter types. However, microbial community composition and diversity at SPA2 were not significantly different from other locations. Abiotic factors may thus have stimulated the microbial decomposer activity at this site. We found a significant correlation between mass loss and soil pH (Table S3) indicating that study sites with higher soil pH accounted for the highest mass loss, as it is the case of SPA2 (pH = 7.8). It has been reported that the activity of phenol oxidase and peroxidase generally increase as soil pH increase, with a peak at pH ~8, which could in part explain the higher decomposition rates at this site (Sinsabaugh, 2010). We are aware that soil pH values extracted from maps, as in our case, are less accurate than direct measures on soil samples. However, since we focused on relative pH differences across study sites instead of absolute values, we preferred to increase data comparability between samples. In fact, the averaged values provided by maps limited the uncertainty derived from seasonal variability and habitat patchiness, which might affect the consistency of soil data.

The prediction of decomposition patterns related to microbial activities are of key relevance for understanding how soil nutrient dynamics may shift in response to global changes (McGuire and Treseder, 2010). So far, few models incorporated inter-kingdom interactions as possible drivers of microbial diversity or activity thus influencing ecosystem functioning. Our results indicated that both positive and negative co-occurrences among decomposers can reflect stable interactions between taxa and may further explain the decomposition dynamics in different litter types. Interactions between fungi and bacteria have been extensively studied in vitro (de Boer et al., 2005; Romaní et al., 2006; de Boer and van der Wal, 2008), and have been found to be both positive (in case of resource partitioning or facilitation) and negative (competition or successive replacement) with varying consequences for nutrient cycling (Fischer et al., 2006). Positive interactions between microbial taxa with different functional roles can significantly affect process rates (McGuire and Treseder, 2010). One example is the release of simple compounds from the degradation of heterogeneous substrates (e.g. wood) after the breakdown of more recalcitrant materials, which could facilitate the growth of other species. This is possible because the activity of more efficient decomposers (e.g. white-rot fungi)

Table 6
Pearson's correlation coefficients between litter mass loss, bacterial (bac) and fungal (fun) diversity indices and positive (pos) and negative (neg) species co-occurrences. Asterisks denote significance levels (*P < 0.05, **P < 0.01, ***P < 0.001). S= OTU richness; H' = Shannon diversity; J = evenness.

	Mass loss	S_{fun}	H'_{fun}	J_{fun}	S_{bac}	H'_{bac}	J_{bac}
Pos. c-oc $_{fun\text{-}fun}$	0.36***	0.16	0.13	0.05	−0.02	−0.05	0.03
Neg. c-oc $_{fun\text{-}fun}$	−0.28***	−0.09	−0.18*	−0.15	0.08	0.14	0.01
Pos. c-oc $_{fun\text{-}bac}$	−0.22*	0.08	0.15	0.08	0.03	−0.01	0.07
Neg. c-oc $_{fun\text{-}bac}$	−0.11	0.08	0.16	0.11	0	−0.04	0.05
Pos. c-oc $_{bac\text{-}bac}$	−0.22**	0.08	0.15	0.08	0.03	−0.01	0.07
Neg. c-oc $_{bac\text{-}bac}$	−0.22**	0.08	0.15	0.08	0.03	−0.01	0.07
Mass loss		−0.06	0.08	0.23**	−0.14	−0.16	−0.09

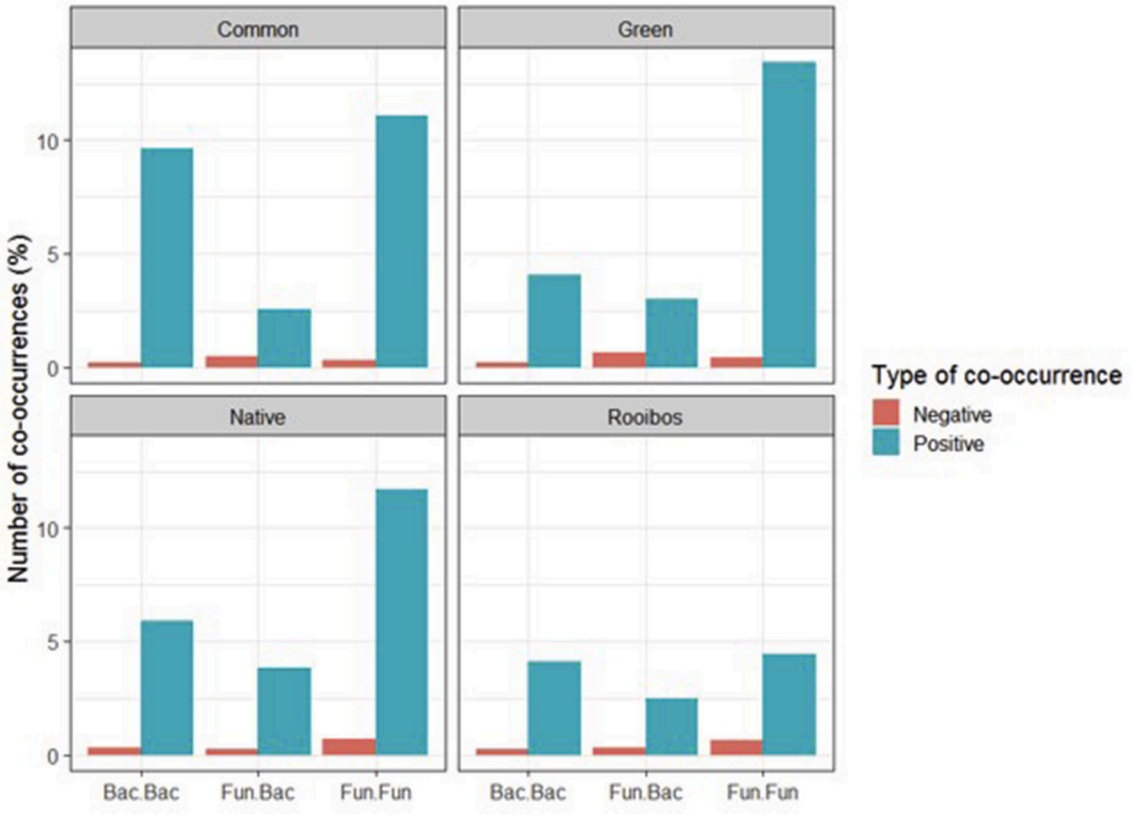

Fig. 6. Total percentage of positive and negative co-occurrences between fungi and bacteria in different litter types. Random co-occurrences are not displayed. Bac.Bac = co-occurrences between bacteria; Fun.Bac = co-occurrences between fungi and bacteria; Fun.Fun = co-occurrences between fungi.

ensures the persistence of groups of fungi and bacteria targeting more labile compounds (McGuire and Treseder, 2010). Other laboratory experiments have revealed a clear dependency of bacterial growth on the enzymatic activity of fungi, which increases the availability of easily accessible resources (Romaní et al., 2006). The coexistence of multiple microbial groups degrading specific structural polymers can potentially result in increased litter decomposition rates. We observed that the type of interaction largely depended on litter type, with green tea characterized by the largest number of positive co-occurrences between fungi (Gessner et al., 2010). The high number of positive interactions on green tea could have contributed to a more efficient mass loss as observed for this litter type. Indeed, we found a positive significant correlation between positive fungal co-occurrences and mass loss, confirming our fourth hypothesis. However, surprisingly, positive co-occurrences between fungi-bacteria and bacteria-bacteria negatively affected mass loss dynamics. As such, some of the fungal-bacterial interactions that we detected, may reflect parasitic relationships (Purahong et al., 2016) which can reduce the fungal decomposition efficiency. Similarly, the positive interaction between bacteria may involve taxa whose functional role is not related with the degradation of organic matter. Negative co-occurrences can indicate a result of competitive exclusion (Pan and May 2009). In this study, we observed few negative co-occurrences indicating that competitive exclusion may not be common in our communities. However, we found that the percentage of all negative co-occurrences was negatively correlated with mass loss. Competitive interactions can potentially lead to functional stress decreasing nutrient uptake and enzymatic production, and eventually reducing the availability of carbon used for an individual's growth (Maynard et al., 2017). Since the superior competitor is not necessarily the most efficient decomposer, decay rates can be reduced due to competition (McGuire and Treseder, 2010). In general, all the mechanisms that promote species coexistence are known to play a role in the maintenance of high community diversity (Kennedy, 2010). Interestingly, we found that negative co-occurrences between fungi negatively affected the diversity of fungi itself, which might also be expected from competitive exclusion. Although the effect of interspecific competition on fungal diversity is still controversial, some laboratory studies have found a decrease in fungal abundance as a result of competition (Engelmoer et al., 2014; Thonar et al., 2014). However, it is less clear how these changes affect species diversity and ecosystem functioning in natural environments.

5. Conclusions

In this study we investigated whether, and to what extent, litter types with different traits represent selective substrates for microbial diversity, and how resulting decomposer communities are related to climatic and soil conditions. We provide a standard protocol that improves the ability to compare microbial community diversity and decomposition dynamics across multiple ecosystems worldwide. We found that the standardized exotic litter types used in our study selected specialized communities of bacteria and fungi, with the most labile litter having the greatest difference with soil community. As we observed some differences between native and other (exotic) litters in microbial colonization, our findings can have relevant implication considering the effects of climate change on litter decomposition and other ecosystem functions, as this co-occurs with the spread of exotic plant species. The driving factors of community structure along the European transect varied according to litter type and were primarily related to soil micro-climatic conditions and properties (moisture, temperature and C/N ratios). Our study provides strong support for the hypothesis that interactions between bacteria and fungi have a substantial impact on litter decomposition, which should be accounted when predicting the patterns of microbial degradation. Identification of the different taxa involved in these interactions along with a deeper characterization of soil and litter

traits are key aspects that could provide valuable insights into microbial ecology and help to develop indicators of ecosystem functioning.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study is part of a pan-European multisite project supported by the ALTER-Net consortium, Europe's Ecosystem Research Network; it is co-financed within their multisite experiment programme. JMS acknowledges (a) the Strategic theme Sustainability of Utrecht University, sub-theme Water, Climate, and Ecosystems and (b) the Swedish research council (VR) for funding. We warmly acknowledge Corrado Leone, Rebecca Magnusson, Pascal Courtois and Johannes Tiwari, at SITES (VR) and Matthias Cuntz, Joost Keuskamp, Bernat Claramunt Lopez, Joan Pino, Heide Spiegel for their contribution in the field activities. Finally, we thank Luca Agea and Carlotta Mazzone of the high school Rainerum in Bolzano, and Michele Fiorese and Martina Verdone from the high school Galileo Galilei in Bolzano for practical help in the nucleic acid extractions within the school-work didactic alternation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2020.107778.

References

Allison, S.D., Lu, Y., Weihe, C., Goulden, M.L., Martiny, A.C., Treseder, K.K., Martiny, J. B.H., 2013. Microbial abundance and composition influence litter decomposition response to environmental change. Ecology 94, 714–725.
Althuizen, I.H.J., Lee, H., Sarneel, J.M., Vandvik, V., 2018. Long-Term climate regime modulates the impact of short-term climate variability on decomposition in alpine grassland soils. Ecosystems 21, 1580–1592.
Aneja, M.K., Sharma, S., Fleischmann, F., Stich, S., Heller, W., Bahnweg, G., Munch, J.C., Schloter, M., 2006. Microbial colonization of beech and spruce litter–influence of decomposition site and plant litter species on the diversity of microbial community. Microbial Ecology 52, 127–135.
Averill, C., Waring, B.G., Hawkes, C.V., 2016. Historical precipitation predictably alters the shape and magnitude of microbial functional response to soil moisture. Global Change Biology 22, 1957–1964.
Bani, A., Borruso, L., Fornasier, F., Pioli, S., Wellstein, C., Brusetti, L., 2018a. Microbial decomposer dynamics: diversity and functionality investigated through a transplantation experiment in boreal forests. Microbial Ecology 76, 1030–1040.
Bani, A., Pioli, S., Ventura, M., Panzacchi, P., Borruso, L., Tognetti, R., Tonon, G., Brusetti, L., 2018b. The role of microbial community in the decomposition of leaf litter and deadwood. Applied Soil Ecology 126, 75–84.
Bezemer, T.M., Fountain, M., Barea, J., Christensen, S., Dekker, S., Duyts, H., Van Hal, R., Harvey, J., Hedlund, K., Maraun, M., 2010. Divergent composition but similar function of soil food webs of individual plants: plant species and community effects. Ecology 91, 3027–3036.
Blaško, R., Högberg, P., Bach, L.H., Högberg, M.N., 2013. Relations among soil microbial community composition, nitrogen turnover, and tree growth in N-loaded and previously N-loaded boreal spruce forest. Forest Ecology and Management 302, 319–328.
Boddy, L., 2000. Interspecific combative interactions between wood-decaying basidiomycetes. FEMS Microbiology Ecology 31, 185–194.
Bouskill, N.J., Lim, H.C., Borglin, S., Salve, R., Wood, T.E., Silver, W.L., Brodie, E.L., 2013. Pre-exposure to drought increases the resistance of tropical forest soil bacterial communities to extended drought. The ISME Journal 7, 384.
Brockett, B.F., Prescott, C.E., Grayston, S.J., 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biology and Biochemistry 44, 9–20.
Cavicchioli, R., Ripple, W.J., Timmis, K.N., Azam, F., Bakken, L.R., Baylis, M., Behrenfeld, M.J., Boetius, A., Boyd, P.W., Classen, A.T., 2019. Scientists' warning to humanity: microorganisms and climate change. Nature Reviews Microbiology 1.
Chen, Y., Liu, Y., Zhang, J., Yang, W., He, R., Deng, C., 2018. Microclimate exerts greater control over litter decomposition and enzyme activity than litter quality in an alpine forest-tundra ecotone. Scientific Reports 8, 14998.
Classen, A.T., Sundqvist, M.K., Henning, J.A., Newman, G.S., Moore, J.A.M., Cregger, M. A., Moorhead, L.C., Patterson, C.M., 2015. Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: what lies ahead? Ecosphere 6 art130.
TeaTime4Schools consortium, 2018. Project Mid-term Report. Austrian Agency for Health and Food Safety (AGES).
de Boer, W., van der Wal, A., 2008. Interactions between Saprotrophic Basidiomycetes and Bacteria. British Mycological Society Symposia Series. Elsevier, pp. 143–153.
de Boer, W.d., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29, 795–811.
Didion, M., Repo, A., Liski, J., Forsius, M., Bierbaumer, M., Djukic, I., 2016. Towards harmonizing leaf litter decomposition studies using standard tea bags—a field study and model application. Forests 7, 167.
Engelmoer, D.J., Behm, J.E., Toby Kiers, E., 2014. Intense competition between arbuscular mycorrhizal mutualists in an in vitro root microbiome negatively affects total fungal abundance. Molecular Ecology 23, 1584–1593.
Evans, S.E., Wallenstein, M.D., 2012. Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? Biogeochemistry 109, 101–116.
Evans, S.E., Wallenstein, M.D., Burke, I.C., 2014. Is bacterial moisture niche a good predictor of shifts in community composition under long-term drought? Ecology 95, 110–122.
Feng, X., Simpson, M.J., 2009. Temperature and substrate controls on microbial phospholipid fatty acid composition during incubation of grassland soils contrasting in organic matter quality. Soil Biology and Biochemistry 41, 804–812.
Fichtner, A., Von Oheimb, G., Härdtle, W., Wilken, C., Gutknecht, J., 2014. Effects of anthropogenic disturbances on soil microbial communities in oak forests persist for more than 100 years. Soil Biology and Biochemistry 70, 79–87.
Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences 103, 626–631.
Fischer, H., Mille-Lindblom, C., Zwirnmann, E., Tranvik, L.J., 2006. Contribution of fungi and bacteria to the formation of dissolved organic carbon from decaying common reed (Phragmites australis). Archiv für Hydrobiologie 166, 79–97.
Freschet, G.T., Weedon, J.T., Aerts, R., van Hal, J.R., Cornelissen, J.H., 2012. Interspecific differences in wood decay rates: insights from a new short-term method to study long-term wood decomposition. Journal of Ecology 100, 161–170.
Garcia-Pichel, F., Loza, V., Marusenko, Y., Mateo, P., Potrafka, R.M., 2013. Temperature drives the continental-scale distribution of key microbes in topsoil communities. Science 340, 1574–1577.
Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular Ecology 2, 113–118.
Gartner, T.B., Cardon, Z.G., 2004. Decomposition dynamics in mixed-species leaf litter. Oikos 104, 230–246.
Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H., Hattenschwiler, S., 2010. Diversity meets decomposition. Trends in Ecology & Evolution 25, 372–380.
Glassman, S.I., Weihe, C., Li, J., Albright, M.B., Looby, C.I., Martiny, A.C., Treseder, K.K., Allison, S.D., Martiny, J.B., 2018. Decomposition responses to climate depend on microbial community composition. Proceedings of the National Academy of Sciences 115, 11994–11999.
Gleeson, D.B., Clipson, N., Melville, K., Gadd, G.M., McDermott, F.P., 2005. Characterization of fungal community structure on a weathered pegmatitic granite. Microbial Ecology 50, 360.
Gray, S.B., Classen, A.T., Kardol, P., Yermakov, Z., Michael Mille, R., 2011. Multiple climate change factors interact to alter soil microbial community structure in an old-field ecosystem. Soil Science Society of America Journal 75, 2217–2226.
Griffith, D.M., Veech, J.A., Marsh, C.J., 2016. Cooccur: probabilistic species Co-occurrence analysis in R. Journal of Statistical Software 69.
Hättenschwiler, S., Tiunov, A.V., Scheu, S., 2005. Biodiversity and litter decomposition in terrestrial ecosystems. Annual Review of Ecology, Evolution and Systematics 36, 191–218.
Hoppe, B., Purahong, W., Wubet, T., Kahl, T., Bauhus, J., Arnstadt, T., Hofrichter, M., Buscot, F., Krüger, D., 2015. Linking molecular deadwood-inhabiting fungal diversity and community dynamics to ecosystem functions and processes in Central European forests. Fungal Diversity 1–13.
Kennedy, P., 2010. Ectomycorrhizal fungi and interspecific competition: species interactions, community structure, coexistence mechanisms, and future research directions. New Phytologist 187, 895–910.
Keuskamp, J.A., Dingemans, B.J.J., Lehtinen, T., Sarneel, J.M., Hefting, M.M., Muller-Landau, H., 2013. Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems. Methods in Ecology and Evolution 4, 1070–1075.
Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology and Biochemistry 40, 2407–2415.
Marschner, P., 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biology and Biochemistry 35, 453–461.
Martinez, P., 2019. pairwiseAdonis: Pairwise Multilevel Comparison Using Adonis. R Package Version 0.3.
Mathieu, Y., Gelhaye, E., Dumarcay, S., Gerardin, P., Harvengt, L., Buee, M., 2013. Selection and validation of enzymatic activities as functional markers in wood biotechnology and fungal ecology. Journal of Microbiological Methods 92, 157–163.
Maynard, D.S., Crowther, T.W., Bradford, M.A., 2017. Fungal interactions reduce carbon use efficiency. Ecology Letters 20, 1034–1042.
McGuire, K.L., Treseder, K.K., 2010. Microbial communities and their relevance for ecosystem models: decomposition as a case study. Soil Biology and Biochemistry 42, 529–535.

Naimi, B., 2015. R package version. Usdm: Uncertainty Analysis for Species Distribution Models, 1, pp. 1–12.
Nielsen, U.N., Ayres, E., Wall, D.H., Bardgett, R.D., 2011. Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity-function relationships. European Journal of Soil Science 62, 105–116.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Package 'vegan'. Community Ecology Package, Version 2.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., 2014. Package 'vegan'. Community Ecology Package, R Package Version 2.
Pan, J.J., May, G., 2009. Fungal-fungal associations affect the assembly of endophyte communities in maize (Zea mays). Microbial Ecology 58, 668–678.
Pioli, S., Antonucci, S., Giovannelli, A., Traversi, M.L., Borruso, L., Bani, A., Brusetti, L., Tognetti, R., 2018. Community fingerprinting reveals increasing wood-inhabiting fungal diversity in unmanaged Mediterranean forests. Forest Ecology and Management 408, 202–210.
Prescott, C.E., 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry 101, 133–149.
Prescott, C.E., Grayston, S.J., 2013. Tree species influence on microbial communities in litter and soil: current knowledge and research needs. Forest Ecology and Management 309, 19–27.
Purahong, W., Schloter, M., Pecyna, M.J., Kapturska, D., Däumlich, V., Mital, S., Buscot, F., Hofrichter, M., Gutknecht, J.L., Krüger, D., 2014. Uncoupling of microbial community structure and function in decomposing litter across beech forest ecosystems in Central Europe. Scientific Reports 4, 7014.
Purahong, W., Wubet, T., Lentendu, G., Schloter, M., Pecyna, M.J., Kapturska, D., Hofrichter, M., Kruger, D., Buscot, F., 2016. Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. Molecular Ecology 25, 4059–4074.
R Core Team, 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Ramette, A., 2009. Quantitative community fingerprinting methods for estimating the abundance of operational taxonomic units in natural microbial communities. Applied and Environmental Microbiology 75, 2495–2505.
Romaní, A.M., Fischer, H., Mille-Lindblom, C., Tranvik, L.J., 2006. Interactions of bacteria and fungi on decomposing litter: differential extracellular enzyme activities. Ecology 87, 2559–2569.
Rousk, J., Bååth, E., 2007. Fungal and bacterial growth in soil with plant materials of different C/N ratios. FEMS Microbiology Ecology 62, 258–267.
Rousk, J., Bååth, E., 2011. Growth of saprotrophic fungi and bacteria in soil. FEMS Microbiology Ecology 78, 17–30.
Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry 42, 391–404.
Talbot, J.M., Treseder, K.K., 2011. Ecology: dishing the dirt on carbon cycling. Nature Climate Change 1, 144.
Talbot, J.M., Treseder, K.K., 2012. Interactions among lignin, cellulose, and nitrogen drive litter chemistry–decay relationships. Ecology 93, 345–354.
Thonar, C., Frossard, E., Šmilauer, P., Jansa, J., 2014. Competition and facilitation in synthetic communities of arbuscular mycorrhizal fungi. Molecular Ecology 23, 733–746.
Tiunov, A.V., Scheu, S., 2005. Facilitative interactions rather than resource partitioning drive diversity-functioning relationships in laboratory fungal communities. Ecology Letters 8, 618–625.
van der Wal, A., Geydan, T.D., Kuyper, T.W., de Boer, W., 2013. A thready affair: linking fungal diversity and community dynamics to terrestrial decomposition processes. FEMS Microbiology Reviews 37, 477–494.
Veech, J.A., 2013. A probabilistic model for analysing species co-occurrence. Global Ecology and Biogeography 22, 252–260.
Wallenstein, M.D., McNulty, S., Fernandez, I.J., Boggs, J., Schlesinger, W.H., 2006. Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. Forest Ecology and Management 222, 459–468.
Wang, Y., Naumann, U., Wright, S.T., Warton, D.I., 2012. mvabund- anRpackage for model-based analysis of multivariate abundance data. Methods in Ecology and Evolution 3, 471–474.
White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications, 18, pp. 315–322.
Wilhelm, R.C., Singh, R., Eltis, L.D., Mohn, W.W., 2019. Bacterial contributions to delignification and lignocellulose degradation in forest soils with metagenomic and quantitative stable isotope probing. The ISME Journal 13, 413.
Wohl, D.L., Arora, S., Gladstone, J.R., 2004. Functional redundancy supports biodiversity and ecosystem function in a closed and constant environment. Ecology 85, 1534–1540.
Xiao, W., Chen, H.Y., Kumar, P., Chen, C., Guan, Q., 2019. Multiple interactions between tree composition and diversity and microbial diversity underly litter decomposition. Geoderma 341, 161–171.
Zhou, J., Deng, Y., Shen, L., Wen, C., Yan, Q., Ning, D., Qin, Y., Xue, K., Wu, L., He, Z., 2016. Temperature mediates continental-scale diversity of microbes in forest soils. Nature Communications 7, 12083.