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Fast bacterial succession associated with the decomposition of *Quercus wutaishanica* litter on the Loess Plateau

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Abstract Understanding plant litter decomposition in broad-leaved forests is important because it influences the geochemical cycles of nutrients and represents a vital link in the global carbon cycle. Bacteria play an important role in litter decomposition, especially late in the decomposition process, when they become abundant. In this paper, we investigate bacterial community composition and diversity during about 1 year of *Quercus wutaishanica* litter decomposition using a molecular approach, to fill the gaps in knowledge about bacterial communities during decomposition. The results showed that the phyla Proteobacteria (Alpha and Betaproteobacteria), Actinobacteria, Bacteroidetes, and Acidobacteria were the most dominant throughout the experiment. As decomposition progressed, a dynamic succession of community and diversity was observed for different decomposition periods. Decay stages and seasonal shifts occurred by successful replacement of copiotrophic bacterial groups such as Betaproteobacteria in the early stage. During the entire process of decomposition, litter decomposition selectively stimulated the relative abundance of Alphaproteobacteria (*Sphingomonas*, *Rhizobium*, and *Methylobacterium*) and Bacteroidetes, but reduced the abundance of *Massilia* (Betaproteobacteria), Acidobacteria, and Actinobacteria. Among the abiotic factors, litter N and P content was the main factor driving the succession of litter bacteria. These results indicate the changes in decomposition stages in terms of the bacterial groups and elucidate the microbial community underpinnings of nutrient cycling in forest ecosystems.

Keywords Bacterial succession · Forest · Litter decomposition · Litter properties · Loess Plateau

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Introduction

Broad-leaved forest ecosystems represent a significant global carbon sink (Janssens et al. 2003). Consequently, understanding their ecology is very important for predicting and managing global nutrient and carbon cycling (Lladó et al. 2017; Berg 2014;

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Keiluweit et al. 2015). Microbes play critical roles in the sequestration and cycling of carbon, as they are the main drivers of C efflux in forest ecosystems (Drake et al. 2011). Bacteria are considered important decomposers, especially in the later decomposition stages. However, the role of bacteria during litter decomposition is still under debate and not fully understood (Purahong et al. 2016). As pointed out in a review by Lladó et al. (2017), we need to pay special attention to bacterial function in response to the changes in litter properties during decomposition, especially for individual taxa and different functional groups.

Litter decomposition is a dynamic process; to understand the microbial processes of litter decomposition, it is essential to address patterns of microbial community change through decomposition. While litter bacteria and fungi are assumed to be important decomposers during the decomposition process (Wal et al. 2013; Purahong et al. 2016), some studies indicate that bacteria may play a more important role in leaf litter decomposition processes than previously thought (Romaní et al. 2006; Keiblinger et al. 2012; Liu et al. 2015; Purahong et al. 2016; Lladó et al. 2017). Recent studies have demonstrated the important role of bacteria in litter decomposition and humus transformation. Many bacterial taxa can decompose cellulose, hemicellulose, and lignin (Šnajdr et al. 2011). Indeed, past work has shown that differences in microbial community composition can have implications for the process of decomposition (Cleveland et al 2007). Berlemont et al. (2014) found microbial communities in leaf litter displayed strong seasonal patterns, which illustrated the important role of litter bacteria during the litter decomposition, and some previous studies demonstrated that the biomass and diversity of bacteria increased gradually during decomposition (Purahong et al. 2014; Urbanová et al. 2014; Tláskal et al. 2016). The bacteria in plant litter can be affected by many factors, including leaf age, leaf type, and various other biotic factors. Abiotic factors also alter leaf bacterial communities. For example, the bacterial community in soil and litter showed significant seasonal dynamics in forest ecosystems in response to soil moisture and temperature (López-Mondéjar et al. 2015). Some previous litter decomposition studies revealed that Proteobacteria, Actinobacteria, Bacteroidetes, and Acidobacteria were the main taxa present in litter (Purahong et al. 2016; Tláskal et al. 2016). Despite these findings, bacterial community dynamics during litter decomposition and understandings of functionally important taxa are still poorly understood.

Bacteria play multiple important ecological roles in the forest ecosystem, such as the decomposition of organic matter and element cycles (Lladó et al. 2017). In the present study, we set up a litter decomposition experiment to explore the relationships between bacteria and litter properties at different decay stages. Thus, we aimed to: (1) investigate the successional changes in bacterial communities during the decomposition of *Quercus wutaishanica* leaf litter over 342 days using a molecular method and (2) test the correlation of physicochemical properties of leaf litter on successional changes in bacterial communities. We hypothesized that (1) litter microbial communities will shift primarily with continuing litter decomposition, as the nutrients and chemical properties are significantly altered during decomposition; (2) this will occur directionally with some variability due to seasonal dynamics. The variations in leaf litter quality may result in changes in the functional groups of bacteria, such as copiotrophic and oligotrophic bacterial groups and N-fixation groups. These groups play a critical role in the recycling of elements, especially for C recycling, which has important implications for global change.

Materials and methods

Site description

The study site was in the Fuxian Observatory for Soil Erosion and Eco-environment, a secondary forest region (Zheng et al. 2005). The mean annual temperature of the study area is 10.7 °C, and the mean annual precipitation is approximately 469 mm. Approximately 60% of the precipitation occurs from July to September (Zheng et al. 2005). *Quercus wutaishanica* was the predominant tree, playing an important role in maintaining the stability and functioning of the ecosystem in this area (Fan et al. 2006; Guo et al. 2010). We established three sites with similar topographical conditions in *Quercus wutaishanica* forests, to investigate the annual litter fall using the method described by Ukonmaanaho and Starr (2001). According to data from the 2 years of observations, the annual litter fall of *Quercus wutaishanica* was

about 200 g/m^2/year (Zeng et al. 2017b). The fresh fallen leaves were collected from the litter collectors in the study site in 2015. The leaf litters were air-dried for 2 weeks until constant weight was achieved, for the decomposition experiment.

Litter decomposition experiments

The experiment was performed using the litterbag method in situ. A total of 36 litterbags (20 cm × 20 cm, 2-mm nylon size) were used for the decomposition experiment. Each litterbag contained 10 g *Quercus wutaishanica* leaf litter, which was calculated based on the annual litter fall. We placed the litterbags horizontally at the surface of litter layer of the studied sites in June 2016. Each plot had three replicates (Fig. 1). There were four sampling dates: in 2016, July (31 days, summer), October (125 days, autumn), and in 2017, January (257 days, winter) and May (342 days, spring). The leaf litter from the litterbags of the same site was harvested and pooled to yield a composite sample (Purahong et al. 2015). Thus, there were three composite samples from each sampling date. Each composite sample was cut into 2.5-cm pieces and divided into three subsamples for the analysis of litter pH, chemical properties, and DNA extraction. The subsample for the extraction of DNA was stored at − 80 °C.

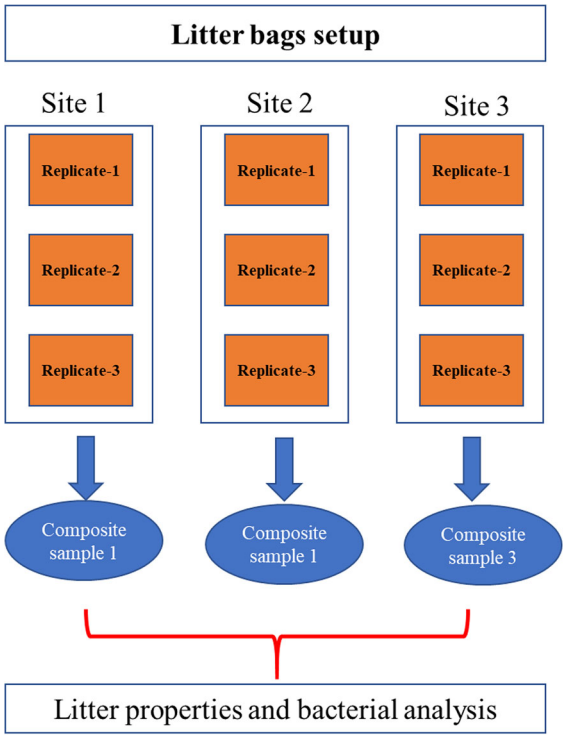


Fig. 1 The setup of the litter decomposition experiment

Analysis of litter properties

The mass loss was determined by the oven drying method at 65 °C for 48 h, until a constant weight was achieved. The dried leaf litter was ground in a ball mill and sieved (0.15-mm pore size). The analysis of the chemical properties (total organic carbon, TC; total nitrogen, TN; total phosphorus, TP; total potassium, TK) of litter was performed as described previously (Zeng et al. 2016). Specially, litter TC was measured by using a modified Mebius method (Nelson and Sommers 1982). Plant litter was digested in concentrated sulphuric acid and hydrogen peroxide ant the digested samples were used to analyze litter N content using calorimetrically determined using the Kjeldahl acid-digestion method, litter total P content using calorimetric analysis and litter K content using an adsorption spectrophotometer. The pH was determined using a 1:10 weight ratio of oven-dried litter to deionized water. The TC, TN, TP, and TK contents were expressed as g/kg and the results are showed in Fig. 2.

Litter DNA extraction, PCR amplification, and Illumina Miseq sequencing

The litter DNA was extracted from 100 mg plant litter sample using a Soil Microbe DNA MiniPrep Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) based on the instructions (Zeng et al. 2017a). The concentration and purity of DNA were determined photometrically using a UV spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Based on the concentration, DNA samples were diluted to 1 ng/μL with sterile water to reduce the effects of PCR inhibitors. For bacteria, the V5–V7 gene of 16S rRNA was amplified using primer set 799F/1193R (Ritpitakphong et al. 2016; Tian and Zhang 2017). The PCR parameters were as follows: full denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 60 s, with a final extension at 72 °C for 5 min. The size and specificity of PCR amplicons were verified using 2% agarose gel

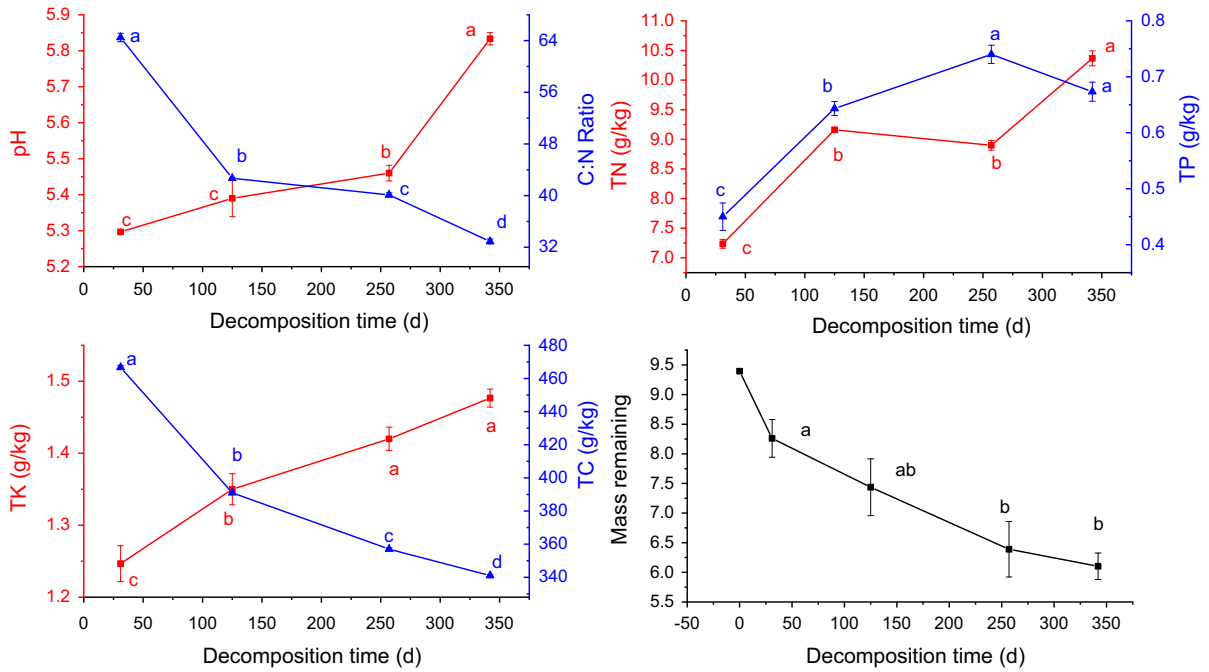


Fig. 2 Plant litter chemical properties and mass remaining across different decomposition times. Different letters indicate significant differences between sampling dates ($P<0.05$)

electrophoresis and samples were then pooled together in equal proportions and purified with Qiagen Gel Extraction Kit (Qiagen, Germany). All PCR reactions were run in duplicate with Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, United States).

Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA), following the manufacturer's recommendations, and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Finally, the sequencing and construction of the 16S rRNA gene clone libraries were carried out at Novogene (Beijing, China), using the Illumina HiSeq2500 platform, and 250-bp paired-end reads were created. Raw sequence data in FASTQ format are accessible from the NCBI SRA with number SRP107086.

Bioinformatics analysis

The QIIME software was used to analyze the sequences data (Caporaso et al. 2010). Raw reads without adapter from the MiSeq sequencer were assigned to different samples based on the barcodes. Paired-end reads with at least a 50-bp overlap and $< 5\%$ mismatches were merged using FLASH software (v1.2.7) (Magoč and Salzberg 2011). A threshold of average quality scores > 30 over 5-bp window size was used to trim the unqualified sequences. After removing the chimeric sequence and all singletons, sequences were clustered into OTU at 97% similarity level using UPASE (V7.0.1001) (Edgar 2010). Taxonomical identities were assigned to the OTUs using the Ribosomal Database Project (RDP) classifier with a 50% confidence score (Cole et al. 2009). The 16S sequences were assigned using the GREENGENES database (McDonald et al. 2017). Representative sequence for each OTU was screened for further annotation. After quality filtering, all samples were normalized to 28,864 sequences per sample which were the smallest among all the samples, to conduct downstream analyses for all samples at the same sequencing depth. OTU abundance information was normalized using a standard sequence number, corresponding to the sample with the least sequences (28,864). Alpha diversity was calculated by analyzing the complexity of species diversity, including

Shannon index (Shannon 1948) and phylogenetic diversity (PD) (Faith 1992). All these indices for our samples were calculated in QIIME (Version 1.7.0) and plotted using R software (Version 2.15.3).

Statistical analysis

The two-dimensional nonmetric multidimensional scaling (NMDS) analysis was used to show the impact of bacterial community succession over time on the relative distribution of the samples on the ordination plot. We used the relative abundance OTU matrices of the bacterial communities and Bray-Curtis dissimilarity distances to obtain the NMDS ordination plot. Significant changes in litter bacterial communities were tested using permutational analysis of variance (PERMANOVA) (Anderson 2001). One-way ANOVA was performed to explore the differences in the leaf litter microbial diversity and compositions across different sampling dates (SPSS version 20.0 for Windows), and comparisons were done using the LSD method ($P = 0.05$). Differences at 0.05 level were deemed statistically significant. Distance-based linear models (DistLM; McArdle and Anderson 2001), associated with dbRDA, was used to explore the relationships between the litter properties and litter bacterial communities, which revealed the effects of the different factors. The NMDS, Cluster, dbRDA, PERMANOVA, and DistLM analyses were performed using PRIMER 7 software (https://www.primer-e.com/) (McArdle and Anderson 2001; Anderson and Walsh 2013).

Results

Shifts in litter quality with the decomposition of plant litter

After 342 days' decomposition, fast decomposition was observed in plant litter (35% mass lass). The decomposition rate decreased as the decomposition processed (Fig. 2). Plant litter total organic carbon (TC) showed similar varied trend with litter mass loss. Litter TN, TP, TK contents and pH showed a significant increase during the whole decomposition period. The significant decrease of TC and increase of TN were observed in the decomposition process. ANOVA showed that sampling dates had significant effects on plant litter C, N, P, pH and C:N ratio. Litter C:N ratio significantly decreased from 65 to 33 from summer to spring. Such results indicated that litter quality seasonally varied with the ongoing decomposition process.

Shifts in bacterial diversity to the decomposition of plant litter

The sequencing and subsequent cleaning and filtering of the reads produced 505,043 useful sequences (42,087 sequences per sample) across all the samples, with an average length of 376 bp (Tables S1 and S2). In total, the sequences belonging to 902 different OTUs were assigned to bacteria and used for further analysis. The numbers of the OTUs ranged from 427 to 686, with a mean of 547 (Table 1). The bacterial diversity indices increased from summer to autumn, and then declined until the end of the experiment (Table 1). The Shannon index increased from 5.7 at summer (after 31 days) to 6.61 (after 125 days) at autumn, and then decreased to 6.32 at the end of the decomposition (342 days, spring). The phylogenetic diversity index (PD) increased significantly between 31 and 125 days and then remained relatively stable (125–342 days). The ANOVA showed that Shannon diversity was significantly lower in summer than other seasons, while the difference between autumn, winter and spring was not significant. The phylogenetic diversity also showed that litter in summer had the lowest diversity than other seasons. Litter bacterial diversity in winter and spring had no significant difference. Such results suggested a clear seasonal succession for the litter bacterial diversity.

Table 1 Plant litter bacterial diversity indices under different decomposition periods

Sampling date	Shannon	OTUs	PD_whole_tree
Sum (31d)	5.7 ± 0.3b	427 ± 38c	26.76 ± 6.9b
Aut (125d)	6.61 ± 0.17a	686 ± 28a	46.17 ± 3.11a
Win (257d)	6.48 ± 0.12a	536 ± 33b	31.22 ± 1.86b
Spr (342d)	6.32 ± 0.13a	540 ± 57b	31.99 ± 3.82b

Different letters indicate significant differences at 0.05 level

Response of the bacterial community composition to the decomposition of plant litter

Actinobacteria (16.8–24.7%), Alphaproteobacteria (38.7–48.4%), Betaproteobacteria (18.4–38.9%), and Acidobacteria (2.3–7.8%) were the most abundant bacterial taxa throughout the entire experiment ($\geq$ 1% in all 12 samples; Fig. 3). Firmicutes, Saccharibacteria, Armatimonadetes, and Chlamydiae were also identified, albeit with lower abundances (<1%), in the present study (Fig. 3). Alphaproteobacteria and Gammaproteobacteria showed no significant difference across the sampling times. The relative abundance of Betaproteobacteria showed a significant decline from 38.9% (31 days) to 18.4% (342 days). The Actinobacteria were mainly represented by Frankiales, Kineosporiales, Micrococcales, and Propionibacteriales.

At the order level, Burkholderiales was the most dominant group, and its relative abundance decreased from 38.6% (after 31 days) to 18.2% (after 342 days). The abundance was followed by Rhizobiales (13.2–27.5%), Sphingomonadales (14.0–18.6%), and Micrococcales (8.4–15.3%) (Fig. 3). Acidobacteriales, Micromonosporales, and Caulobacterales had a increasing abundance during the decomposition, which accounted for 9.7–13.3% of the total reads. The ANOVA showed that there was no significant difference during the decomposition process for Sphingomonadales, Micromonosporales, and Kineosporiales. Burkholderiales showed a significant decrease, whereas Micrococcales significantly increased during the decomposition process.

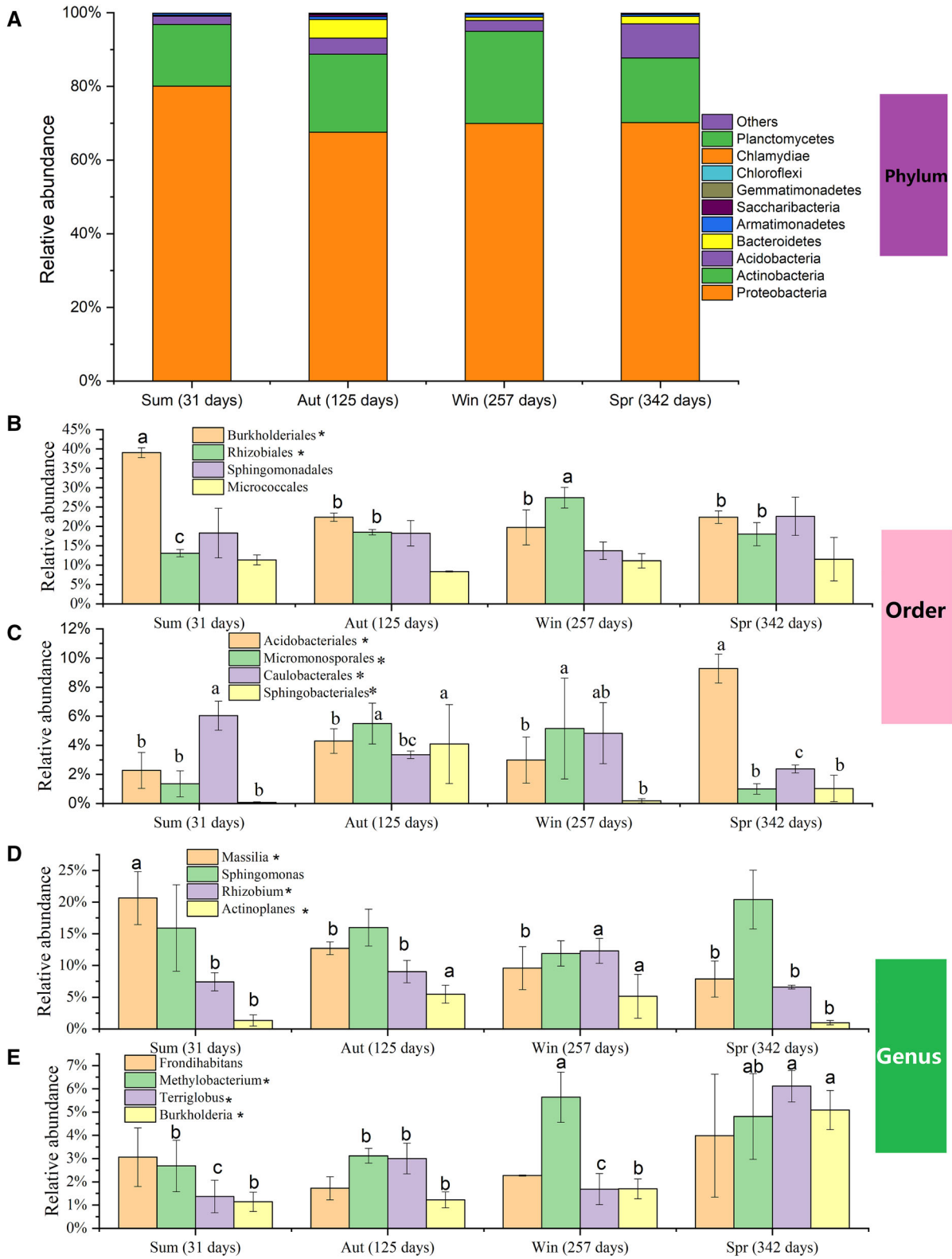
At the genus level, at the early stage (after 31 days), the leaf litter was highly dominated by *Massilia* (22.1%) and *Sphingomonas* (16.1%) (Fig. 3). These two genera remained dominant after 342 days of decomposition, with a relative abundance of 5.8% and 15.0%, respectively, but their relative abundances decreased gradually to 16.3% and 1.1%, respectively, after 342 days. Qualitatively we found that the majority of early-stage dominant bacterial taxa (*Massilia*) decreased in abundance during the later stages. Other abundant genera were *Rhizobium*, *Actinoplanes*, *Frondihabitans*, *Terriglobus*, *Methylobacterium*, *Burkholderia*, *Amnibacterium*, *Rhodopseudomonas*, and *Kineosporia*, which accounted for 22.1–38.9% of the total reads.

Fig. 3 The composition of litter bacteria during the decomposition process. Different letters indicate significant differences between sampling dates. *Significant difference between different seasons or decomposition time

Clear differences in bacterial communities in plant litter were observed at different decomposition times (Fig. 4), suggesting a distinct succession of litter bacterial structure. As expected, the close clustering of plant litter samples at winter and autumn sampling dates indicated similar bacterial community compositions. The samples at summer were distinctly separated from the other sampling dates. As the decomposition progressed, the average Bray-Curtis dissimilarity of bacterial communities increased from 24.35% (after 31 days) to 32.26% (after 342 days). The PERMANOVA analysis (main test) also showed that the bacterial communities were significantly different between the different sampling dates or seasons ($F = 3.7064$, $P = 0.001$).

Properties of *Quercus wutaishanica* leaf litters during decomposition and their effects on the bacterial community

To determine if each individual factor accounted for a different or similar proportion of bacterial community structure, 'forward' DistLM model was computed using the adjusted R^2 criterion. The results showed that litter TN (+ 25.2%; $P = 0.001$), litter pH (+ 19.1%; $P = 0.004$), and litter TP (+ 11.6%; $P = 0.007$) accounted for significant and distinct proportions of bacterial community structure (Table 2). The dbRDA analysis revealed that the first two axes explained 47.9% and 32.8% of the variance, respectively, for the fitted model, and 26.8% and 18.4%, respectively, of the total variation. Together these three variables (litter pH, TN, and TP) explained 80.7% of the fitted variation (Fig. 5). The samples separated clearly between sampling dates, indicating that the bacterial decompositions were distinct at different decomposition times. Results from DistLM and dbRDA suggested that litter TN, pH, and TP were the most relevant variables to the affect litter bacterial community structure (Table 3).


A
Relative abundance
100%
80%
60%
40%
20%
0%
Sum (31 days)
Aut (125 days)
Win (257 days)
Spr (342 days)
Others
Planctomycetes
Chlamydiae
Chloroflexi
Gemmatimonadetes
Saccharibacteria
Armatimonadetes
Bacteroidetes
Acidobacteria
Actinobacteria
Proteobacteria
Phylum
B
Relative abundance
Burkholderiales *
Rhizobiales *
Sphingomonadales
Micrococcales
Order
C
Relative abundance
Acidobacteriales *
Micromonosporales *
Caulobacterales *
Sphingobacteriales*
D
Relative abundance
Massilia *
Sphingomonas
Rhizobium*
Actinoplanes *
Genus
E
Relative abundance
Frondihabitans
Methylobacterium*
Terriglobus*
Burkholderia *

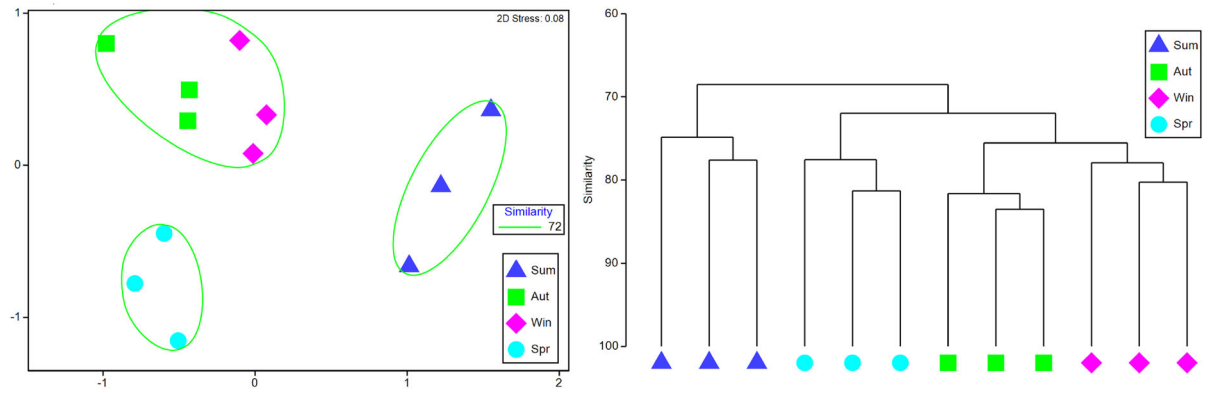


Fig. 4 The two-dimensional NMDS and cluster plots based on the OUT data between different sampling dates

Table 2 Proportion of, and cumulative bacterial community accounted for by individual litter properties by sequential DistLM

Variable	Adj R^2	Pseudo-F	P	Prop.	Cumul.
+ TN	0.1773	3.3706	0.001	0.25209	0.25209
+ pH	0.32038	3.1052	0.004	0.19185	0.44394
+ TP	0.39428	2.0981	0.007	0.11553	0.55948

Prop percentage variance explained by that variable, *Cum.* cumulative percentage of variance explained, *TN* total nitrogen, *TP* total phosphorus

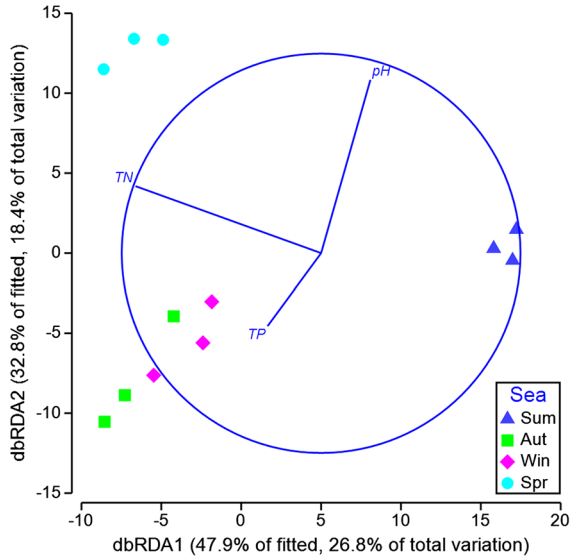


Fig. 5 dbRDA analysis of the variation in litter bacterial community structure as explained by litter properties. Vectors represent the correlations of variables with community structure along the first two dbRDA axes

Discussion

Litter bacterial communities indicated some clear seasonal variations throughout the decomposition in the present study. Generally, at the early stage of the litter decomposition, microbial communities become more active and experience a rapid growth, which results in their higher diversity and biomass (Purahong et al. 2014). In the present study, as the decomposition processed, the bacteria became more diverse during 31–125 days, which was in agreement with previous results (Tláskal et al. 2016).

Throughout the decomposition, the dominant taxa were consistent with the previous studies in the forest ecosystem (López-Mondéjar et al. 2015). At the genus level, the dominant genera were *Massilia*, *Sphingomonas*, *Rhizobium*, *Actinoplanes*, *Frondihabitans*, *Terriglobus*, *Methylobacterium*, and *Burkholderia*. Most of these members were detected in litters during the decomposition in other forest ecosystems (López-Mondéjar et al. 2015; Tláskal et al. 2016). In their studies, *Pseudomonas* and *Mucilaginibacter* had higher relative abundance, which was not consistent with this study. In the present study, Massilia and Sphingomonas were the most dominant with the average relative abundance of 12.8% and 14.8%. The difference in terms of the dominant genera may be explained by difference in the tree species and climate conditions compared with other studies. Although their relative abundances were different, these results from previous studies showed that these members were common and widely present in leaf litters and

Table 3 The correlations between bacterial diversity (Shannon diversity, species richness and the scores of the first axis from unweighted NMDS) and litter TC, TN and TP

Variations	TN	TP	TC
Species	0.543	0.573	− 0.711**
Shannon	0.650*	0.814**	− 0.700*
PD	0.336	0.314	− 0.223
NMDS1	0.685*	0.654*	− 0.623*

TC total organic carbon, *TN* total nitrogen, *TP* total phosphorus, *NMDS1* the scores of the first axis from unweighted NMDS

*Significant correlations at 0.05 level, **significant correlations at 0.01 level

had an active role in the decomposition of the litter (López-Mondéjar et al. 2015; Tláskal et al. 2016).

Sphingomonas were dominant in the early decay litters (∼ 16%), as they may be able to produce proteolytic or cellulolytic enzymes (Aislabie et al. 2006). This pattern was consistent with the bacterial succession during the decomposition of European beech litters (Purahong et al. 2014). Sphingomonas, along with Massilia, Rhizobium, and Actinoplanes, i.e., bacteria which can use some complex substrates or fix nitrogen, were the main members of the litter bacterial community in this study and varied during the decomposition process (Aislabie et al. 2006; López-Guerrero et al. 2013; Purahong et al. 2016). It was also interesting that Streptomyces became more abundant at the later decay stage, which is known to have a potential for transforming humus and lignin (Purahong et al. 2016). Rhizobium was dominant during the entire decomposition period, especially at winter (after 257 days). Overall, these results suggest that the composition of microbial communities sensitively responds to the decomposition process in the forest ecosystem.

The DistLM analysis showed that the litter chemical variables significantly shaped community composition during the decomposition, by explaining a large fraction of observed variance (55.9% at OTU level). Litter decomposition drove the dynamics of the nutrients along with the decomposition process (Berg 2000; Liu et al. 2016; Pourhassan et al. 2016). Our work supports that the nutrients of litter changes shifted the bacterial compositions, which is consistent with past research results that have demonstrated bacterial community shifts along with nutrient availability in chronosequence studies (Philippot et al. 2011; Freedman and Zak 2015; Zhang et al. 2016; Zeng et al. 2017a). The patterns observed in this work are consistent with past research that has shown that microbes may shift rapidly along with the changes in nutrient conditions during litter decomposition, and that some functional taxa are dominant in the community at particular times (Prosser et al. 2007). The results of the present study showed that the leaf litter properties, including N and P nutrients and pH, significantly influenced the compositions of the bacteria, especially for some specific bacterial taxa. Similarly, Purahong et al. (2016) found that the abundance of the N-fixing bacteria changed with time during the decomposition, which may explain the variations in bacterial composition at different decomposition times (Prosser et al. 2007). In our study, the TN and TP contents of the leaf litter drove the bacterial succession during decomposition and which is consistent with previous studies (Purahong et al. 2014, 2015). In addition, pH was a significant factor that altered bacterial community composition and diversity in our study and this is consistent with past studies that show pH may impact the relative abundance of many functional groups (Nicol et al. 2008; Griffiths et al. 2011; Zhalnina et al. 2015). Similar findings were observed for the plant litters in the present study and confirmed by some previous studies (Matulich et al. 2015; Purahong et al. 2015). The variations in bacterial community composition appeared to be driven by the quantities of the labile nutrients.

In our study, litter decomposition caused the increase in N in the litter environment. A similar result was found in another plant litter decomposition study (Purahong et al. 2016). The higher N content in plant litter caused a significant decrease in C:N ratio in plant litter. Compared with the initial C:N ratio in plant litter, it significantly decreased from 64 to 33. The decrease in the C:N ratio may involve two mechanisms. First, the rapid growth of microbes in the plant litter needs to utilize C resources, resulting in the decline of C content. The loss of carbon due to mineralization lead to the decrease in the C:N ratio. Second, litter microbes can obtain nitrogen from the environment for their growth and reproduction, resulting in a net increase in litter N (Berg and Staaf 1981). Some studies on litter decomposition also reported that the existence of N-fixing microbes results in the

accumulation of the plant litter N (Crews et al. 2000; Kowalchuk and Stephen 2001). In this study, *Sphingomonas*, *Methylobacterium*, and *Amnibacterium* have been found to be significantly related with litter TN content ($P < 0.05$). Indeed, the members of genus *Burkholderia* are highly versatile with respect to their ecological niches and lifestyles. The genera *Burkholderia*, *Rhodopseudomonas*, *Caulobacter*, *Stenotrophomonas* and *Azospirillum* were free-living N-fixing bacteria which may enhance the content of N (Estrada-De Los Santos et al. 2001; Pepe et al. 2013; Baldani et al. 2014). These genera were highly distributed in the plant litters which may accounted for the increase of total N content.

In addition, temperature may be another factor affecting the seasonal variations for litter bacterial communities. Previous studies showed temperature was one of the most important environmental factors affecting the bacterial community (Pietikäinen et al. 2005). It seems clear that the communities don't change much from autumn to winter (Figs. 4 and 5) and this is probably related to lower temperatures. Temperature can also influence bacterial activity via substrate availability (Adams et al. 2010). At colder temperatures (Winter and Autumn), bacteria begin to lose substrate affinity despite available sources in the plant litters (Nedwell 1999). From summer to autumn, higher temperature may have enhanced the shift of litter bacteria resulting the increase of bacterial diversity and some bacterial groups. However, litter pH, TN and TP contents are not seen to change cyclically with seasonality. Indeed, litter pH and nutrients have been shown to be a dominant control in succession beyond seasonal dynamics (Knelman et al. 2014). Therefore, the succession of bacterial communities in litter may have been affected by the interactions between seasonal factors and litter nutrients due to decomposition in this study. While this study suggests the connection between microbial succession and decomposition and that seasonality may simply speed up or slow down this process, further investigation is needed to fully understand the extent to which microbial changes are driven by successional vs. seasonal variability, or the interaction of the two.

These findings suggested that the shifts in the leaf litter bacterial communities were largely driven by bacteria life strategy caused by the remaining nutrients in litter. This variation may lead to the changes of the copiotroph and oligotroph relative abundances. Betaproteobacteria and Actinobacteria are assumed as copiotrophs, which can fast grow in high resource environments (Bonanomi et al. 2016; Fierer et al. 2007). The decrease of copiotrophic species may be explained by the leaching and consumption by the microorganisms of the liable substrates. Acidobacteria are usually considered oligotrophs (Zeng et al. 2017a, b; Fierer et al. 2007). During the decomposition, the relative abundance of Acidobacteria significantly increased with the decline of the liable resources. Fierer et al. (2007) reported that Acidobacteria were lowered in an individual soil amended with high concentrations of organic C. With the loss of labile nutrients, we observed that copiotrophic bacteria (Betaproteobacteria) became less abundant and the abundance of oligotrophic bacteria (Acidobacteria) significantly increased with the ongoing decomposition process. The declines we observed in Betaproteobacteria have indeed been observed in past research on bacterial succession, where these microbes may exploit existing nutrients early in succession due to their life history strategy but decline in later successional environments (Nemergut et al 2007; Knelman et al. 2018). These results were consistent with a meta-analysis analyzed by Fierer et al. (2007). They reported that the Acidobacteria was less abundant, and the β-Proteobacteria (as well as the α- and γ-Proteobacteria) was more abundant in rhizosphere soils than in the comparatively C-poor bulk soils. Such findings corroborate our study's findings that declines in betaproteobacteria over time, for example, may be driven by bacteria life strategy alongside shifts in nutrient availability of litter during the decomposition process. However, not all the copiotrophic bacteria were found to decrease as decomposition progressed, as in the case of Alphaproteobacteria. Indeed, this study supports emerging research that has shown across a variety of microbial successional environments, bacterial life history strategy is a primary control on the assembly of communities through time (Nemergut et al. 2015).Therefore, future studies need to focus on the specific function of the genus or species in response to the decomposition process, which would provide new insights into the understanding of geochemical cycles and the global changes caused by the microbes in forest ecosystems.

Conclusions

In the present study, we conducted a litterbag experiment using a molecular method to analyze the bacterial succession during leaf litter decomposition in a secondary forest ecosystem in the Loess Plateau, China. Our work provides insights into bacterial community dynamics during the decomposition process. In conclusion, the sequence results of this study clearly showed that bacterial community undergoes defined patterns of succession during microbial decomposition which might reflect the variations of litter quality. The results demonstrated that higher alpha-diversity indices were observed at the decomposition of 125 days. After 31 days of decomposition, some copiotrophic bacterial groups, such as Alphaproteobacteria and Actinobacteria, became more abundant and were dominant in the later stage of the decomposition. The substrates (C, N, and P contents in plant litter) control the succession of litter bacteria and led to the increase in copiotrophic bacterial groups and the decline of oligotrophs. Microbial community succession may contribute to plant litter decomposition, but further research is needed to connect observed patterns in bacterial community composition through decomposition and the decomposition process itself. Such findings would enhance the understanding of the microbial mechanisms of litter decomposition and the cycling of litter elements.

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References

Adams HE, Crump BC, Kling GW (2010) Temperature controls on aquatic bacterial production and community dynamics in arctic lakes and streams. Environ Microbiol 12(5):1319–1333

Aislabie JM, Broady PA, Saul DJ (2006) Culturable aerobic heterotrophic bacteria from high altitude, high latitude soil of La Gorce Mountains (86 30'S, 147 W), Antarctica. Antarct Sci 18:313–321

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Aust J Ecol 26:32–46

Anderson MJ, Walsh DC (2013) PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? Ecol Monogr 83:557–574

Baldani JI, Reis VM, Videira SS, Boddey LH, Baldani VLD (2014) The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: a practical guide for microbiologists. Plant Soil 384:413–431

Berg B (2000) Litter decomposition and organic matter turnover in northern forest soils. For Ecol Manag 133:13–22

Berg B (2014) Decomposition patterns for foliar litter—a theory for influencing factors. Soil Biol Biochem 78:222–232

Berg B, Staaf H (1981) Leaching, accumulation and release of nitrogen in decomposing forest litter. Ecol Bull 33:163–178

Berlemont R, Allison SD, Weihe C, Lu Y, Brodie EL, Martiny JB, Martiny AC (2014) Cellulolytic potential under environmental changes in microbial communities from grassland litter. Front Microbiol 5:639

Bonanomi G, De Filippis F, Cesarano G, La Storia A, Ercolini D, Scala F (2016) Organic farming induces changes in soil microbiota that affect agro-ecosystem functions. Soil Biol Biochem 103:327–336

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336

Cleveland CC, Nemergut DR, Schmidt SK, Alan RT (2007) Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. Biogeochemistry 82(3):229–240

Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen A, Mcgarrell DM, Marsh T, Garrity GM (2009) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res 37:D141–D145

Crews TE, Farrington H, Vitousek PM (2000) Changes in asymbiotic, heterotrophic nitrogen fixation on leaf litter of *Metrosideros polymorpha* with long-term ecosystem development in Hawaii. Ecosystems 3:386–395

Drake JE, Gallet-Budynek A, Hofmockel KS, Bernhardt ES, Billings SA, Jackson RB, Johnsen KS, Lichter J, Mccarthy HR, Mccormack ML (2011) Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. Ecol Lett 14:349–357

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461

Estrada-De Los Santos P, Bustillos-Cristales R, Caballero-Mellado J (2001) Burkholderia, a genus rich in plant-associated nitrogen fixers with wide environmental and geographic distribution. Appl Environ Microbiol 67:2790–2798

Faith DP (1992) Conservation evaluation and phylogenetic diversity. Biol Conserv 61:1–10

Fan WY, Wang XA, Guo H (2006) Analysis of plant community successional series in the Ziwuling area on the Loess Plateau. Acta Ecol Sin 26:706–714

Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. Ecology 88:1354–1364

Freedman Z, Zak DR (2015) Soil bacterial communities are shaped by temporal and environmental filtering: evidence from a long-term chronosequence. Environ Microbiol 17:3208–3218

Griffiths RI, Thomson BC, James P, Bell T, Bailey M, Whiteley AS (2011) The bacterial biogeography of British soils. Environ Microbiol 13:1642–1654

Guo H, Wang XA, Zhu ZH, Wang SX, Guo JC (2010) Seed and microsite limitation for seedling recruitment of *Quercus wutaishanica* on Mt. Ziwuling, Loess Plateau, China. New For 41:127–137

Janssens IA, Freibauer A, Ciais P, Smith P, Nabuurs G-J, Folberth G, Schlamadinger B, Hutjes RW, Ceulemans R, Schulze E-D (2003) Europe's terrestrial biosphere absorbs 7 to 12% of European anthropogenic CO_2 emissions. Science 300:1538–1542

Keiblinger K, Schneider T, Roschitzki B, Schmid E, Eberl L, Hämmerle I, Leitner S, Richter A, Wanek W, Riedel K (2012) Effects of stoichiometry and temperature perturbations on beech leaf litter decomposition, enzyme activities and protein expression. Biogeosciences 9:4537

Keiluweit M, Nico P, Harmon ME, Mao J, Pett-Ridge J, Kleber M (2015) Long-term litter decomposition controlled by manganese redox cycling. Proc Natl Acad Sci USA 112:E5253–E5260

Knelman JE, Schmidt SK, Lynch RC, Darcy JL, Castle SC, Cleveland CC, Nemergut DR (2014) Nutrient addition dramatically accelerates microbial community succession. PLoS ONE 9(7):e102609

Knelman JE, Graham EB, Prevéy JS, Robeson MS, Kelly P, Hood E, Schmidt SK (2018) Interspecific plant interactions reflected in soil bacterial community structure and nitrogen cycling in primary succession. Front Microbiol 9:128

Kowalchuk GA, Stephen JR (2001) Ammonia-oxidizing bacteria: a model for molecular microbial ecology. Annu Rev Microbiol 55:485–529

Liu D, Li M, Xi B, Zhao Y, Wei Z, Song C, Zhu C (2015) Metaproteomics reveals major microbial players and their biodegradation functions in a large-scale aerobic composting plant. Microb Biotechnol 8:950–960

Liu C, Liu Y, Guo K, Zhao H, Qiao X, Wang S, Zhang L, Cai X (2016) Mixing litter from deciduous and evergreen trees enhances decomposition in a subtropical karst forest in southwestern China. Soil Biol Biochem 101:44–54

Lladó S, López-Mondéjar R, Baldrian P (2017) Forest soil bacteria: diversity, involvement in ecosystem processes, and response to global change. Microbiol Mol Biol Rev 81:e00063-00016

López-Guerrero MG, Ormeño-Orrillo E, Rosenblueth M, Martinez-Romero J, Martínez-Romero E (2013) Buffet hypothesis for microbial nutrition at the rhizosphere. Front Plant Sci 4:188

López-Mondéjar R, Voříšková J, Větrovský T, Baldrian P (2015) The bacterial community inhabiting temperate deciduous forests is vertically stratified and undergoes seasonal dynamics. Soil Biol Biochem 87:43–50

Magoč T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963

Matulich KL, Weihe C, Allison SD, Amend AS, Berlemont R, Goulden ML, Kimball S, Martiny AC, Martiny JB (2015) Temporal variation overshadows the response of leaf litter microbial communities to simulated global change. ISME J 9:2477–2489

Mcardle BH, Anderson MJ (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82:290–297

Nedwell DB (1999) Effect of low temperature on microbial growth: lowered affinity for substrates limits growth at low temperature. FEMS Microbiol Ecol 30(2):101–111

Nemergut DR, Anderson SP, Cleveland CC, Martin AP, Miller AE, Seimon A, Schmidt SKJME (2007) Microbial community succession in an unvegetated, recently deglaciated soil. Microb Ecol 53:110–122

Nemergut DR et al (2015) Decreases in average bacterial community rRNA operon copy number during succession. ISME J 10(5):1147

Nicol GW, Leininger S, Schleper C, Prosser JI (2008) The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environ Microbiol 10:2966–2978

Pepe O, Ventorino V, Blaiotta G (2013) Dynamic of functional microbial groups during mesophilic composting of agro-industrial wastes and free-living (N_2)-fixing bacteria application. Waste Manag 33:1616–1625

Philippot L, Tscherko D, Bru D, Kandeler E (2011) Distribution of high bacterial taxa across the chronosequence of two alpine glacier forelands. Microb Ecol 61:303–312

Pietikäinen J, Pettersson M, Bååth E (2005) Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. FEMS Microbiol Ecol 52(1):49–58

Pourhassan N, Bruno S, Jewell MD, Shipley B, Roy S, Bellenger J-P (2016) Phosphorus and micronutrient dynamics during gymnosperm and angiosperm litters decomposition in temperate cold forest from Eastern Canada. Geoderma 273:25–31

Prosser JI, Bohannan BJ, Curtis TP, Ellis RJ, Firestone MK, Freckleton RP, Green JL, Green LE, Killham K, Lennon JJ (2007) The role of ecological theory in microbial ecology. Nat Rev Microbiol 5:384

Purahong W, Schloter M, Pecyna MJ, Kapturska D, Däumlich V, Mital S, Buscot F, Hofrichter M, Gutknecht JL, Krüger D (2014) Uncoupling of microbial community structure and function in decomposing litter across beech forest ecosystems in Central Europe. Sci Rep 4:7014

Purahong W, Kapturska D, Pecyna MJ, Jariyavidyanont K, Kaunzner J, Juncheed K, Uengwetwanit T, Rudloff R, Schulz E, Hofrichter M (2015) Effects of forest management practices in temperate beech forests on bacterial and fungal communities involved in leaf litter degradation. Microb Ecol 69:905–913

Purahong W, Wubet T, Lentendu G, Schloter M, Pecyna MJ, Kapturska D, Hofrichter M, Kruger D, Buscot F (2016) Life in leaf litter: novel insights into community dynamics

of bacteria and fungi during litter decomposition. Mol Ecol 25:4059–4074

Ritpitakphong U, Falquet L, Vimoltust A, Berger A, Métraux JP, L'Haridon F (2016) The microbiome of the leaf surface of Arabidopsis protects against a fungal pathogen. New Phytol 210:1033–1043

Romaní AM, Fischer H, Mille-Lindblom C, Tranvik LJ (2006) Interactions of bacteria and fungi on decomposing litter: differential extracellular enzyme activities. Ecology 87:2559–2569

Shannon CE (1948) A mathematical theory of communication, Part I, Part II. Bell Syst Tech J 27:623–656

Šnajdr J, Cajthaml T, Valášková V, Merhautová V, Petranková M, Spetz P, Leppanen K, Baldrian P (2011) Transformation of *Quercus petraea* litter: successive changes in litter chemistry are reflected in differential enzyme activity and changes in the microbial community composition. FEMS Microbiol Ecol 75:291–303

Stackebrandt E, Goebel B (1994) Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Evol Microbiol 44:846–849

Tian XY, Zhang CS (2017) Illumina-based analysis of endophytic and rhizosphere bacterial diversity of the coastal halophyte *Messerschmidia sibirica*. Frontiers in microbiology 8:2288

Tláskal V, Voříšková J, Baldrian P (2016) Bacterial succession on decomposing leaf litter exhibits a specific occurrence pattern of cellulolytic taxa and potential decomposers of fungal mycelia. FEMS Microbiol Ecol 92:fiw177

Ukonmaanaho L, Starr M (2001) The importance of leaching from litter collected in litterfall traps. Environ Monit Assess 66:129–146

Urbanová M, Šnajdr J, Brabcová V, Merhautová V, Dobiášová P, Cajthaml T, Vaněk D, Frouz J, Šantrůčková H, Baldrian P (2014) Litter decomposition along a primary post-mining chronosequence. Biol Fertil Soils 50:827–837

Wal A, Geydan TD, Kuyper TW, Boer W (2013) A thready affair: linking fungal diversity and community dynamics to terrestrial decomposition processes. FEMS Microbiol Rev 37:477–494

Zeng Q, Xin L, Dong Y, An S, Darboux F (2016) Soil and plant components ecological stoichiometry in four steppe communities in the Loess Plateau of China. CATENA 147:481–488

Zeng Q, An S, Liu Y (2017a) Soil bacterial community response to vegetation succession after fencing in the grassland of China. Sci Total Environ 609:2–10

Zeng Q, Liu Y, An S (2017b) Impact of litter quantity on the soil bacteria community during the decomposition of Quercus wutaishanica litter. PeerJ 5:e3777

Zhalnina K, Dias R, De Quadros PD, Davis-Richardson A, Camargo FA, Clark IM, Mcgrath SP, Hirsch PR, Triplett EW (2015) Soil pH determines microbial diversity and composition in the park grass experiment. Microb Ecol 69:395–406

Zhang C, Liu G, Xue S, Wang G (2016) Soil bacterial community dynamics reflect changes in plant community and soil properties during the secondary succession of abandoned farmland in the Loess Plateau. Soil Biol Biochem 97:40–49

Zheng F, He X, Gao X, Zhang C, Tang K (2005) Effects of erosion patterns on nutrient loss following deforestation on the Loess Plateau of China. Agric Ecosyst Environ 108(1):85–97