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Title: Temporal turnover of the soil microbiome composition is guild-specific

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Data accessibility statement: Sequencing data have been deposited in the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under PRJNA701565. R-script and the datasets necessary to run the analysis are available as Supplementary File 1 and Supplementary File 2, respectively.

Abstract

Although spatial and temporal variation are both important components structuring microbial communities, the exact quantification of temporal turnover rates of fungi and bacteria has not been performed to date. In this study, we utilized repeated resampling of bacterial and fungal communities at specific locations across multiple years to describe their patterns and rates of temporal turnover. Our results show that microbial communities undergo temporal change at a rate of 0.010-0.025 per year (in units of Sorensen similarity), and the change in soil is slightly faster in fungi than in bacteria, with bacterial communities changing more rapidly in litter than soil. Importantly, temporal development differs across fungal guilds and bacterial phyla with different ecologies. While some microbial guilds show consistent responses across regional locations, others show site-specific development with weak general patterns. These results indicate that guild-level resolution is important for understanding microbial community assembly, dynamics and responses to environmental factors.

Introduction

Communities of organisms assemble following basic processes related to dispersal, drift, competitive interactions, and environmental filtering (Vellend, 2010; Leibold and Chase, 2018). However, the relative role of deterministic and stochastic processes in driving the development of communities across temporal and spatial scales is not sufficiently understood (Shade et al., 2013; Leibold and Chase, 2018). As a consequence, accurately predicting species distributions and abundances is difficult, particularly for microbial communities with cryptic lifestyles, where most species are still undescribed (Thompson et al., 2017; Baldrian et al., 2021). The important role of microorganisms in soil processes affecting the global climate, such as carbon storage or greenhouse gas emissions, has been widely recognized in microbial ecology (Crowther et al., 2019). Ecosystem functions, including nutrient cycling, are thus highly dependent on the resilience of the microbial communities and their metabolic activity in a constantly changing environment (Baldrian, 2017a; Jacoby et al., 2017). Because of the involvement of microbes in nutrient cycling, the spatial distribution of nutrient stocks is known to largely reflect the composition, abundance and activity of the soil microbiome (Averill et al., 2014; Štursová et al., 2016; Kivlin and Hawkes, 2020)□.

The community dynamics of several groups of organisms creates patterns not only in space but also in time (Leibold and Chase, 2018). This concept is well illustrated by a number of plant species (Verstraeten et al., 2013; in 't Zandt et al., 2021). Plants respond to temporal variations in nutrient availability by various mechanisms, such as by switching to a different nutrient source at the expense of their growth in times of increased competition (Boutin et al., 2017; Lü et al., 2017; Lu et al., 2018)□. Similar information is not yet available for microorganisms, whose spatial patterns have been widely studied on both the local (Bahram et al., 2016; Štursová et al., 2016; Karimi et al., 2018)□ and global scales (Lozupone and Knight, 2007; Põlme et al., 2014; Tedersoo et al., 2014; Větrovský et al., 2019)□; however, their dynamics at relevant timescales have not yet been relevantly addressed (Kivlin and Hawkes, 2020).

Recent methodological advances have made it possible to obtain time series of microbial community composition (Stevenson et al., 2014; Buscardo et al., 2018; Wang et al., 2019; Goldmann et al., 2020a; Goldmann et al., 2020b)□, which indicates that their temporal dynamics is rather complex. The amount of temporal variation and the respective change in microbial communities can be driven by a number of environmental factors, including site-specific factors, such as soil chemistry, nutrient availability, and vegetation, and global factors, such as climate (Averill et al., 2019; Kivlin and Hawkes, 2020).

The dynamics of microbial communities in soils should also reflect the stratification of soil properties and microbial activity. Forest topsoils show especially high levels of such stratification (Jumpponen et al., 2010; Voříšková et al., 2014; Lladó et al., 2017)□. Typically, the decrease in the quality of organic matter from litter to deeper soil horizons is followed by a decrease in microbial diversity, biomass and activity (Tedersoo et al., 2003; Lindahl et al., 2007; Šnajdr et al., 2008; Lladó et al., 2017)☐. While fungal communities in litter are driven mainly by litter chemistry (Kivlin and Hawkes, 2016; Bahnmann et al., 2018; Santonja et al., 2018)☐, in deeper soil horizons, additional abiotic factors such as pH, moisture or carbon content, also contribute (Bahnmann et al., 2018; Burke et al., 2019)☐. Microbial communities in litter are also exposed to larger fluctuations in climate and resource quality compared to communities in soil.

Our knowledge and understanding of the microbial response to global climate change and its influence on microbe-mediated biogeochemical processes is still limited (Jansson and Hofmockel, 2020)☐. To be able to predict such a response, the present temporal stability of the soil microbiome has to be assessed in undisturbed soils to provide a baseline for future predictions. To obtain such baseline data, we addressed temporal variations in soil microbial community composition by repeatedly sampling fungal and bacterial communities in soil and litter in 2013, 2016 and 2017 at 31 locations in a Central European temperate forest during the same growing season. Extensive replication in space and precise resampling within same locations were performed to (1) determine how fungal and bacterial community composition shifts over time and if the rates of temporal turnover differ between these two domains, (2) assess whether the rates of temporal community turnover vary among locations, (3) compare the rates of turnover in litter and soil, and (4) determine whether the rates of temporal turnover are affected by site-specific characteristics (e.g., nutrient content or vegetation).

We hypothesized that the bacterial communities would exhibit faster turnover rates than the fungal communities because of the faster growth rates of bacteria and the long-term association of certain fungi with their plant hosts (Peay et al., 2016; Koch et al., 2018). Owing to their common traits, such as unicellular morphology and low cost of biomass production, yeast communities were expected to follow the patterns of bacteria and filamentous fungi were expected to be less dynamic. Free living saprotrophs have a more generalist ecology and are able to adapt to a broad range of environmental conditions, while ectomycorrhizal fungi re-establish mutualistic symbiotic associations every season; therefore, we expected ectomycorrhizal communities to undergo more dynamic temporal changes. The differences in turnover rates between litter and soil should reflect the higher rates of microbial activity and faster change of environmental conditions in litter, making litter more dynamic than soil. Within litter, its rate of decomposition, which is plant species-specific, is expected to affect the dynamics of microbial communities with faster decomposition, implying faster change.

Materials and Methods

Study area and sample collection and processing. Samples were collected in the Training Forest Enterprise Masaryk Forest Křtiny of Mendel University in Brno, a managed mixed temperate forest located north of Brno, Czech Republic (latitude 49°19' N, longitude 16°45' E). The forest covers 102 km^2 spread across altitudes ranging from 210 to 575 m a.s.l. and is characterized by a wide range of natural conditions and environmental factors. The forest is mostly mixed woods with 46% coniferous and 54% deciduous tree species. The mean annual temperature is 7.5 °C, and the mean annual precipitation is 610 mm (Supplementary Table 1).

The study area was divided into sixty-four 1 km^2 squares covering an approximately 8 x 8 km grid. Sampling sites were located in the centers of each square or nearby if the center of the square did not fall into forest. The sites were sampled in 2013 and resampled in 2016 and 2017. In the period between the first and the last sampling, several sites experienced serious disturbance or other change of environmental conditions (windthrow, clearcutting, thinning, etc.) and were thus omitted from sampling, resulting in the recovery of 31 out of the initial 64 sites in all the sampling years (Supplementary Fig. 1, Supplementary Table 2).

Five soil cores of 4-cm diameter were collected at each location and time, including a central core and four additional cores located at a distance of exactly 2 m to the north, south, east and west from the central core, and they were pooled to create a composite sample. The distance between the central (and additional) soil cores in the respective sampling years was 20 cm, i.e., each of the 5 cores in subsequent years were collected by shifting the exact positions 20 cm in the same direction (Fig. 1). In 2017, a second set of samples (2017b) was collected at each location to quantify the share of between-year variation in community composition caused by the shift of sampling in space by 20 cm. By following this sampling design, samples collected in 2017 (samples a and b, same year), 2016 and 2017a/b (1 year difference), 2013 and 2016 (3 years difference), and 2013 and 2017a/b (4 years difference) were all sampled at equal spatial distances from each other.

To the best of our knowledge, spatial sampling of microbiomes has not been replicated by resampling the same locations at exactly defined positions. Microbial communities are extremely spatially heterogeneous, with fungal communities observed at a scale of centimeters to meters (Mummey and Rillig, 2008; Kadowaki et al., 2014)□ and bacterial communities observed at an even smaller scale (Nunan et al., 2003)□**.** Thus, within-site spatial variability in the total spatiotemporal dynamics of soil microbial communities must be discussed at this scale and sample dissimilarity at the same sampling time must be considered, such as by collecting two independent samples in 2017. Furthermore, since microbial communities in topsoil show seasonal changes in composition (Voříšková et al., 2014; López-Mondéjar et al., 2015; Zhang et al., 2020) that follow trends at timescales longer than one year; thus, sampling must be performed at exactly the same time to consider vegetation phenology. Here, sampling of the temperate forest region was performed in the first days of October each year, which phenologically corresponds to the onset of tree senescence (the first senescent leaves of *Fagus sylvatica* start to fall).

Soil sampling and vegetation survey. The soil cores were stored at 4 °C and processed within 24 h after collection. Litter was collected from the soil cores, and the upper 10 cm of the soil was used for further processing. Material from the five cores per site was combined to create a composite sample of both litter and soil. Litter was cut, and soil was sieved through a 5-mm sieve. Two grams of fresh soil and litter for DNA extractions were stored at -80 °C, while the rest of the material was freeze-dried. The water content was measured as a loss of mass after freeze-drying, the organic matter content was measured after combustion at 650 °C, and the pH was measured in distilled water (1:10). Measurements of organic carbon (C) and nitrogen (N) contents were performed in an external laboratory. The same procedure was repeated in 2013, 2016 and 2017, and average values were calculated for each location. Additionally, within each plot, all vascular plant species were recorded and cover abundance was estimated. A vegetation survey was conducted in 2013. At each site, vegetation was sampled in a 5 × 5 m plot around the area sampled using soil cores. Within each 25 m^2 plot, all vascular plant species were recorded, including those rooted outside the border but with branches extending over the sides of the plot. The cover abundance of plant species was estimated on the nine-degree Braun-Blanquet scale for the tree layer, shrub layer and herbal layer (Westhoff and Van Der Maarel, 1978) (Supplementary Table 3). To summarize the plant community composition, a principal component analysis was applied to the Hellinger-transformed community matrix, and principal component 1 (hereafter Plant_PC1) included vegetation in posterior statistical analyses. Plant_PC1 was positively associated with the broadleaved tree species *Fagus sylvatica*, *Acer platanoides* and *Fraxinus excelsior* and negatively associated with the conifer *Picea abies*.

DNA extraction and amplicon-based sequencing. DNA was extracted in triplicate from 250 mg of fresh soil and litter following a modified Miller method (Sagova-Mareckova et al., 2008). The extracted DNA was cleaned using a Geneclean Turbo Kit (MP Biomedicals). Triplicate extracts were combined into one sample that was used as a template for PCR amplification. PCR was performed in triplicate to reduce PCR bias. The fungal ITS2 region was amplified using barcoded gITS7 and ITS4 primers (Ihrmark et al., 2012), and the bacterial hypervariable V4 region of the 16S rRNA gene was amplified using barcoded primers 515F and 806R (Caporaso et al., 2012). Triplicate PCRs contained 5 µL of 5x Q5 Reaction Buffer, 0.25 µL of Q5 High-Fidelity DNA polymerase, 1.5 µL of BSA (10 mg/mL), 0.5 µL of PCR Nucleotide Mix (10 nM), 0.5 µL of each primer (10 pmol/µL), 1 µL of template DNA (concentration approximately 5-50 ng/µL) and sterile ddH2O up to 25 µL. Conditions for fungal ITS2 amplification were 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for

30 s, and a final setting of 72 °C for 10 min. Conditions for V4 16S amplification were 94 °C for 4 min, 25 cycles of 94 °C for 45 s, 50 °C for 60 s and 72 °C for 75 s, with a final setting of 72 °C for 10 min. Amplicons were pooled, purified, and sequencing libraries were produced using the TruSeq PCR-free Kit (Illumina). Sequencing was performed in house using an Illumina MiSeq system (2 x 250 base reads).

Bioinformatic analysis. Amplicon sequencing data processing was performed using the pipeline Seed 2.0.3. (Větrovský et al., 2018) as described previously (Žifčáková et al., 2016). Briefly, paired-end reads were joined using fastq-join (Aronesty, 2013). For the ITS2 marker, the ITS2 region was extracted using ITSx 1.0.8 (Nilsson et al., 2010) before performing clustering and a chimera check, while for the 16S marker, primer sequences were removed. Chimeric sequences were detected using Usearch 8.0.1623 (Edgar, 2010) and deleted, and sequences were clustered into operational taxonomic units (OTUs) using UPARSE implemented within USEARCH (Edgar, 2013) at a 97% similarity level. The most abundant sequence of each OTU was used to represent it.

The identification of bacterial and fungal sequences was performed using BLASTn against the Ribosomal Database Project (Cole et al., 2014) and UNITE (Nilsson et al., 2019), respectively. Sequences identified as nonbacterial or nonfungal were discarded. The OTU tables were randomly subsampled to 6300 sequences for 16S and 3150 sequences for ITS2. Fungal ecology was determined using the FungalTraits database (Põlme et al., 2020): fungal genera with the yeast body form were classified as yeasts, all remaining fungal genera were classified based on their primary ecology. For bacteria, ecological guild classification is not available, but some traits appear to be phylogenetically conserved on the level of phyla (Martiny et al., 2015; Kielak et al., 2016; Lladó et al., 2017). For this reason, bacterial classification to phyla was used in analyses.

Statistical analysis. All analyses were performed on presence-absence community matrices because this method allows for easy interpretation of community turnover as species replacement over time.

When the same analyses were performed using OTU relative abundance data, qualitatively similar results were obtained. Fungal and bacterial OTU compositions in soil and litter were analyzed to explore the overall temporal dynamics of these groups. Then, we analyzed the three most abundant fungal ecological guilds (saprotrophs, ECM and yeasts) and six most abundant bacterial phyla (Proteobacteria, Actinobacteria, Planctomycetes, Bacteroidetes, Verrucomicrobia and Acidobacteria) separately for soil and litter to determine whether fungi and bacteria are ecologically homogenous groups that do not present within-domain variations in temporal dynamics or whether they are composed of ecologically heterogeneous subgroups.

We expected to capture three kinds of year-to-year variation: directional temporal trend, non-directional temporal trend and stochastic temporal variation. To distinguish between them, we applied PERMANOVA (Anderson, 2001) analysis to each microbial community in two different ways: i) including sampling year as numerical explanatory variable (linear effect) and ii) including it as categorical explanatory variable (non-linear effect). A significant linear effect of sampling year would indicate the presence of directional temporal trend. Since categorical effect includes the possibility of linear effect, categorical year would yield similar explanatory power (measured as R^2) in presence of just directional effect. On the other hand, a superior explanatory power of the analysis with categorical sampling year would indicate the presence of a non-directional (non-linear) temporal trend. Lastly, no (or little) effect of year, in presence of temporal community turnover, would indicate stochastic temporal variation (i.e. with no systematic changes in time across spatial locations). PERMANOVAs were fitted with adonis() function of the *vegan* package (Oksanen et al., 2018) in R (R Core Team, 2018, v 3.3.2). Spatial location was included as strata in the models to account for the fact that samples of different years were repeated measures within spatial locations. *P*-values were computed with 999 permutations. Additionally, to visualize the fungal and bacterial community structure in soil and litter, we applied the unconstrained ordination method nonmetric multidimensional scaling (NMDS) to the sampling units using the Sorensen dissimilarity. NMDS was performed using the metaMDS() function of the *vegan* package (Oksanen et al., 2018) in R.

Significant explanatory variables were added to the ordinations as arrow vectors using the envfit() function of the *vegan* package (Oksanen et al., 2018). The significance of the variables was tested with 999 permutations. All NMDS ordinations and PERMANOVAs were applied on Sorensen dissimilarity matrices of community composition.

To quantify the rate of community turnover over time, we computed the Sorensen similarity (as 1-dissimilarity) between the samples collected in the same location in different years and we built distance decay plots (Nekola and White, 1999) by opposing the Sorensen similarity to the distance in time between samples. Sorensen index takes values from 0 to 1, thus it is easily interpreted as percent difference in community similarity. Then, using linear mixed effect models as implemented in the *lme4* package (Bates et al., 2015) in R, we fitted a model for each studied community using the Sorensen similarity between samples as a response variable and temporal distance between samples as a fixed explanatory variable. The estimate of the slope of this relationship represents the overall rate of community turnover. We computed bootstrap confidence intervals (with 999 resamplings with replacement) for the regression parameters using confint.merMod() function of *lme4* package. The null hypothesis of no temporal community turnover was rejected when the 95% confidence interval of the slope did not overlap zero. To model between site variations in community turnover we fitted site-level random effects as random intercepts and slopes (random = ~ time|location). Moreover, to quantify how much of the temporal turnover was the result of species losses or gains and how much due to replacement of species without changes in species richness, we performed the partitioning of beta diversity (measured as Sorensen similarity) into species replacement effect and species richness effect (species gain/loss), as described in (Carvalho et al., 2013). The partitioning was performed with beta() function of package *BAT* (Cardoso et al., 2021) in R.

Last, to assess whether site characteristics, such as vegetation, nutrient content and species diversity, influence the rate of temporal turnover, the interactions of time with Plant_PC1, total N (for soil communities), litter C/N ratio (for litter communities) and species richness (the species richness of the specific community being modeled) were included in the mixed models and tested for significance. Significance was assessed with the above bootstrap confidence interval procedure: the null hypothesis of no effect was rejected when the 95% confidence interval of the parameter associated to each interaction did not overlap zero. A significant interaction between time and these variables would mean that temporal community turnover depends on the site vegetation type, nutrient content or species diversity. All covariates excluding temporal distance were scaled to a mean zero and unit variance; thus, the model intercepts represent within-year or baseline similarity (i.e., community similarity between 2017a and 2017b).

Results

All microbial groups showed systematic differences between the studied years, as indicated by the significant effects of study year found in PERMANOVA analyses (Table 1). However, the strength of the temporal patterns varied widely between taxa: soil fungi showed the strongest pattern, followed by litter fungi, soil bacteria and litter bacteria (Table 1). Evidence for a directional trend was stronger in fungi than in bacteria: for fungi, the non-lineal analysis yielded near 33% higher R^2 as compared to the lineal analysis; whereas for bacteria, the non-lineal analysis yielded near 66% higher R^2 values. Moreover, the directional temporal trend was clearly visible in soil fungi; samples from 2013 and 2017 tended to separate in independent clusters at opposing ends of the ordination, and samples from 2016 clustered somewhere in the middle (Fig. 2a). By contrast, soil bacteria showed non-directional between-year differences; samples from 2016 tended to separate from the rest and, samples from 2013 and 2017 tended to cluster together (Fig. 2b). Temporal trends in litter fungal and bacterial communities were not clearly visible in the ordinations (Fig. 2c, d). Several environmental variables correlated to the main patterns in community composition, but only Plant_PC1 and pH (from either soil or litter) were significantly associated with the composition of all studied communities (Fig. 2).

There was also a clear difference in the rates of temporal community turnover between fungi and bacteria as well as between soil and litter. Soil fungi, which showed a strong temporal pattern in community composition, exhibited the fastest temporal turnover of community composition (i.e., the steepest slope in the time decay plot), and the rate was approximately 2.5 times faster than that of soil bacteria. Moreover, the temporal turnover of litter bacteria was significantly faster than that of soil bacteria (Fig. 3). Litter fungi showed no significant relationship between changes in community similarity and distance over time (Fig. 3). However, the baseline similarity modeled by the intercept was already remarkably low for litter fungi: 0.332 in units of Sorensen similarity compared to 0.451 for soil fungi, 0.410 for litter bacteria and 0.416 for soil bacteria (Fig. 3). The partitioning of beta diversity (temporal community turnover) into species replacement effect and species richness effect (species gain/loss) revealed that species replacement was the main driver of the temporal change (Supplementary Fig. 2).

The differences in community composition across time were apparent within fungal ecological guilds. The temporal trend observed in soil fungi appeared equally strong in the soil saprotroph community (Supplementary Fig. 3a), with samples from 2013 and 2017 clearly separating into independent clusters and samples from 2016 clustering in the middle. Saprotrophs and ECM in both soil and litter showed evidence of directional temporal trend, with non-lineal and lineal effects of sampling year yielding similar R^2 values (Table 1). However, soil and litter yeasts showed strong non-directional trend, with non-lineal analyses yielding near three times as large R^2 values (Table 1). The overall temporal pattern of the soil yeast community resembled the pattern of soil bacteria, with samples from 2016 tending to cluster separately from the rest (Supplementary Fig. 3c, Fig. 2b). The patterns were not evident in the ordinations of soil ECM and litter fungal guilds (Supplementary Fig. 3b, d, e, f).

Differences in community composition trends over time were also present among bacterial phyla. Directional temporal trend was rather weak in all bacterial phyla in soil and litter (Table 1). In line with domain-level patterns, non-lineal analyses yielded considerably higher R^2 values pointing to non-directional trends (Table 1). The temporal pattern observed for the whole soil bacterial community, where 2016 samples tended to separate from the rest, was visible in Proteobacteria and, to a limited extent, in Bacteroidetes and Verrucomicrobia (Supplementary Fig. 4a, d, e). None of the bacterial phyla in litter showed clear between-year differences upon visual inspection (Supplementary Fig. 5).

Differences in the rates of temporal community turnover were evident among fungal ecological guilds and bacterial phyla (Fig. 4). Despite weaker systematic temporal trend, soil ECM fungi showed slightly faster temporal turnover than soil saprotrophs. The temporal community turnover of yeasts again behaved differently from that of other fungal guilds (Fig. 4a). Low baseline similarities observed in litter fungi were clear in all ecological guilds, and no significant temporal trends were observed (Fig. 4b). Among soil bacteria, Planctomycetes and Verrucomicrobia showed the lowest temporal turnover. All other soil bacterial phyla showed similar, slightly higher rates (Fig. 4a). Overall, litter bacterial phyla had higher rates of community turnover than soil bacterial phyla, but there were between-phylum differences, with Planctomycetes and Verrucomicrobia showing slower temporal turnover rates than other phyla (Fig. 4a).

Lastly, temporal turnover of species was strongly dependent on local conditions, with site-level random effects explaining a considerable fraction of the variance in most of the studied communities, including whole domains as well as specific fungal guilds and bacterial phyla (Fig. 4c). None of the available predictors of nutrient content (C and N content) or litter turnover rate (vegetation composition) had a significant effect on the turnover rates of whole communities of fungi or bacteria. The only significant interactions between the time effect and site characteristics were found for soil saprotrophs (*time × total N soil* parameter estimate[CI] = -0.006[-0.012,-0.001]) and litter Proteobacteria (*time × plant PC1*, parameter estimate[CI] = 0.004[0.000,0.008]). The community of saprotrophic fungi in soil showed faster turnover in forest stands with high soil N content, whereas litter Proteobacteria turned over faster in stands dominated by spruce than in stands dominated by broadleaved species (Supplementary Fig. 6).

Discussion

Our study revealed that patterns of community change and rates of temporal community turnover vary widely between and within fungi and bacteria, as well as between soil and litter. Soil fungi exhibited strong temporal patterns and the highest rate of temporal turnover. As expected, the temporal turnover of bacterial communities was higher in litter, which experiences larger fluctuations in environmental conditions (e.g., climate), than in soil and is more often subject to seasonal change (Baldrian, 2017b). On the other hand, litter fungi showed no significant temporal community turnover and baseline similarity modeled by the intercept was remarkably low. This is mainly because litter fungi, unlike bacteria, show a high level of spatial variation, even at scales of <2 m (Štursová et al., 2016). Differences in litter communities were previously observed at centimeter distances within a single tree leaf (Navrátilová et al., 2017). Here, multiple composite litter samples collected at the same time at a distance of 20 cm showed similarity at <0.20 Sorensen units (Fig. 2). Due to this level of spatial variation and the fact that tree litter undergoes profound seasonal changes representing bottleneck events due to seasonal litter input, the rates of temporal turnover appear to be too fast to be measured (Voříšková et al., 2014). Species replacement was the main driver of temporal community turnover in all studied communities, whereas species loss (or gain) had little effect; this is not surprising, since several long-term studies of temporal community change of multiple taxa have shown that species diversity can remain unvarying even in presence of species community turnover (Brown et al., 2001).

The three types of temporal patterns observed in community composition may indicate different processes that affect community development. First, we observed gradual development of microbial communities that followed similar trends over time among all studied sites. This pattern was strongest in fungal saprotrophic communities (Supplementary Fig. 3a) and may indicate directional shifts in community composition caused by environmental changes on a regional scale, such as climate. Climate was identified as an important driver of fungal species distribution (Tedersoo et al., 2014; Větrovský et al., 2019). Moreover, members of fungal ecological guilds showed different sensitivities to climate (Větrovský et al., 2019). In our study site, a strong increasing trend in accumulated precipitation was observed between 2013 and 2017 (Supplementary Fig. 7b), however, whether such effects are able to change community composition in the short term and from year to year is not clear.

Second, the separation of years with no gradual pattern but similar trends among the studied sites (Supplementary Fig. 3c) might indicate the response to short-term climatic conditions. Similar to fungi, bacteria also seem to respond to temperature (Thompson et al., 2017). Interestingly, this second pattern was observed in bacteria and yeasts that were previously reported to share some similarity in ecology due to their unicellular nature, which makes them dependent on local resources within microhabitats (Mašínová et al., 2018). Compared to filamentous multicellular organisms, communities of unicellular microbes may undergo more rapid development (Rillig et al., 2017). A few winters with mild temperatures preceded the sampling year in 2016, which might has influenced 2016 community compositions (Supplementary Fig. 7b). Nevertheless, we recognize that the conclusion is speculative and this observation may well be a coincidence.

The third pattern indicates random changes in local communities that are site specific. This pattern was observed mainly in soil ECM fungi (Supplementary Fig. 3b), which showed the weakest temporal patterns together with the fastest rates of temporal turnover among fungal guilds. One of the potential explanations would be the stochasticity of recolonization of fine tree roots newly emerging every year. Compared to saprotrophic fungi, the activity of ECM fungi is largely reduced during winters in temperate regions; therefore, their communities have to re-establish every season (Žifčáková et al., 2016). The flush of carbon to the new roots in the spring season can allow some ECM fungal taxa to capture a disproportionate fraction of the resource and become dominant in the soil, which may lead to high temporal variability of the ECM fungal community. The importance of priority effects on the composition of ECM fungal communities has been repeatedly documented

(Kennedy et al., 2009; Livne-Luzon et al., 2017). Although the importance of stochasticity on the assembly of mycorrhizal fungal communities at the beginning of the growth season has been previously hypothesized (Dumbrell et al., 2011), our study provides the first direct support for this hypothesis.

Planctomycetes and Verrucomicrobia showed the lowest temporal turnover among bacterial phyla in soil and litter, which is partly due to their slow growth. Bacterial taxa indeed show differences in growth rates in soil (Lladó et al., 2019; Blazewicz et al., 2020); in grassland soils, Planctomycetes and Verrucomicrobia showed slower growth than other bacterial phyla, including Proteobacteria, Acidobacteria and Actinobacteria (Blazewicz et al., 2020). The rate of growth is one of the obvious potential drivers of temporal community turnover. However, our hypothesis that a faster growth rate of bacteria may result in a more rapid change in their communities was not confirmed. A possible explanation is that the share of inactive (dormant or slow growing) bacteria in soils is relatively high (Lennon and Jones, 2011), and these microbes represent the slow fraction of community turnover. Moreover, it has been reported that the choice of data type (presence-absence vs. abundance) and similarity index (e.g. Sorensen index vs. Bray-Curtis index) may influence distance-decay relationships by giving different weights to rare vs. abundant or dormant vs. active species in the community (Locey et al., 2020). Therefore, to ensure the robustness of our results and conclusions we repeated all analyses based on abundance data and using Bray-Curtis dissimilarity index, obtaining qualitatively similar results (results not shown).

Although temporal turnover of species was strongly dependent on local conditions, none of the available predictors could explain a substantial fraction of these between site variations. The community of saprotrophic fungi in soil showed slightly faster turnover in forest stands with high soil N content; this is in line with previous evidence that community assembly processes leading to stochasticity may increase with productivity (Chase, 2010). Previous studies observed greater temporal changes in litter communities of fungi in hardwood forests with rapidly decomposing litter (Voříšková et al., 2014) than in coniferous forests with slowly decomposing litter (Žifčáková et al., 2016). However, as stated above, the temporal turnover of fungi in litter was impossible to detect, which was likely due to high spatial variation in community composition. On the other hand, litter Proteobacteria showed the opposite trend, turning over slightly faster in stands dominated by spruce than in stands dominated by broadleaved species.

Our results indicate that microbial communities undergo temporal change at a rate of 0.010-0.025 Sorensen units per year, which is slightly faster in fungi than in bacteria in soil, while bacterial communities in litter change more rapidly than in soil. Importantly, the rate of turnover and patterns of temporal development were not uniform across fungal guilds and bacterial phyla with different ecologies. While some microbial guilds showed consistent responses across regional locations, others showed site-specific development without general patterns. Short-term climatic conditions are one of the possible drivers of community development. Moreover, a guild-level resolution is important for understanding microbial community assembly, dynamics and response to environmental factors.

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Conflict of Interest. All authors declare that there are no competing interests.

Tables:

Table 1. Summary of PERMANOVA analyses on the effect of time (coded as numeric continuous variable or factor with three levels) on fungal and bacterial species compositions, as well as, fungal guild and bacterial phyla compositions, in soil and litter. *P*-values were computed using 999 permutations.

Community	**Effect**	**Df_{num}/Df_{den}**	**Adjusted R^2**	***p*-value**
Soil fungi				
	Study year (numeric)	1/92	0.04	0.001
	Study year (factor)	2/91	0.06	0.001
Soil bacteria				
	Study year (numeric)	1/92	0.01	0.001
	Study year (factor)	2/91	0.03	0.001
Litter fungi				
	Study year (numeric)	1/92	0.03	0.001
	Study year (factor)	2/91	0.05	0.001
Litter bacteria				
	Study year (numeric)	1/92	0.01	0.001
	Study year (factor)	2/91	0.03	0.001
Soil Saprotrophs				
	Study year (numeric)	1/92	0.06	0.001
	Study year (factor)	2/91	0.08	0.001
Soil ECM				
	Study year (numeric)	1/92	0.03	0.001
	Study year (factor)	2/91	0.04	0.001
Soil Yeasts				
	Study year (numeric)	1/92	0.04	0.001
	Study year (factor)	2/91	0.12	0.001
Litter Saprotrophs				
	Study year (numeric)	1/92	0.04	0.001
	Study year (factor)	2/91	0.06	0.001
Litter ECM				

	Study year (numeric)	1/92	0.04	0.001
	Study year (factor)	2/91	0.05	0.001
Litter Yeasts				
	Study year (numeric)	1/92	0.02	0.001
	Study year (factor)	2/91	0.05	0.001
Soil Proteobacteria				
	Study year (numeric)	1/92	0.01	0.001
	Study year (factor)	2/91	0.03	0.001
Soil Actinobacteria				
	Study year (numeric)	1/92	0.01	0.001
	Study year (factor)	2/91	0.02	0.001
Soil Planctomycetes				
	Study year (numeric)	1/92	0.01	0.003
	Study year (factor)	2/91	0.03	0.001
Soil Bacteroidetes				
	Study year (numeric)	1/92	0.02	0.001
	Study year (factor)	2/91	0.04	0.001
Soil Verrucomicrobia				
	Study year (numeric)	1/92	0.02	0.001
	Study year (factor)	2/91	0.05	0.001
Soil Acidobacteria				
	Study year (numeric)	1/92	0.01	0.001
	Study year (factor)	2/91	0.02	0.001
Litter Proteobacteria				
	Study year (numeric)	1/92	0.01	0.002
	Study year (factor)	2/91	0.03	0.001
Litter Actinobacteria				
	Study year (numeric)	1/92	0.01	0.006
	Study year (factor)	2/91	0.02	0.001
Litter Planctomycetes				
	Study year (numeric)	1/92	0.01	0.001
	Study year (factor)	2/91	0.03	0.001
Litter Bacteroidetes				
	Study year (numeric)	1/92	0.02	0.001

	Study year (factor)	2/91	0.03	0.001
Litter Verrucomicrobia				
	Study year (numeric)	1/92	0.01	0.001
	Study year (factor)	2/91	0.03	0.001
Litter Acidobacteria				
	Study year (numeric)	1/92	0.01	0.014
	Study year (factor)	2/91	0.02	0.001

Figure legends:

Figure 1: Sampling design. Location of soil cores at each sampling location. The distance between cores 2013, 2016 and 2017a and between cores 2017a and 2017b was always exactly 20 cm.

Figure 2: NMDS ordination plots of fungal and bacterial communities. NMDS ordination plots showing the main patterns in community structure in soil fungi (a), soil bacteria (b), litter fungi (c), and litter bacteria (d). Arrow vectors depict covariates significantly associated with patterns in community structure.

Figure 3: Time decay in the similarity of fungal and bacterial communities. Temporal decay in community similarity showing the rates of community turnover for fungi and bacteria in soil and litter.

Figure 4. Forest plots with estimates of the slope and intercept and explanatory power of fixed and random effects of linear mixed-effect models. Estimates of slopes (a) intercepts (b) and explanatory powers of time effect and site-level random effects (c) of linear mixed-effect models relating community similarity and distance in time of fungal and bacterial communities in soil and litter. The slopes represent the overall rates of community turnover. The intercepts represent the baseline

community similarity when temporal distance equals zero. Blue and red colors indicate soil and litter communities, respectively. The error bars represent bootstrap confidence intervals based on 999 resamplings with replacement.

Supplementary Figure legends

Supplementary Figure 1. Map of the study area showing the location of study sites.

Supplementary Figure 2. Partition of total temporal community turnover (i.e. beta diversity) within spatial locations, measured as Sorensen similarity, into species replacement effect and species richness (species loss/gain) effect in a) soil fungi, b) soil bacteria, c) litter fungi and d) litter bacteria.

Supplementary Figure 3: NMDS ordination plots of fungal ecological guilds. NMDS ordination plots showing the main patterns in community structure for soil saprotrophs (a), ECM (b) and yeasts (c) and litter saprotrophs (d), ECM (e) and yeasts (f). Arrow vectors depict covariates significantly associated with patterns in community structure.

Supplementary Figure 4: NMDS ordination plots of bacterial phyla in soil. NMDS ordination plots showing the main patterns in community structure for soil Proteobacteria (a), Actinobacteria (b) Planctomycetes (c), Bacteroidetes (d), Verrucomicrobia (e) and Acidobacteria (f). Arrow vectors depict covariates significantly associated with patterns in community structure.

Supplementary Figure 5. NMDS ordination plots of bacterial phyla in litter. NMDS ordination plots showing the main patterns in community structure for litter Proteobacteria (a), Actinobacteria (b)

Planctomycetes (c), Bacteroidetes (d), Verrucomicrobia (e) and Acidobacteria (f). Arrow vectors depict covariates significantly associated with patterns in community structure.

Supplementary Figure 6. Effect of site characteristics on temporal community turnover. (a) Effect of soil total N on temporal species turnover of soil saprotrophs (*time × total N soil* parameter estimate[CI] = -0.006[-0.012,-0.001]) ; and b) effect of the plant PC1 axis on temporal species turnover of litter Proteobacteria (*time × plant PC1*, parameter estimate[CI] = 0.004[0.000,0.008]).

Supplementary Figure 7. Average temperatures and total precipitations of the study years. a) Average temperatures of the study years. Solid line depicts monthly mean temperatures; winter season (Oct-Mar) in blue and summer season (Apr-Sep) in red. Points and dashed lines indicate summer (red) and winter (blue) mean temperatures. b) Accumulated precipitations of the study years. Solid line indicates monthly-accumulated precipitations. Points and dashed lines indicate yearly-accumulated precipitations. Data were collected from a weather station on the southern edge of the study area (Meteosat, 2019: https://meteostat.net/en/station/11723).

<u>Supplementary Table legends</u>

Supplementary Table 1. Average temperature and total precipitation of the study years. Data were collected from a weather station on the southern edge of the study area (Meteosat, 2019: https://meteostat.net/en/station/11723).

Supplementary Table 2. Location and properties of the study sites.

Supplementary Table 3. Vegetation survey of the studied sites. Species coverage is expressed in %. Coding of vegetation layers: 1 = tree layer, 2 = shrub layer, 3 = nonwoody plants in herb layer, 7 =

juveniles (seedlings or young trees and shrubs). The cover abundance of plant species estimated on the nine-degree Braun-Blanquet scale is here replaced by the mid-percentage value for each degree.

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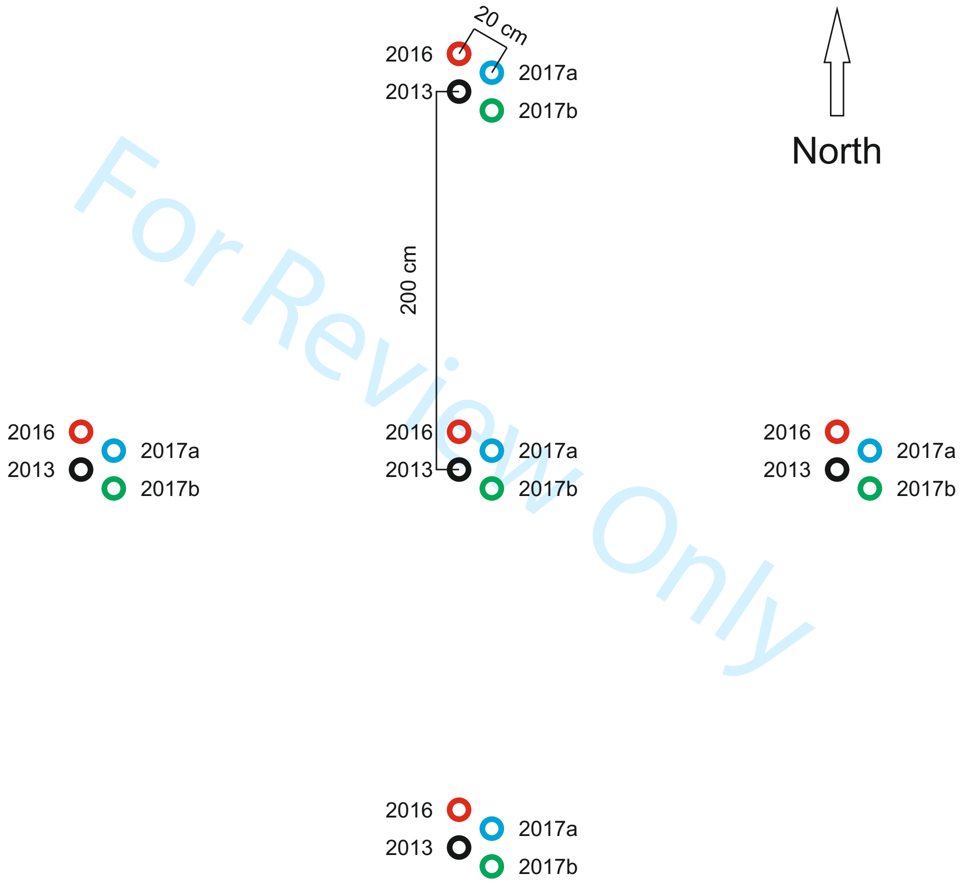
20 cm
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North
2016
2013
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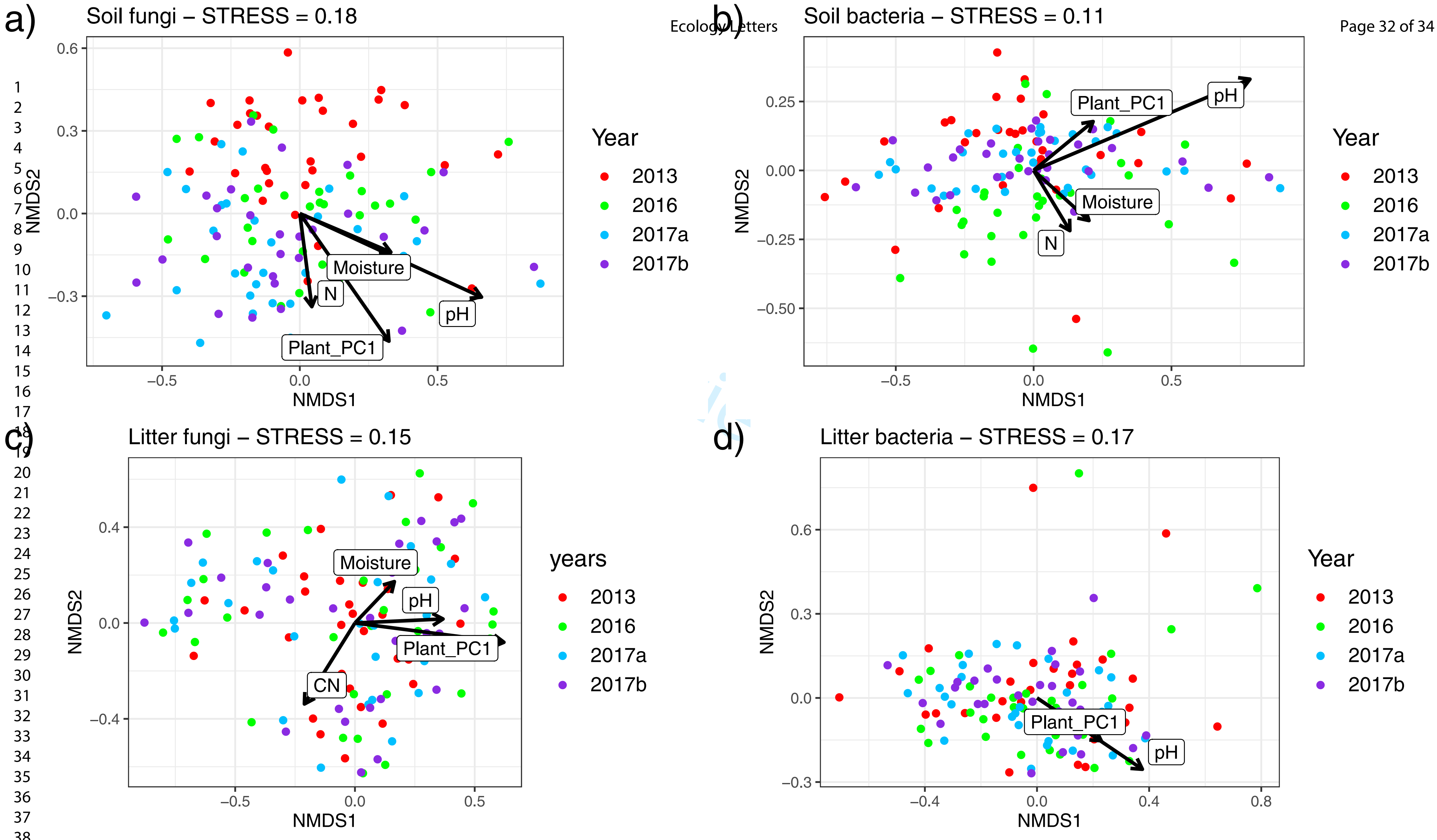
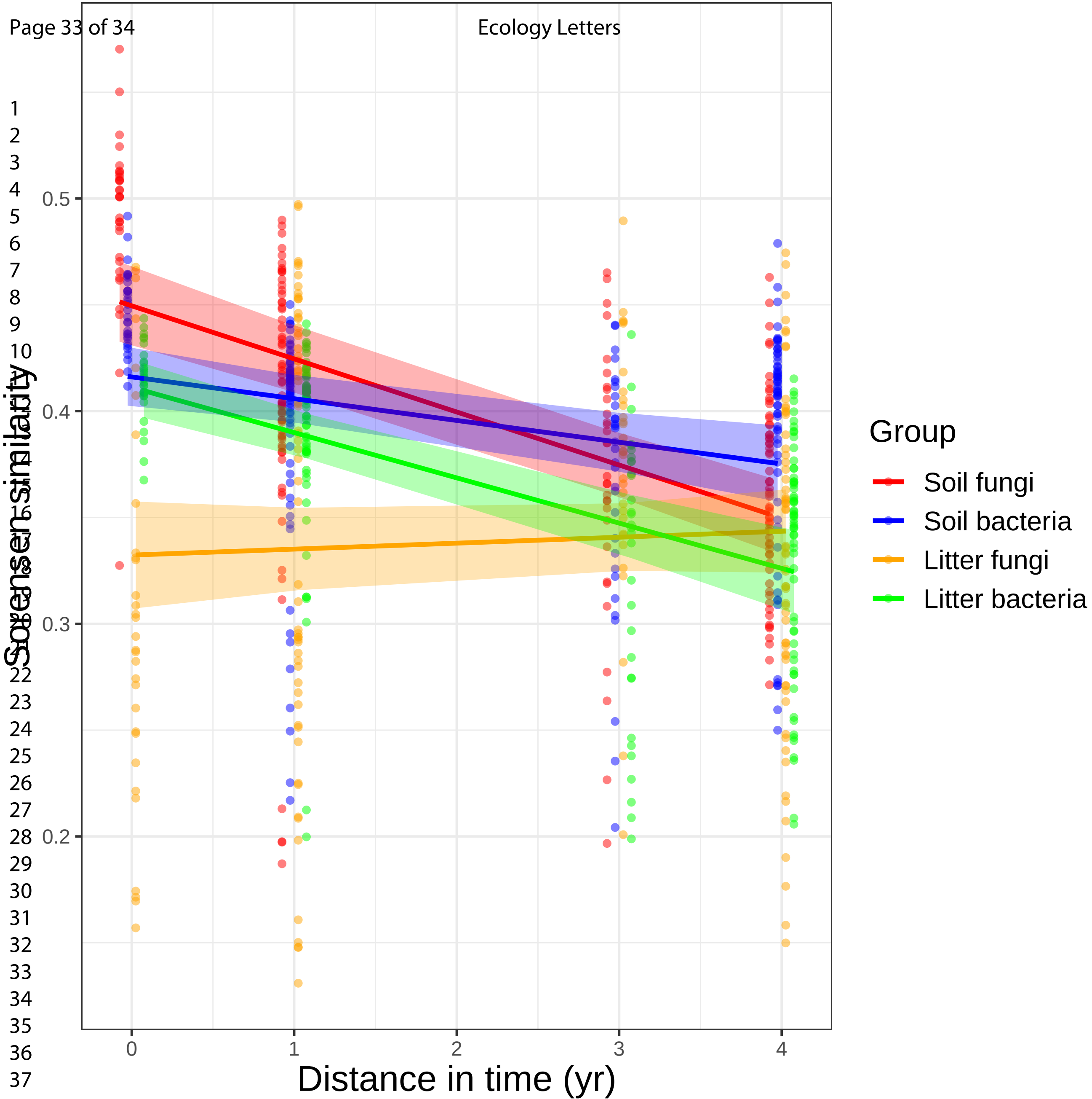
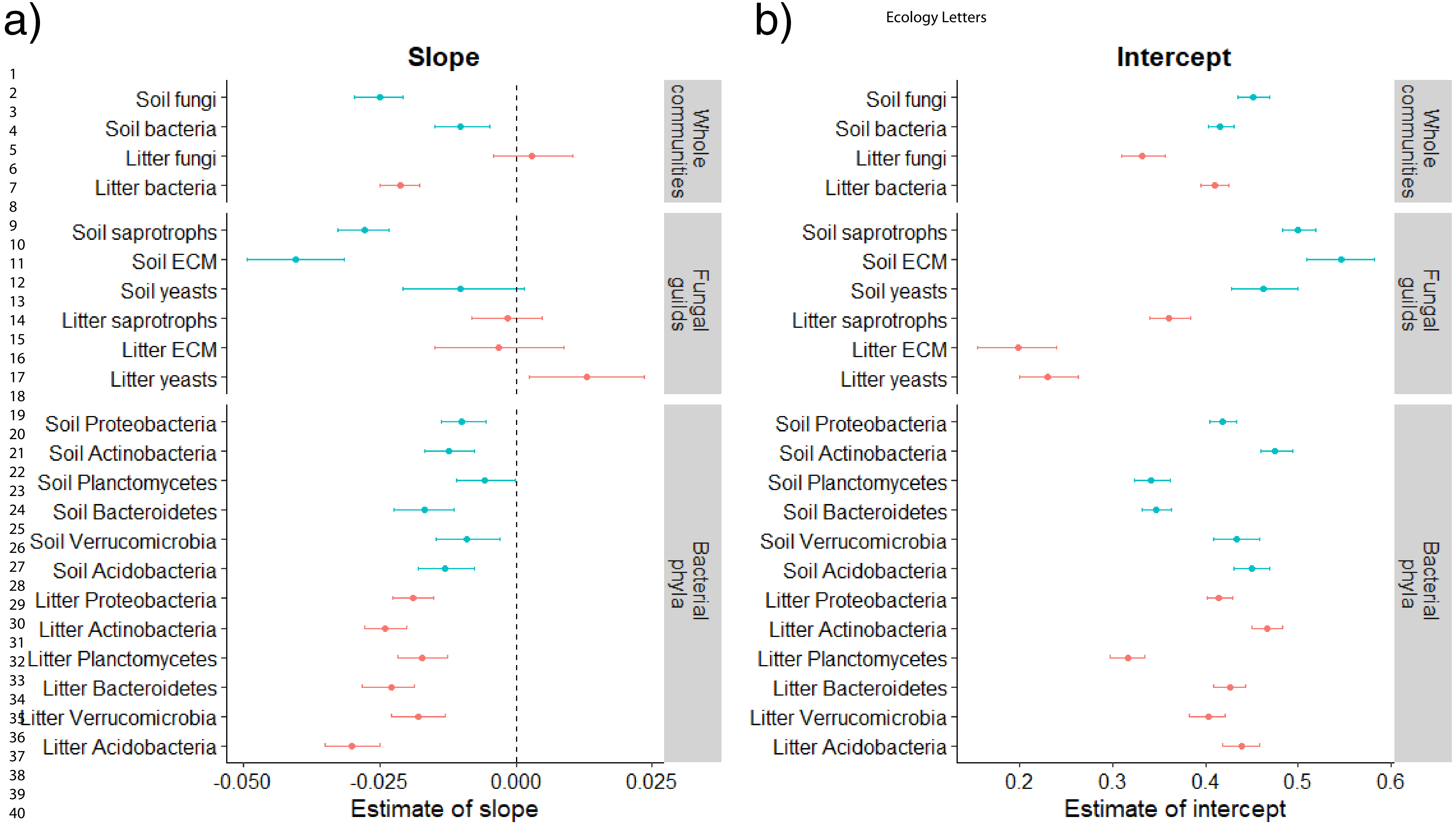

a)
Soil fungi – STRESS = 0.18
NMDS2
NMDS1
0.6
0.3
0.0
−0.3
−0.5
0.0
0.5
Moisture
N
pH
Plant_PC1
Year
2013
2016
2017a
2017b
b)
Soil bacteria – STRESS = 0.11
NMDS2
NMDS1
0.25
0.00
−0.25
−0.50
−0.5
0.0
0.5
Plant_PC1
pH
Moisture
N
Year
2013
2016
2017a
2017b
c)
Litter fungi – STRESS = 0.15
NMDS2
NMDS1
0.4
0.0
−0.4
−0.5
0.0
0.5
Moisture
pH
Plant_PC1
CN
years
2013
2016
2017a
2017b
d)
Litter bacteria – STRESS = 0.17
NMDS2
NMDS1
0.6
0.3
0.0
−0.3
−0.4
0.0
0.4
0.8
Plant_PC1
pH
Year
2013
2016
2017a
2017b


Sorensen similarity
0.5
0.4
0.3
0.2
0
1
2
3
4
Distance in time (yr)
Group
Soil fungi
Soil bacteria
Litter fungi
Litter bacteria



c)

R² time effect	R² site-level random effect
0.30	0.28
0.09	0.31
0.01	0.10
0.31	0.40
0.32	0.31
0.21	0.45
0.02	0.25
0.01	0.18
0.02	0.25
0.08	0.11
0.09	0.28
0.12	0.38
0.03	0.39
0.16	0.19
0.04	0.54
0.12	0.44
0.27	0.37
0.34	0.41
0.18	0.49
0.25	0.39
0.17	0.47
0.31	0.41