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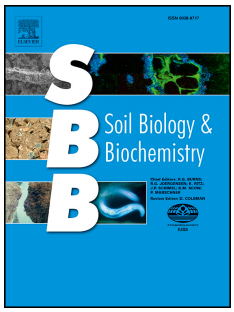

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Linking plant litter microbial diversity to microhabitat conditions, environmental gradients and litter mass loss: insights from a European study using standard litter bags

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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.

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.

Keywords: abiotic drivers, litter decomposition, microbial communities' diversity, microbial co-occurrences, molecular fingerprinting, pan-European study.

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

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

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

126

127 **Materials and methods**

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 Figure 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).

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 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 grams 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

153 of approximately 1 gram. We further determined the AUR of each native litter by acid fractionation
154 using a Soxhlet extractor, following the method described in Keuskamp et al. (2013). The AUR
155 represents the recalcitrant fraction of the material, which contains a high amount of lignin and other
156 aromatic material. Native litter C/N ratio was determined by combustion on about 4 mg of finely
157 ground and dried litter using a CHN-analyzer at Utrecht University (EA NA 1110; Carlo Erba,
158 Milan, Italy). C/N ratios and AUR for the exotic litter types were determined before the
159 experimental period and reported in Table 2. Sequencing of the starting material showed a
160 negligible microbial load in a parallel study (TeaTime4Schools consortium, 2018).

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

175

176 *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).

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 grams fresh soil after drying the soil for 48h 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.

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 Figure S1 for indices formulas) in R version 3.4.1 (R Development 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 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 micro-climatic 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.

Results

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 (Figure 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 (Figure 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 (Figure 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 (Figure S2 and Figure 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 (Figure 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 fungi (Table 3).

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 (Figures 4 - 5). 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.

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, Figure 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).

Species co-occurrences

Intra- and inter-kingdom interactions were investigated through co-occurrences matrices for each litter type across sites (Figure 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).

Discussion

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).

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), 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 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.

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

422 case of resource partitioning or facilitation) and negative (competition or successive replacement)
423 with varying consequences for nutrient cycling (Fischer et al., 2006). Positive interactions between
424 microbial taxa with different functional roles can significantly affect process rates (McGuire and
425 Treseder, 2010). One example is the release of simple compounds from the degradation of
426 heterogeneous substrates (e.g. wood) after the breakdown of more recalcitrant materials, which
427 could facilitate the growth of other species. This is possible because the activity of more efficient
428 decomposers (e.g. white-rot fungi) ensures the persistence of groups of fungi and bacteria targeting
429 more labile compounds (McGuire and Treseder, 2010). Other laboratory experiments have revealed
430 a clear dependency of bacterial growth on the enzymatic activity of fungi, which increases the
431 availability of easily accessible resources (Romaní et al., 2006). The coexistence of multiple
432 microbial groups degrading specific structural polymers can potentially result in increased litter
433 decomposition rates. We observed that the type of interaction largely depended on litter type, with
434 green tea characterized by the largest number of positive co-occurrences between fungi (Gessner et
435 al., 2010). The high number of positive interactions on green tea could have contributed to a more
436 efficient mass loss as observed for this litter type. Indeed, we found a positive significant
437 correlation between positive fungal co-occurrences and mass loss, confirming our fourth
438 hypothesis. However, surprisingly, positive co-occurrences between fungi-bacteria and bacteria-
439 bacteria negatively affected mass loss dynamics. As such, some of the fungal-bacterial interactions
440 that we detected, may reflect parasitic relationships (Purahong et al., 2016) which can reduce the
441 fungal decomposition efficiency. Similarly, the positive interaction between bacteria may involve
442 taxa whose functional role is not related with the degradation of organic matter. Negative co-
443 occurrences can indicate a result of competitive exclusion (Pan and May, 2009). In this study, we
444 observed few negative co-occurrences indicating that competitive exclusion may not be common in
445 our communities. However, we found that the percentage of all negative co-occurrences was
446 negatively correlated with mass loss. Competitive interactions can potentially lead to functional
447 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.

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.

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FIGURE CAPTION

Figure 1 – Map of the study sites. Bold circles represent overlapping study sites.

Figure 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.

Figure 3 – NMDS ordinations of bacterial and fungal communities per litter type across the European transect. Different colours represent different litters/soil. Centroids and standard errors are displayed. Stress = stress value

Figure 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.

Figure 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.

Figure 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

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

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 **

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

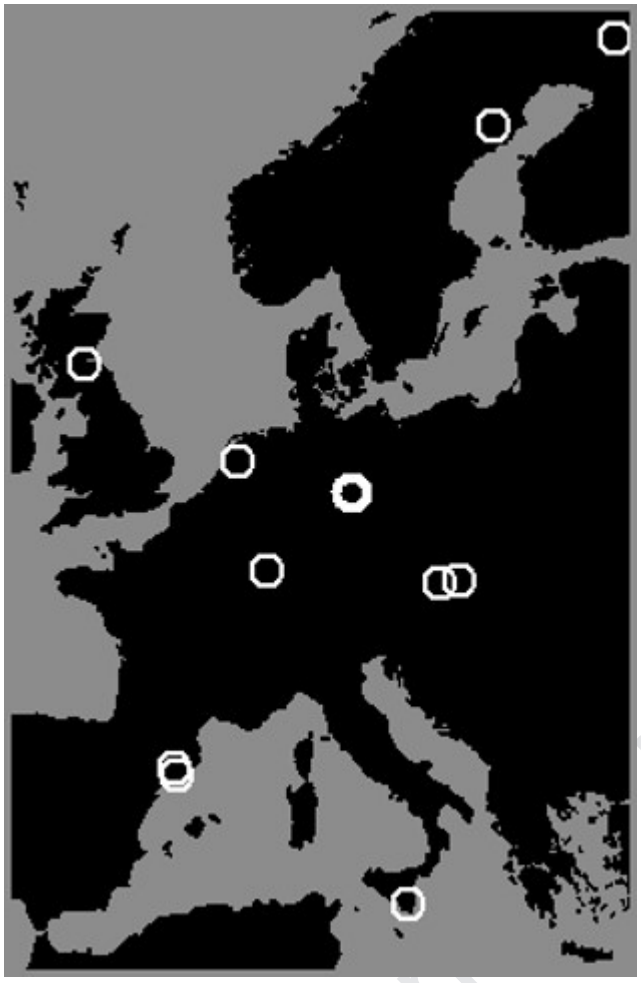
b	**Fungi**	**AIC**
Model 1	Soil moisture***	20006
Model 2	Soil temp***	20009
Model 3	Soil pH***	20001
Model 4	**Soil C/N*** **	**19997**
Model 5	Soil moisture*** + Soil temp***	20032
Model 6	Soil moisture*** + Soil pH***	20010
Model 7	Soil moisture*** + Soil C/N**	20050
Model 8	Soil temp ***+ Soil pH***	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

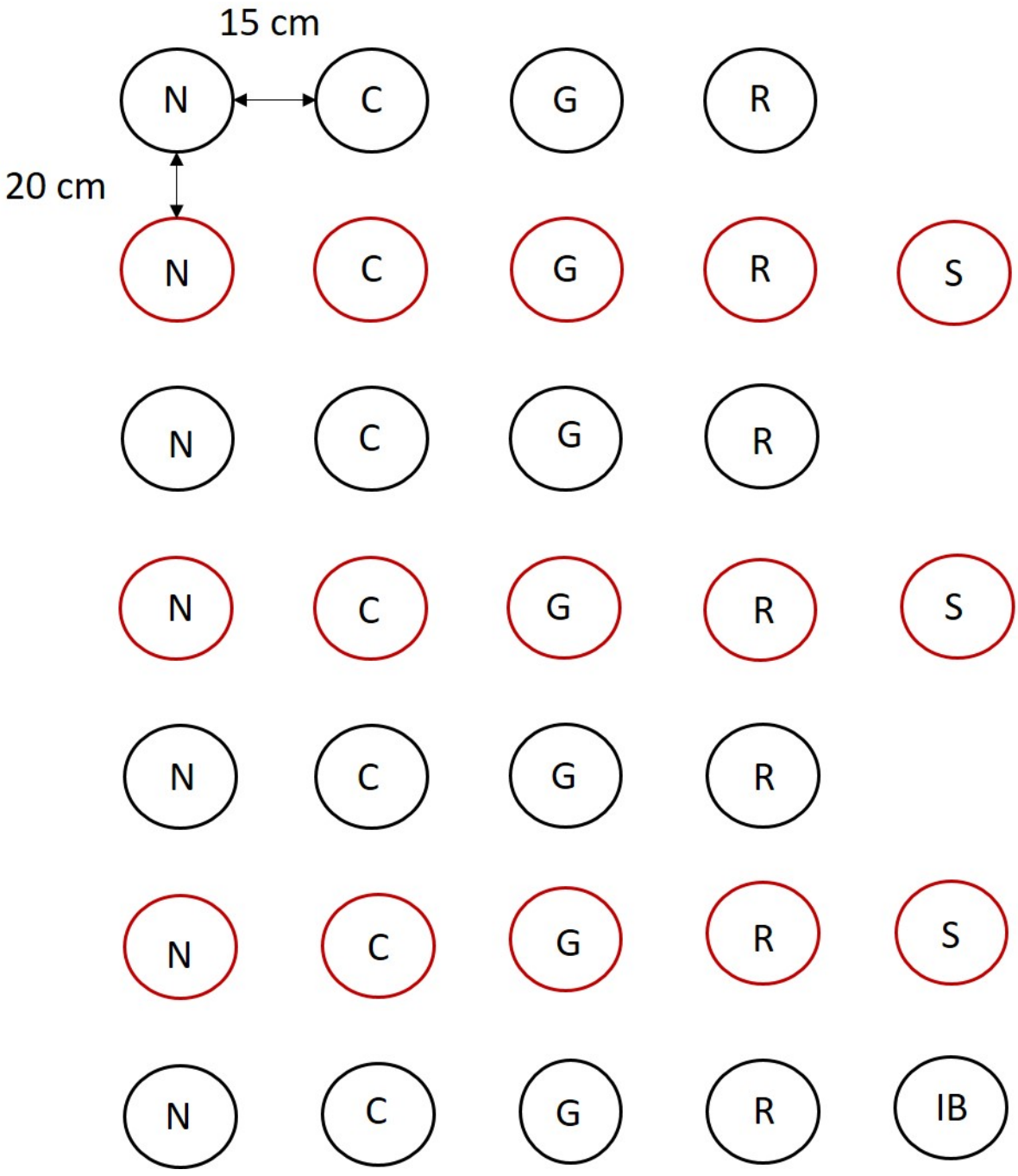
Table 5 – Mean values and standard deviation (n=4) of mass loss fractions per litter types at all sites. Different letters indicate significant differences between litters (P <0.05).

	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

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 $_{fun-fun}$	0.36***	0.16	0.13	0.05	-0.02	-0.05	0.03
Neg. c-oc $_{fun-fun}$	-0.28***	-0.09	-0.18*	-0.15	0.08	0.14	0.01
Pos. c-oc $_{fun-bac}$	-0.22*	0.08	0.15	0.08	0.03	-0.01	0.07
Neg. c-oc $_{fun-bac}$	-0.11	0.08	0.16	0.11	0	-0.04	0.05
Pos. c-oc $_{bac-bac}$	-0.22**	0.08	0.15	0.08	0.03	-0.01	0.07
Neg. c-oc $_{bac-bac}$	-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




15 cm
20 cm
N
C
G
R
N
C
G
R
S
N
C
G
R
N
C
G
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S
N
C
G
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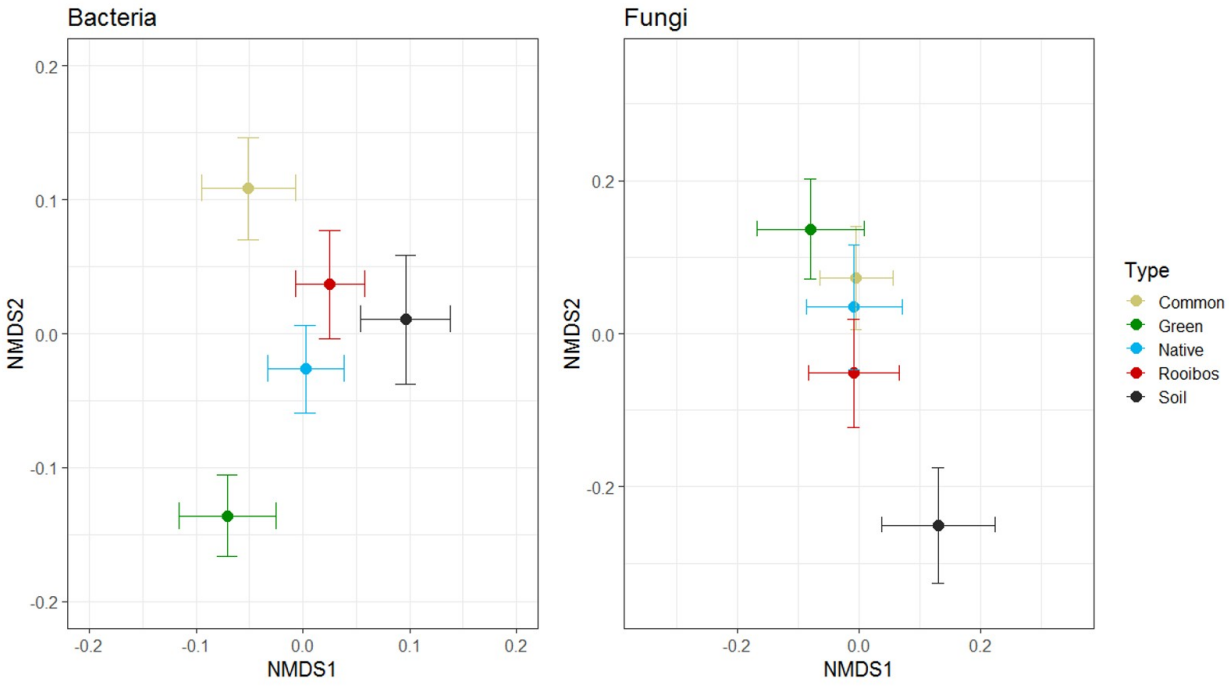
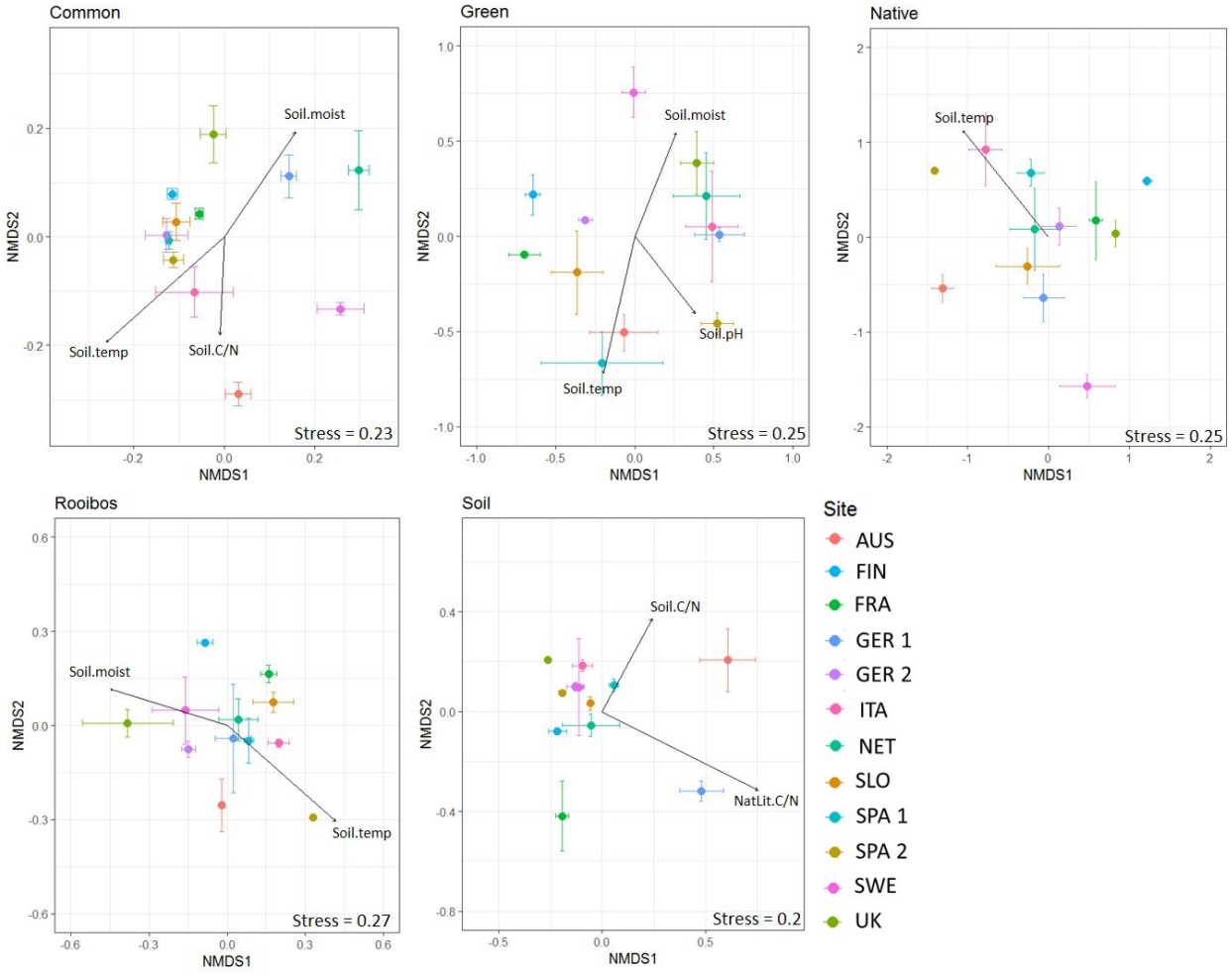
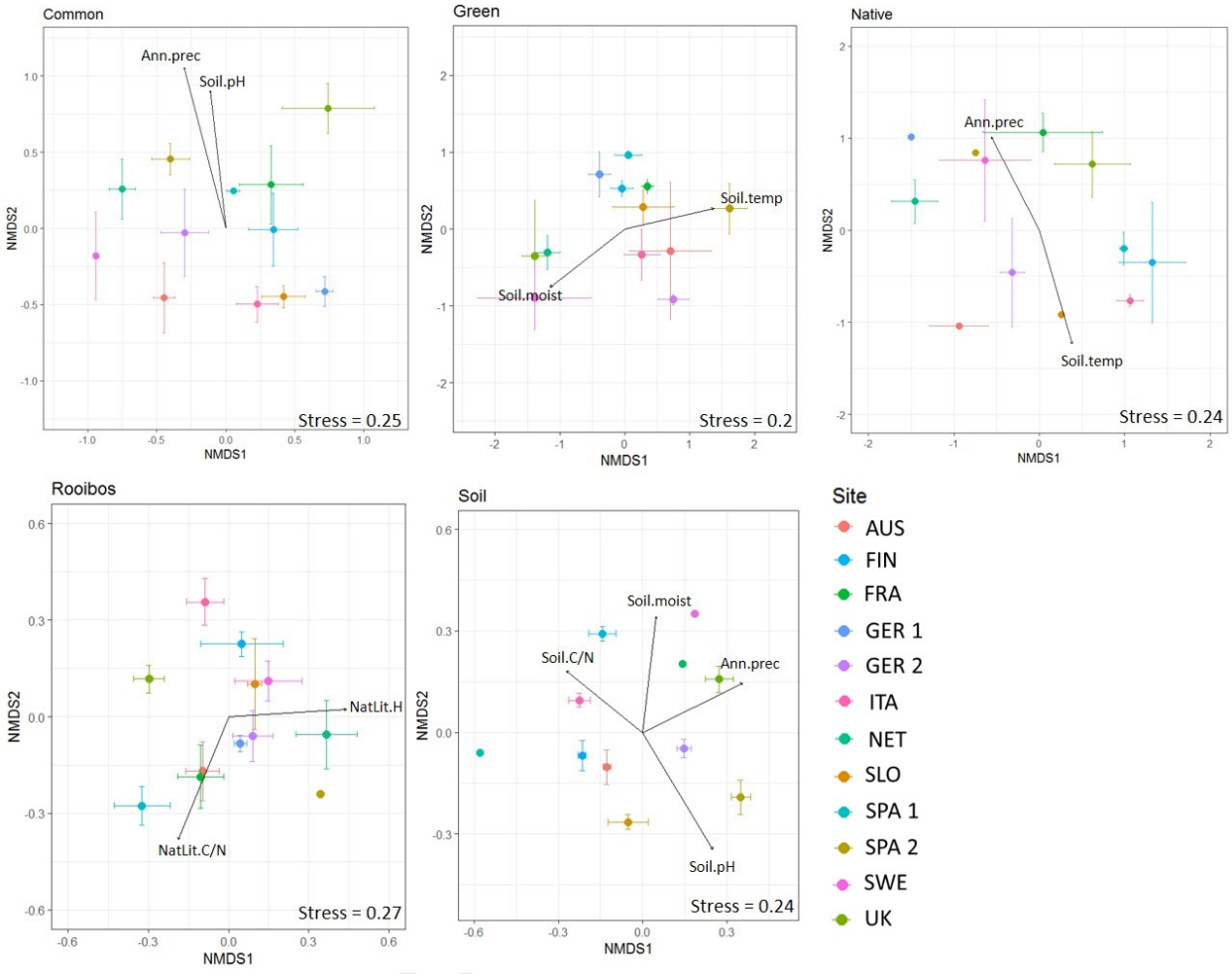
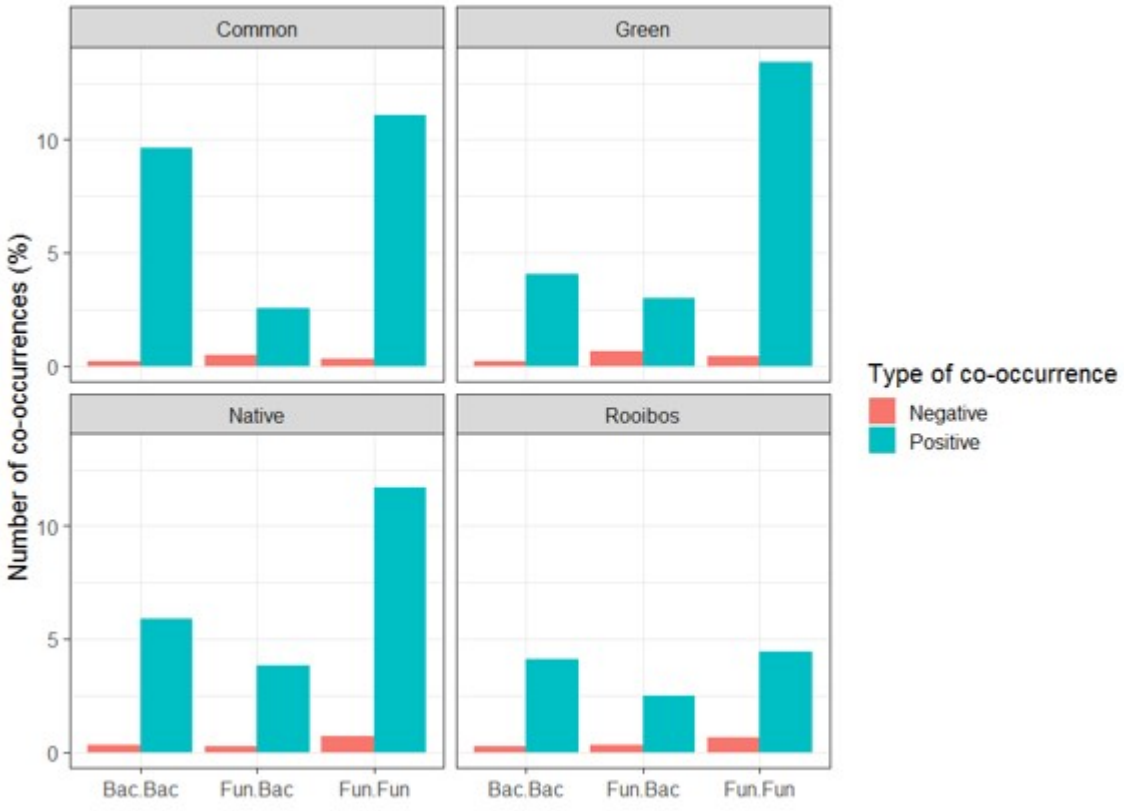
N
C
G
R
S
N
C
G
R
IB


Bacteria
Fungi
NMDS1
NMDS2
0.2
0.1
0.0
-0.1
-0.2
Type
Common
Green
Native
Rooibos
Soil


Common
Soil.moist
Soil.temp
Soil.C/N
Stress = 0.23
NMDS1
NMDS2
Green
Soil.moist
Soil.pH
Soil.temp
Stress = 0.25
Native
Soil.temp
Stress = 0.25
Rooibos
Soil.moist
Soil.temp
Stress = 0.27
Soil
Soil.C/N
NatLit.C/N
Stress = 0.2
Site
AUS
FIN
FRA
GER 1
GER 2
ITA
NET
SLO
SPA 1
SPA 2
SWE
UK


Common
Ann.prec
Soil.pH
Stress = 0.25
NMDS1
NMDS2
Green
Soil.temp
Soil.moist
Stress = 0.2
Native
Ann.prec
Soil.temp
Stress = 0.24
Rooibos
NatLit.H
NatLit.C/N
Stress = 0.27
Soil
Soil.moist
Soil.C/N
Ann.prec
Soil.pH
Stress = 0.24
Site
AUS
FIN
FRA
GER 1
GER 2
ITA
NET
SLO
SPA 1
SPA 2
SWE
UK


Common
Green
Native
Rooibos
Number of co-occurrences (%)
0
5
10
Bac.Bac
Fun.Bac
Fun.Fun
Type of co-occurrence
Negative
Positive

Highlights

- Litter types with different traits act as ecological filters for bacteria and fungi
- Edaphic conditions exert a major influence on microbial diversity
- Litter decomposition is related to fungal diversity and microbial co-occurrences
- Using standard litter types allow comparisons across wide geographic ranges

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: