Impact of litter quantity on the soil bacteria community during the decomposition of *Quercus wutaishanica* litter

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The forest ecosystem is the main component of terrestrial ecosystems. The global climate and the functions and processes of soil microbes in the ecosystem are all influenced by litter decomposition. The effects of litter decomposition on the abundance of soil microorganisms remain unknown. Here, we analyzed soil bacterial communities during the litter decomposition process in an incubation experiment under treatment with different litter quantities based on annual litterfall data (normal quantity, 200 g/(m2/yr); double quantity, 400 g/(m2/yr) and control, no litter). The results showed that litter quantity had significant effects on soil carbon fractions, nitrogen fractions, and bacterial community compositions, but significant differences were not found in the soil bacterial diversity. The normal litter quantity enhanced the relative abundance of Actinobacteria and Firmicutes and reduced the relative abundance of Bacteroidetes, Plantctomycets and Nitrospiare. The Beta-, Gamma-, and Deltaproteobacteria were significantly less abundant in the normal quantity litter addition treatment, and were subsequently more abundant in the double quantity litter addition treatment. The bacterial communities transitioned from Proteobacteria-dominant (Beta-, Gamma-, and Delta) to Actinobacteria-dominant during the decomposition of the normal quantity of litter. A cluster analysis showed that the double litter treatment and the control had similar bacterial community compositions. These results suggested that the double quantity litter limited the shift of the soil bacterial community. Our results indicate that litter decomposition alters bacterial dynamics under the accumulation of litter during the vegetation restoration process, which provides important significant guidelines for the management of forest ecosystems.

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Abstract

The forest ecosystem is the main component of terrestrial ecosystems. The global climate and the functions and processes of soil microbes in the ecosystem are all influenced by litter decomposition. The effects of litter decomposition on the abundance of soil microorganisms remain unknown. Here, we analyzed soil bacterial communities during the litter decomposition process in an incubation experiment under treatment with different litter quantities based on annual litterfall data (normal quantity, 200 g/(m^2/yr); double quantity, 400 g/(m^2/yr) and control, no litter). The results showed that litter quantity had significant effects on soil carbon fractions, nitrogen fractions, and bacterial community compositions, but significant differences were not found in the soil bacterial diversity. The normal litter quantity enhanced the relative abundance of Actinobacteria and Firmicutes and reduced the relative abundance of Bacteroidetes, Plantctomycets and Nitrospiare. The Beta-, Gamma-, and Deltaproteobacteria were significantly less abundant in the normal quantity litter addition treatment, and were subsequently more abundant in the double quantity litter addition treatment. The bacterial communities transitioned from Proteobacteria-dominant (Beta-, Gamma-, and Delta) to Actinobacteria-dominant during the decomposition of the normal quantity of litter. A cluster analysis showed that the double litter treatment and the control had similar bacterial community compositions. These results suggested that the double quantity litter limited the shift of the soil bacterial community. Our results indicate that litter decomposition alters bacterial dynamics under the accumulation of litter during the vegetation restoration process, which provides important significant guidelines for the management of forest ecosystems.

1. Introduction

Plant litter is the main source of soil carbon and nitrogen, and influences the function and development of terrestrial ecosystems (Sauvadet et al. 2016). The interaction between the soil and plant litter microorganism has attracted much attention (Urbanová et al. 2015). Microorganisms provide the link between the soil and plant and plays an important role in the soil biogeochemical recycle, including the recycling of carbon (C), nitrogen (N), phosphorus (P) and other mineral elements (Keiluweit et al. 2015). Plants are the major sources of soil nutrients and affect soil properties via litter decomposition, root exudates and microorganism invasion from litter (Wardle et al. 2004). Litter decomposition is a key process for element recycling and had been studied by many researchers in different areas (Aerts 1997; Fanin et al. 2014; Freschet et al. 2013; Gundel et al. 2016; Kuramae et al. 2013; Sauvadet et al. 2016; van Huysen et al. 2016). Previous studies have shown litter quality and quantity are the main factors that drive the litter decomposition process (Keiluweit et al. 2015). Litter quality includes the C, N, P, Mn, Fe, Ca, Al, cellulose, hemi-cellulose and lignin content in the litter (Aerts 1997; Berg & Mcclaugherty 2014; Keiluweit et al. 2015). Litter represents a major pathway for C cycling between the vegetation and the soil in terrestrial ecosystems, and changes in the aboveground litter quantity and quality could have important consequences for C cycling. Some researchers have reported that litter quantity increased litter decomposition, litter carbon (C) loss and soil respiration, but did not alter soil organic carbon content after 2.5 years in the forest system (Fang et al. 2015). Generally, the total C and N contents of soil is not sensitive to the litter decomposition process, but soil organisms have proved to be a sensitive indicator of the response of vegetation restoration (An et al. 2013; Huang et al. 2011).

The quality of litter inputs determines on both the genetic structure of the soil microbial communities and their substrate use patterns, which may have effects on soil microbial structure (Lamarche et al. 2007; Zhang et al. 2013). Thus, much more attention should be paid to the response of sensitive soil indicators to litter decomposition with the increase of the litter layer.

With the on-going Grain for Green project in China that began in 1999, plant coverage, plant biomass and the litter layer have gradually increased on the Loess Plateau (Deng et al. 2014). Enhanced soil quality and soil carbon storage have been reported by many researchers (An et al. 2013; Cheng et al. 2015; Deng et al. 2013). With the process of vegetation restoration, plant litters gradually accumulate, which may influence the function of soil microorganisms. Litter quantity is a key factor that can influence the function and composition of soil organisms. Higher plant litter quantities usually favor the growth of opportunistic bacterial taxa for the greater labile C compounds from litter (Nemergut et al. 2010). Thus, the accumulation of plant litter should theoretically enhance the biomass of soil microbes, in particular, organisms better that are suited to address the greater availability of C compounds via exploitative resource strategies (Nemergut et al. 2010). However, the relative effects of litter quantity on the soil bacterial structure have rarely been assessed, and to our knowledge, there are no studies disentangling the effects of litter quantity on the soil bacteria during the decomposition processes in forest soils.

With the objective of disentangling the effects of litter quantity on soil bacterial structure and function, we analyzed the soil community structure and diversity in an incubation experiment with different litter quantities, including normal and double levels based on the data from annual litter fall. Illumina Hiseq sequencing was used to determine the response of the soil bacterial community

to different amounts of litter decomposition. We hypothesized that (1) litter decomposition may enhance the soil bacterial diversity and community composition, especially for the oligotrophic bacteria and (2) this trend increase with the increase of litter quantity as more nutrients are available from litter decomposition. Our results provide insights to better understand the process of litter decomposition and to manage forest land with accumulated plant litter.

2. Materials and Methods

2.1 Site description

Soil and litter samples were collected from the Fuxian Observatory for Soil Erosion and Eco-environment, a secondary forest region. *Quercus wutaishanica* was the predominant community, playing an important role in maintaining the stability of the system in this area (Fan et al. 2006; Guo et al. 2010). Therefore, understanding the effects of *Quercus wutaishanica* leaf litter decomposition provides insights into the carbon and nitrogen recycling in the soil-plant system. We established three plots in *Quercus wutaishanica* forests with similar topographical conditions to investigate the annual litter fall using the method described by Ukonmaanaho & Starr (2001) (Ukonmaanaho & Starr 2001). Over two years of observations, the annual litter fall of Quercus wutaishanica was approximately 200 $g/m^2/yr$.

2.2 Soil and litter sampling

Soil samples from 0-20 cm were obtained in September 2015 when most of the leaves had fallen. All roots, stones, small animals and other debris were removed from the soil samples by hand, and the samples were sieved through a 2-mm screen. The mixed soils were used to conduct the litter decomposition experiment in the laboratory. The soil organic carbon and total nitrogen

106 contents were 18.26 g/kg and 1.60 g/kg, respectively. Fresh litter was collected with a litter
107 collector. To avoid damaging the litter structure, the leaves were air-dried for more than two weeks
108 at room temperature to a consistent weight.

109 **2.3 Litter decomposition experiment**

110 Litter decomposition experiment was conducted using the nylon mesh bag technique. There
111 were three treatments, including normal quantity (200 g/(m^2/yr)) litter, double quantity (400
112 g/(m^2/yr)) litter, and control (no litter) (Fig. 1). The litter bags (10 cm × 20 cm size) were
113 constructed out of 1 mm nylon mesh. First, we placed 200 g fresh soils in a 1 L plastic basin and
114 then placed a litter bag (5 g, normal quantity; 10 g, double quantity) on the surface. Each treatment
115 had three replicates. We also conducted a control experiment without litter bags. All basins were
116 incubated at 25 ℃ in an incubator. The soil water content was adjusted using the weighting method
117 every week at a relative humidity of 20%. After 90 days, we collected the soil sample layer below
118 the litter bags to analyze the soil properties and bacterial communities. After harvest, each soil
119 sample was mixed and separated into two parts. One part was air-dried for the evaluation of the
120 soil properties. The other part was frozen at -80 °C (using liquid nitrogen) for subsequent
121 sequencing analysis.

122 **2.4 Analysis of the soil properties**

123 The soil moisture was determined gravimetrically with fresh soils at 105 ℃ for 24 h, and the
124 water content was expressed as a percentage of the dry weight. The fumigation-extraction method
125 was used to determine microbial biomass carbon (MBC) and microbial nitrogen (MBN) (Vance et
126 al. 1987). The dissolved carbon (DOC) and dissolved nitrogen (DON) in the soil were determined

127 by extracting the samples in 0.5 mol/L K_2SO_4. The soil total N (STN), soil organic carbon (SOC),
128 soil nitrate nitrogen (NO_3^--N) and soil ammonia nitrogen (NH_4^+-N) were analyzed using the
129 method described by Zeng et al. (2016) (Zeng et al. 2016).

130 **2.5 Soil NDA extraction and PCR amplification**

131 The DNA of the soil was extracted from a 0.5 g soil sample using the CTAB method. The
132 concentration and purity of the DNA were monitored using 1% agarose gels. According to the
133 concentration, the DNA samples were diluted to 1 ng/μL with sterile water to reduce the effects of
134 the PCR inhibitors. The V4 gene of the 16S rRNA was amplified using the 515F /806R primer
135 sets (Bergmann et al. 2011; Zeng et al. 2017). All PCR reactions were carried out with Phusion®
136 High-Fidelity PCR Master Mix (New England Biolabs). The same volume of 1 × loading buffer
137 (contained SYB green) was mixed with the PCR products, and electrophoresis was conducted on
138 2% agarose gels for detection. The samples with a bright strip between 400-450 bp were chosen
139 for further experiments. The PCR products were mixed at equal density ratios. Then, the PCR
140 mixtures were purified using a Qiagen Gel Extraction Kit (Qiagen, Germany).

141 **2.6 Illumina Miseq sequencing**

142 Sequencing libraries were generated using a TruSeq® DNA PCR-Free Sample Preparation
143 Kit (Illumina, USA) following the manufacturer's recommendations and index codes were added.
144 The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent
145 Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina HiSeq 2500 platform
146 and 250 bp paired-end reads were generated. The 16S rRNA gene amplicon sequencing was
147 conducted at Novogene Bioinformatics Technology Co., Ltd., Beijing, China. The raw sequence

148 data in FASTQ format are accessible from the NCBI SRA with the number of SRP107086.

149 **2.7 Statistical and bioinformatics analysis**

150 QIIME software was used to analyze the sequences data (Caporaso et al. 2010). The
151 sequencing data yielded 569171 raw reads, with 71146 raw reads per sample. After removing the
152 low quality reads and trimming the barcodes and primers, there were 545740 valid reads (average
153 length 253 bp). Clustering sequences at 97% similarity levels were assigned to the same OTUs
154 (Stackebrandt & Goebel 1994). After the removal of chimeric sequences, a total of 4833 different
155 OTUs were recorded. Taxonomy was assigned to each OTU via the Ribosomal Database Project
156 (RDP) classifier (Cole et al. 2009). The representative sequence for each OTU was screened for
157 further annotation. The abundance of OTUs information was normalized using a standard
158 sequence number corresponding to the sample with the fewest sequences. The alpha diversity
159 was applied to analyze the complexity of the species diversity of each sample, including the
160 observed-species index and the Shannon index. All indices in our samples were calculated with
161 QIIME (Version1.7.0) and displayed with R software (Version 2.15.3).

162 The similarities between treatments were measured using a principal coordinate analysis
163 (PCoA) plot. The PCoA was analyzed using the WGCNA, stat and ggplot2 packages in R software
164 (Version 2.15.3). One-way ANOSIM and SIMPER analysis were used to compare the differences
165 in the bacterial composition among the different treatments using the Bray-Curtis method
166 (PRIMER software v 7) (Zeng et al. 2017). A higher R value in ANOSIM indicated a higher
167 separation between the treatments. The linear discriminant analysis effect size (LEfSe) method
168 was used to determine the difference between the normal and the double litter amount treatments

169 (Segata et al. 2011). One-way ANOVA was performed to explore the differences between the soil
170 properties and the soil bacterial compositions under the different treatments (SPSS version 20.0
171 for Windows), and the Student-Newman-Keuls (SKN) method was used for the comparison
172 (P=0.05). The relationships between soil bacterial composition and the environmental factors were
173 tested using Pearson correlation analyses using SPSS 20.0 for Windows.

174 **3. Results**

175 **3.1 Soil chemical properties and the response of microbial biomass to litter**
176 **decomposition**

177 The soil nitrogen fractions, carbon fractions and soil moisture were significantly altered by
178 the addition of litter (Fig. 2). The soil moisture showed a significant decline in the normal treatment
179 and an increase in double treatment. No significant differences were observed among the
180 treatments for soil NH_4^+-N, which ranged from 5.39 to 5.73 mg/kg. The MBN content was
181 significantly higher in the normal treatment and ranged from 43.50 to 124.14 mg/kg, and was in
182 the order of normal>double >control. The DON showed the opposite trend to the MBN, with the
183 highest value measured in the control treatment. The soil nitrate nitrogen ranged from 21.98 to
184 27.90 mg/kg, and there was no significant difference between the normal and the control
185 treatments. The control treatment had the highest MBC and the lowest DOC, and was significantly
186 different from the double treatment. With the increase of litter quantity, the soil nitrate nitrogen,
187 soil moisture, MBC, DOC and DON showed significant reductions in the normal treatment, and a
188 significant increase was observed in the MBN.

189 **3.2 Response of the soil bacterial community activity to litter decomposition**

190 The bacterial diversity indices showed no significant changes between the different
191 treatments (Table 1), but the soil bacterial community compositions demonstrated significant
192 structuring in response to litter addition. The most dominant groups across all soil samples were
193 Proteobacteria (38-42%), Actinobacteria (11-21%), Acidobacteria (18-20%), Gemmatimonadetes
194 (5%), Bacteroidetes (4-6%), Chloroflexi (3%), Firmicutes (1-2%), Verrucomicrobia (2-4%),
195 Planctomycetes (3-4%) and Nitrospirae (2%) (Fig. 3). The relative abundance of Actinobacteria,
196 Bacteroidetes, Planctomycetes, Firmicutes and Nitrospirae in the normal treatment was
197 significantly higher than in the double and control treatments (Fig. 3-A).

198 To explore the dynamics of the major microbial taxa under different mounts of litter
199 treatment, we found that Alpha, Beta, Gamma, and Delta-proteobacteria were the main members
200 of Proteobacteria. Only Alpha-proteobacteria showed no significant differences among the
201 different treatments, and ranged from 15.50 to 17.82%. With the increase of litter quantity, the
202 relative abundance of Bet, Gamma, and Deltaproteobacteria showed a decrease in the normal
203 treatment, and an increase in the double treatment. The Beta, Gamma, and Deltaproteobacteria
204 occupied 5.75%, 6.00%, and 6.93%, respectively, in the normal treatment, which significantly
205 differed from the double and control treatment (Fig 3-B). At the order level, Subgroup_6 and
206 Subgroup_4 were the dominant taxa in the Acidobacteria phylum, and showed no significant
207 changes with the increase of litter quantity. Rhizobiales was the dominant taxa of Alpha-
208 proteobacteria, and ranged from 7.01 to 8.75%, and showed similar variation to those of the Alpha-
209 proteobacteria. Solirubrobacterales, Xanthomonadales, Sphingobacteriales, Myxococcales and
210 Gaiellales had significant differences among the litter addition treatments (Fig. 4). These

differences were only detected between the normal treatment and the double or the control treatment. The cluster analysis and PCoA also indicted these changes (Fig. 3 and Fig. 5). More specifically, the bacterial community profiles in normal treatment trended to group together and were separated from those in the double and control treatments. A t-test showed that the soil bacterial taxa were significantly different between the normal and the double treatments, including Proteobacteria (Xanthomonadales, Salinisphaerales, Legionellales, Chromatiales, Syntrophobacterales, Sh765B-TzT-29, Myxococcales, SC-I-84, Sneathiellales, DB1-14 and Caulobacterales), Planctomycetes (WD2101_soil_group, Phycisphaerales, CCM11a), and Actinobacteria (Micrococcales, Solirubrobacterales, Rubrobacterales and Acidimicrobiales) (Fig. 5).

The ANOSIM based on the OTUs of the 16S rRNA gene sequences indicated that the differences were significant between the different litter addition treatments (ANOSIM Global R = 0.761, P = 0.01). SIMPER analysis revealed that bacterial communities were 76-81% similar between the normal, double and control treatments. The LEfSe analyses identified the significant difference in the abundant taxa between the different litter quantity treatments. Using the LEfSe, we found that Bacteroidetes, Myxococcales and Deltaproteobacteria were primarily different in the high-litter treatment (double). The green color in Fig.6 indicates the significantly different taxa in the normal treatment, and these species could potentially be used as biomarkers in the normal quantity treatment (Fig. 6).

Pearson correlation analysis showed that soil moisture, DON and MBN were the factors that mainly contributed to the significant correlation with bacterial taxa (Table 2). DON was

232 significantly correlated with the relative abundance of Actinobacteria, Bacteroidetes,
233 Verrucomicrobia, Verrucomicrobia, Firmicutes and Nitrospirae, with coefficients of -0.684, 0.812,
234 0.679, 0.669, -0.804 and 0.715, respectively. The SM and MBN were similarly correlated with the
235 bacterial community composition (Table 2). There were no significant correlations with the
236 relative abundance of Acidobacteria, Gemmatimonadetes and Chloroflexi, as the abundance of
237 these taxa was stable among the different treatments.

238 **4. Discussion**

239 Plant litter decomposition is a key process of in the recycling of soil elements (Berg &
240 Mcclaugherty 2014). In this study, the SOC and STN contents were not significantly altered by
241 litter decomposition (Fig. 2). This result is not consistent with other litter decomposition studies.
242 This study was a short-term experiment (only three months), generally, while total C and N
243 accumulation in soil occurs over long term processes with different mechanisms. However, the
244 available nutrients in soil, such as nitrite nitrogen and dissolved nitrogen, were significantly altered
245 by litter decomposition. Litter decomposition altered the available soil N fractions (. i.e., MBN,
246 DON and NO_3-N), and provided N resources for the growth of microbial organisms (Cleveland &
247 Townsend 2006; Wardle et al. 2004). The MBC and DOC also differed between the different
248 treatments. These changes revealed that the available C and N concentrations in the soil were
249 sensitive to litter decomposition, which could help to estimate and evaluate the effects of litter
250 decomposition under global climate change, N deposition, extreme drought and other
251 environmental problems.

252 Litter decomposition altered the bacterial community composition by a greater degree in the

normal quantity treatment than in the double treatment, but the bacterial diversity did not differ significantly (Shannon and observed-species indices). Short-term litter decomposition increased the relative abundance of Actinobacteria, Firmicutes and Thermoleophilia, and decreased the relative abundance of Deltaproteobacteria, Gammaproteobacteria, Betaproteobacteria and Sphingobacteriia, which is most likely a result of the available C and N input via litter deposition caused by soil or litter microorganisms (Cleveland & Townsend 2006; Wardle et al. 2004). Soil copiotrophic Bacteroidetes, α-, β-, and γ-Proteobacteria were relatively more abundant in the control and the double quantity litter treatment soils. The available nutrients released by the litter stimulated the microbial production of extracellular enzymes (Koyama et al. 2013), resulting in increased C and N availability, which also altered the bacterial community composition. Zhang et al. (2016) (Zhang et al. 2016) also observed that soil Proteobacteria increased with succession in Loess Plateau grasslands, as the soil nutrients were enhanced across the succession. In addition, our results indicated that soil water content significantly increased with the quantity of litter (Table 1). Increased water availability should alter soil microbial processes such as litter decomposition and nutrient mineralization (DeAngelis et al. 2015). These results suggest that nutrient and water availability in the soil may help explain why the increase in litter input altered the soil bacterial community composition in the normal and control treatments.

Bacteria play an important role in the litter decomposition process. Most Alphaproteobacteria, Acidobacteria and Actinobacteria can degrade recalcitrant C in plant litter (Barret et al. 2011). Acidobacteria can grow on complex polymers, including plant hemicellulose or cellulose and fungal chitin (Eichorst et al. 2011).With litter addition, the soil bacterial community composition

changed. These changes were indicated between the control and the normal treatments. The cluster tree analysis, PCoA and one-way ANOSIM all indicated that double and control treatments had similar bacterial communities (Figs. 3 and 5, Table 3). These results were consistent with the results of the LEefSe analysis and taxa abundance. Based on the results of LEefSe analysis indicated that Gaiellaes, Solirubrobacterales, Thermoleophilia and Alphaproteobacteria were significantly different in the normal treatment, and Shphingobacteria, Myxococcales and Deltaproteobacteria were significantly different in double treatment, which suggested that litter addition had significant effects on certain bacterial species (Fanin et al. 2014; Mau et al. 2015). The abundance of soil microbes was based on the nutritional preferences and functions of the microbes (Banerjee et al. 2016; Mau et al. 2015). The normal amount of litter addition altered the priming effects of soil bacterial communities, which has been confirmed by other researchers (Banerjee et al. 2016). Litter addition enhanced the decomposition of soil organic matter and altered the abundance of functional groups, as seen by the decline of copiotrophic bacteria. The double litter addition treatment did not alter the soil bacterial composition, as much more liable nutrients from litter decomposition could maintain the growth of copiotrophic bacteria.

Soil available nutrients may be the primary difference caused by these shifts. Zhong et al. (2015) (Zhong et al. 2015) found that N addition caused changes of the soil bacterial and fungal communities in a long term field experiment. The SOC was another main factor that affected the affecting soil bacterial community composition. Liu et al. (2014) found that Actinobacteria was significantly positively related to SOC, and Deltaproteobacteria was significantly negatively related to SOC (Liu et al. 2014). However, similar results were not observed in this study, which

295 was in accordance with the results from Zhong et al. (2015) (Zhong et al. 2015). We also found
296 that soil total N had no significant effect on soil community structure, but soil available N was
297 significantly related to the soil bacterial community. Soil available N is the main resource for soil
298 bacterial growth, which caused the variation in soil bacterial community structure. Zhang et al.
299 (2016) (Zhang et al. 2016) reported that the soil nitrate nitrogen content was significantly related
300 to the soil bacterial community along a natural succession. Yao et al. (2014) (Yao et al. 2014)
301 found that the soil ammonium nitrogen content played an important role in the soil bacterial
302 community compositions in the grass land soils of China. Yuan et al. (2014) (Yuan et al. 2014)
303 also observed similar results in soil on the Tibetan Plateau. All these results confirmed that soil
304 available N content was the main factor that drove these changes in the soil bacterial communities.

305 **5. Conclusion**

306 These results suggested that normal litter quantity could alter soil bacterial community
307 compositions. A higher quantity of litter did not affect the soil microbial community. Beta,
308 Gamma, and Deltaproteobacteria were significantly decreased in the normal quantity litter addition
309 treatment, and subsequently increased in the double quantity litter addition treatment. The bacterial
310 communities transitioned from Proteobacteria-dominant (Beta-, Gamma-, and Delta) to
311 Actinobacteria-dominant during decomposition of the normal quantity of litter. The soil available
312 nutrients and the soil copiotrophic bacterial communities were higher in the control and the double
313 quantity of litter decomposition treatments. These results suggested that litter addition affected the
314 soil bacterial structure, and can provide guidance to manage vegetation restoration with the
315 increase of litter quantity.

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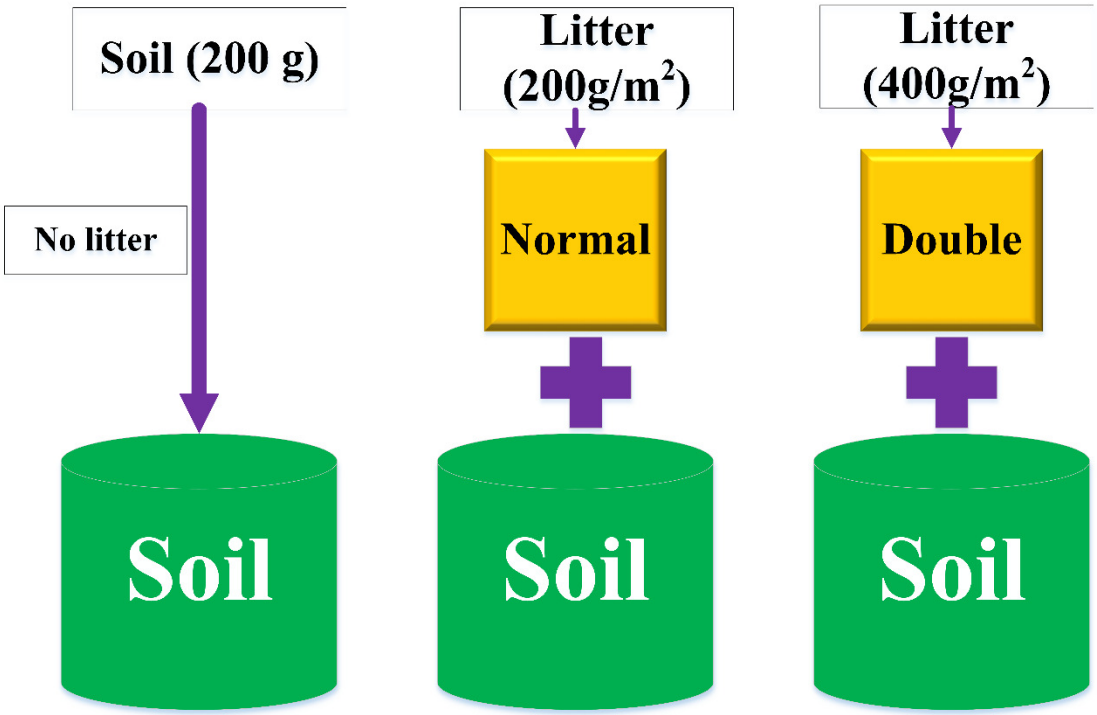
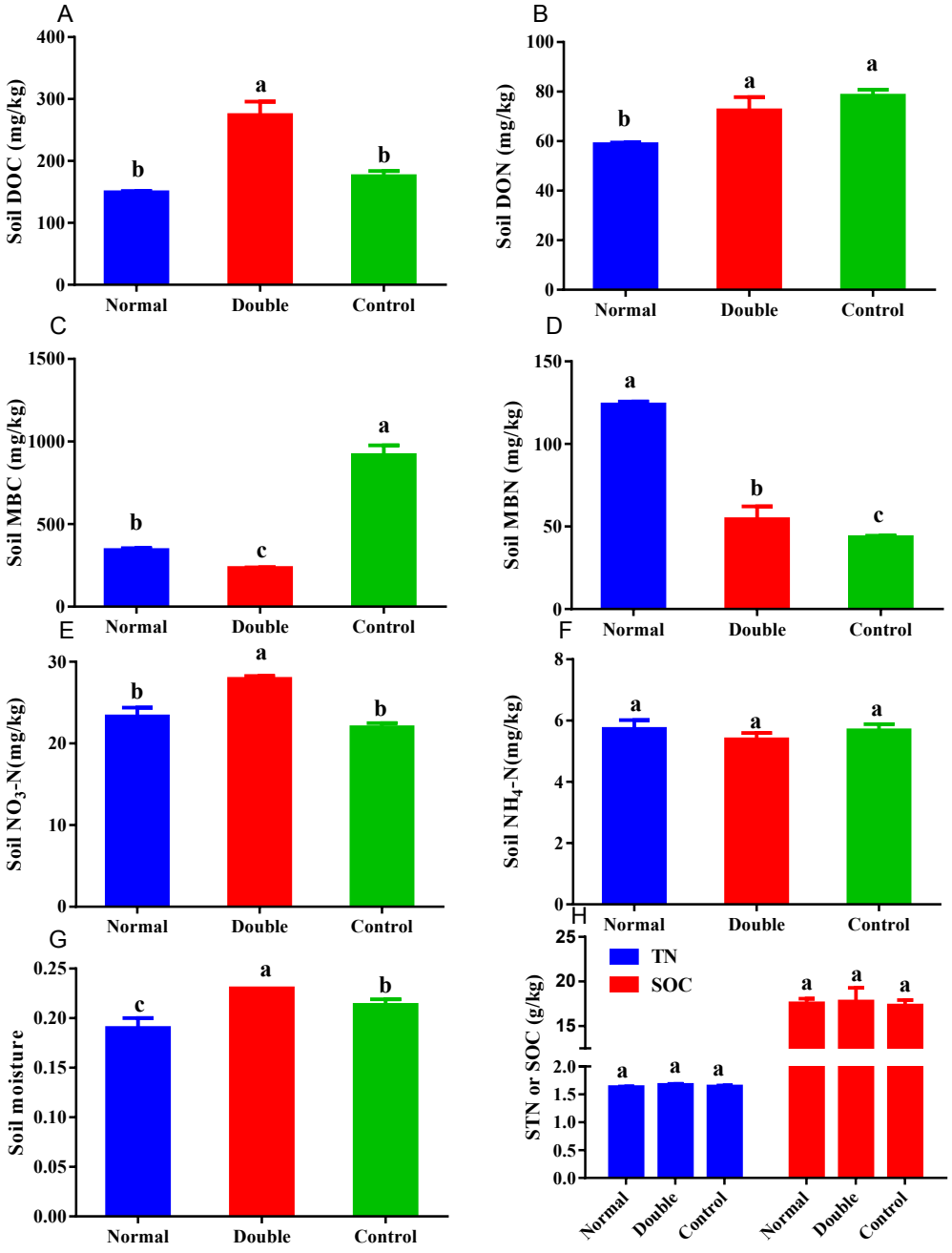


Fig. 1 The setup of the litter decomposition experiment under different litter quantities.



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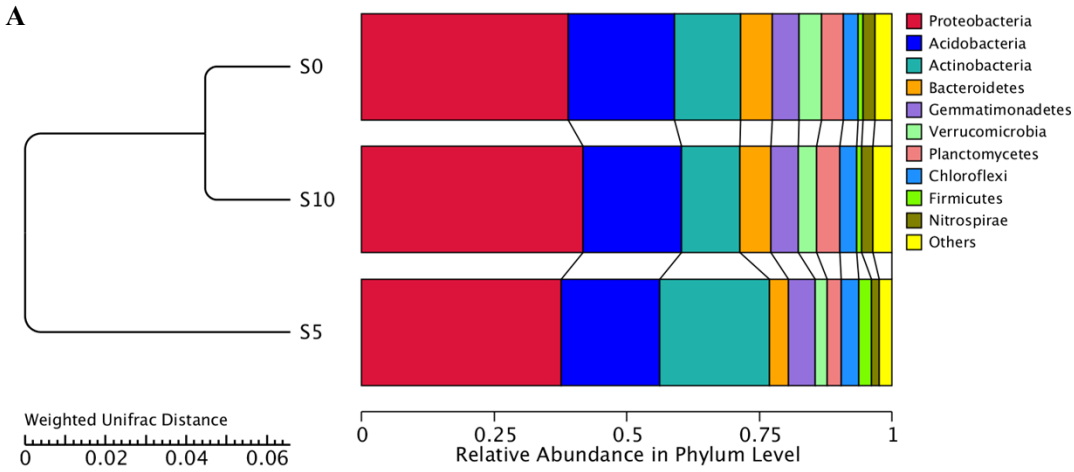
435 Fig. 2 Soil carbon and nitrogen fractions in the different treatments. Different lowercase letters

436 indicate significant differences at the 0.05 level. All data are expressed as means ± SD



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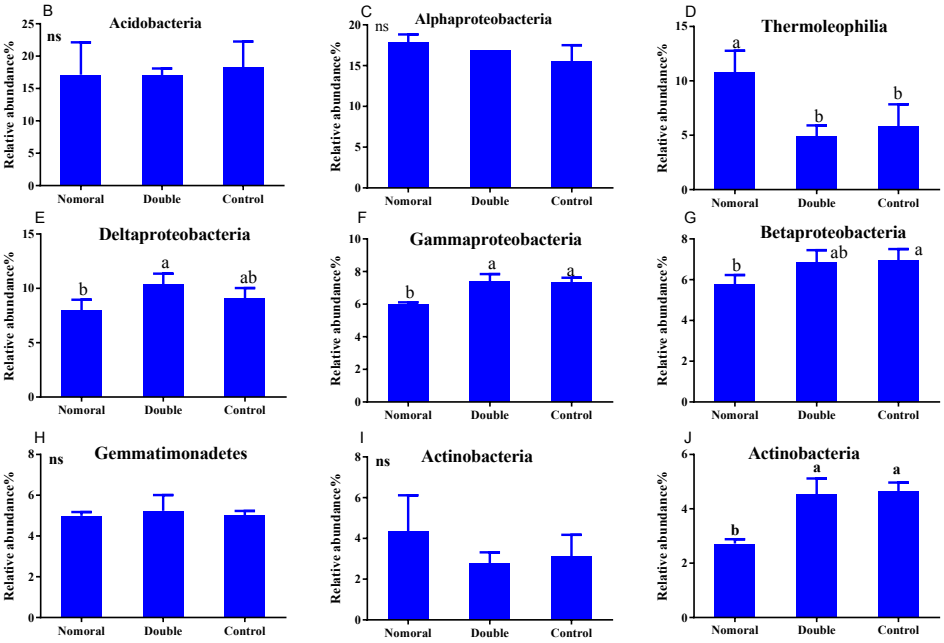

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439 Fig .3 Soil bacterial communities under different litter quantities at the phylum level (A) and

440 class level (B). Different lowercase letters indicate significant differences between the different

441 litter quantity treatments ($P<0.05$); ns indicates that there is no significant difference. All data are

442 expressed as means ± SD. S0, control; S5, normal treatment; S10 double treatment.

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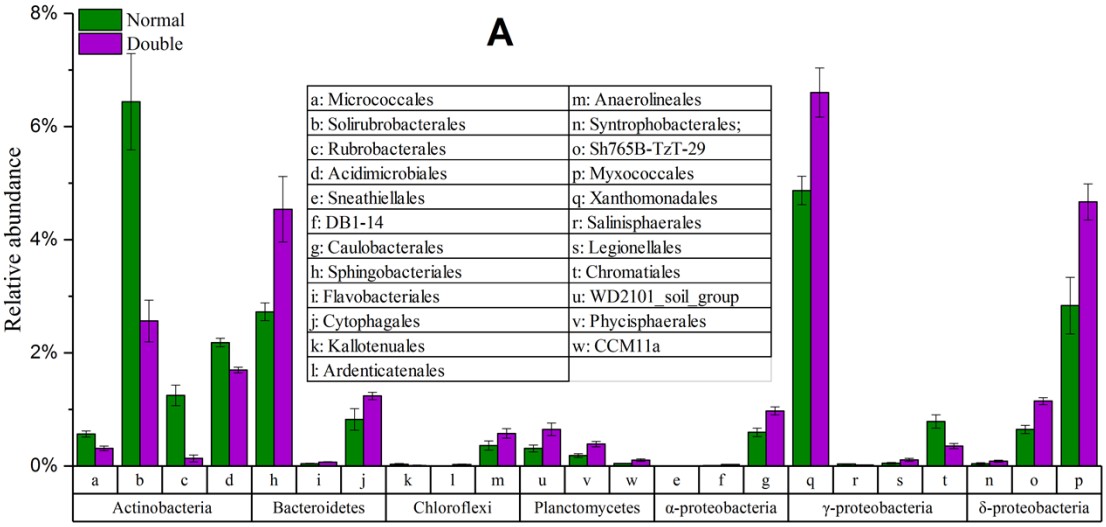

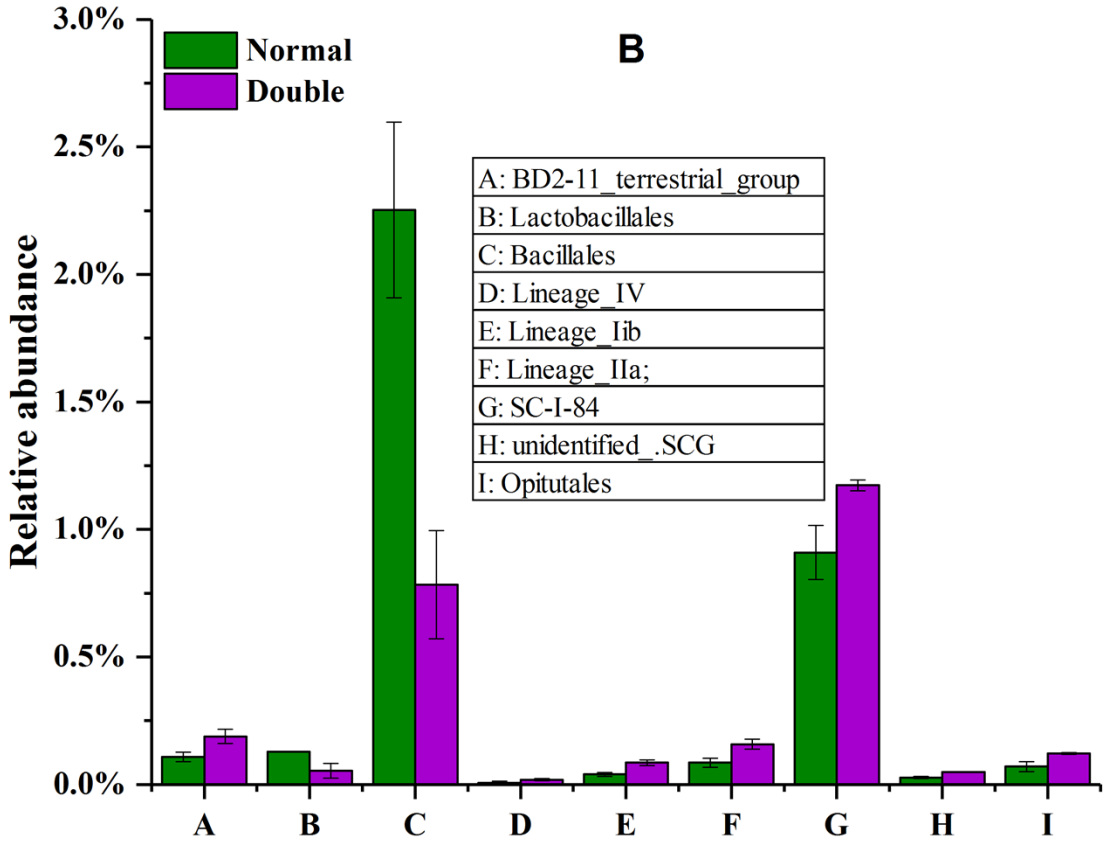

Fig. 4 The significantly different taxa between the normal treatment and the double treatment as determined by a T-test. The taxa shown in the figure were significant at the 0.05 level. All data are expressed as means ± SD

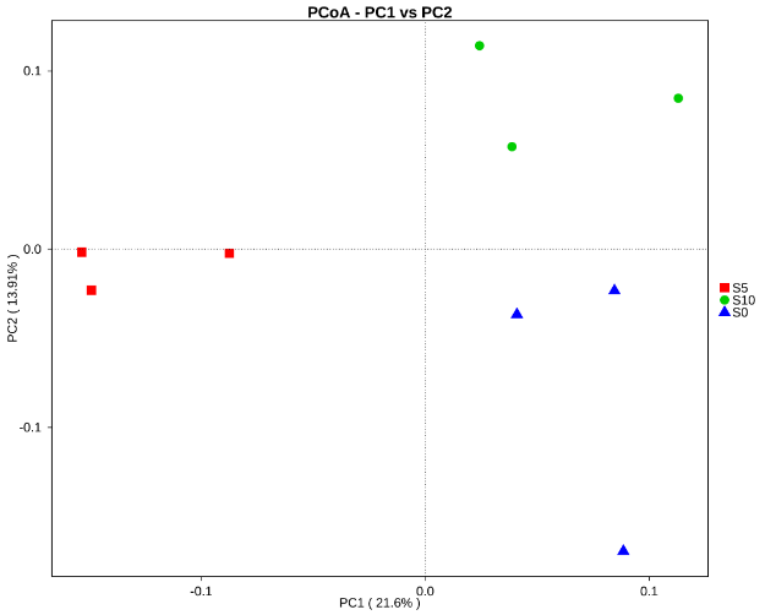

Fig. 5 Principal coordinates analysis (PCoA) of the soil bacterial community composition based on Bray-Curtis distances. S0, control; S5, normal treatment; S10 double treatment.

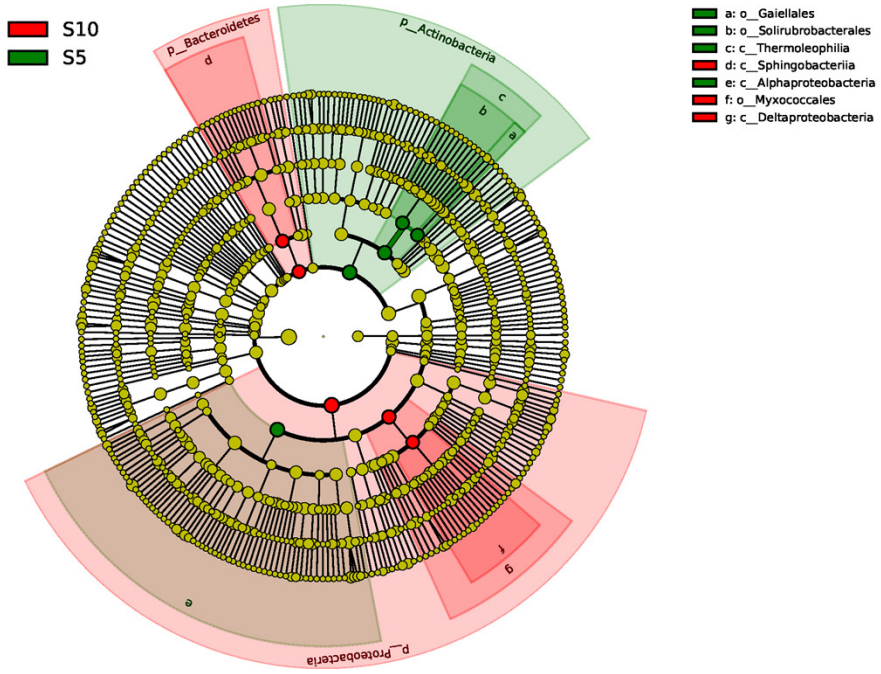

Fig. 6 A linear discriminant analysis effect size (LEsFe) method identifies the significantly different abundant taxa of bacteria under different litter quantity treatments. Taxa with significantly different abundance among treatments are represented by colored dots. S5, normal treatment; S10 double treatment.

Table 1 Soil bacterial alpha diversity indices under different the litter quantity treatment

Treatment	Observed_species	Shannon
Normal	3035±42	9.57±0.11
Double	2962±109	9.59±0.04
Control	2932±62	9.53±0.10

Note: All indices were not significantly different between the different treatments. All data are expressed as means ± SD.

Table 2 The Pearson correlations between the soil properties and the soil bacterial community composition

	DOC	DON	MBC	MBN	SM	NO_3-N	NH_4-N
Proteobacteria	**0.759***	0.302	-0.227	-0.426	**0.676***	0.511	-0.313
Actinobacteria	-0.648	**-0.684***	-0.189	**0.816****	**-0.839****	-0.444	0.514
Bacteroidetes	0.644	**0.812***	0.33	**-0.915****	**0.749***	0.26	-0.306
Verrucomicrobia	0.114	**0.679***	0.511	**-0.674***	0.385	-0.035	-0.343
Verrucomicrobia	0.537	**0.669***	0.201	**-0.785***	**0.674***	0.395	-0.462
Firmicutes	-0.623	**-0.804****	-0.262	**0.897****	**-0.820****	-0.404	0.426
Nitrospirae	0.563	**0.715***	0.307	**-0.797***	0.637	0.239	-0.318

Note: DOC: dissolve organic carbon. DON: dissolve organic nitrogen. MBC: microbial biomass carbon. MBN: microbial biomass nitrogen. SM: soil moisture. NO_3-N: nitrate nitrogen; NH_4-N: ammonia nitrogen. * indicate significance at the 0.05 level, ** indicate significant at the 0.007 level (adjusted by Bonferroni correction).

Table 3 ANOSIM and SIMPER analysis between the different litter treatments

□	SIMPER	ANOSIM

Group A & B	Average similarity %	R value
Normal vs Double	76.28	1
Normal vs Control	76.66	0.889
Double vs Control	81.02	0.296

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