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

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In terrestrial ecosystems, forest ecosystem is the main competent, affecting the world climate and and soil microbial functioning and processes in ecosystem via specific litter decomposition. Effects of litter decomposition on the abundance of soil microorganisms still remain unknown. Here we analyzed soil bacterial communities during the process of litter decomposition in an incubation experiment under different litter quantity (normal quantity, 200 g/(m^2.yr); double quantity, 400 g/(m^2.yr) and control, none litter). The results showed that litter quantity had significant effects on soil carbon fractions, nitrogen fractions, and bacterial community compositions, but no significant effects on soil bacterial diversity. Normal litter quantity enhanced the relative abundance of Actinobacteria and Firmicutes and reduced the the relative abundance of Bacteroidetes, Plantctomycets and Nitrospiare. Beta-, Gamma-, and Deltaproteobacteria showed significantly decreased at the normal quantity litter addition, and subsequently increased at the double quantity litter addition. Bacterial communities transitioned from Proteobacteria-dominant (Beta-, Gamma-, and Delta) to Actinobacteria-dominant during the litter decomposition with normal quantity. Cluster analysis showed that double litter treatment and control had similar bacterial community compositions. These results suggested double quantity litter limited the shift of soil bacterial community. Our results indicate that litter decomposition has altered bacterial dynamics under the accumulation of litter in the process of vegetation restoration, which provided significant guidelines for the management of forest ecosystem.

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Abstract

In terrestrial ecosystems, forest ecosystem is the main competent, affecting the world climate and and soil microbial functioning and processes in ecosystem via specific litter decomposition. Effects of litter decomposition on the abundance of soil microorganisms still remain unknown. Here we analyzed soil bacterial communities during the process of litter decomposition in an incubation experiment under different litter quantity (normal quantity, 200 g/(m^2.yr); double quantity, 400 g/(m^2.yr) and control, none litter). The results showed that litter quantity had significant effects on soil carbon fractions, nitrogen fractions, and bacterial community compositions, but no significant effects on soil bacterial diversity. Normal litter quantity enhanced the relative abundance of Actinobacteria and Firmicutes and reduced the the relative abundance of Bacteroidetes, Plantctomycets and Nitrospiare. Beta-, Gamma-, and Deltaproteobacteria showed significantly decreased at the normal quantity litter addition, and subsequently increased at the double quantity litter addition. Bacterial communities transitioned from Proteobacteria-dominant (Beta-, Gamma-, and Delta) to Actinobacteria-dominant during the litter decomposition with normal quantity. Cluster analysis showed that double litter treatment and control had similar bacterial community compositions. These results suggested double quantity litter limited the shift of soil bacterial community. Our results indicate that litter decomposition has altered bacterial dynamics under the accumulation of litter in the process of vegetation restoration, which provided significant guidelines for the management of forest ecosystem.

Key words: Carbon fractions; Nitrogen fractions; Litter decomposition; Soil bacteria

1. Introduction

Plant litter is the main source of soil carbon and nitrogen, affecting the function and development of terrestrial system (Sauvadet et al. 2016). The interaction between soil and plant litter microorganism has attracted much attention (Urbanová et al. 2015). Microorganism was the link between soil and plant which played an important role in soil biogeochemical recycle, including carbon (C), nitrogen (N), phosphorus (P) and other mineral elements recycles (Keiluweit et al. 2015). Plants, as the major resource of soil nutrients, affecting soil properties via litter decomposition, root exudates and microorganism invasion from litter (Wardle et al. 2004). Litter decomposition was a key process for element recycle and had been studied by many researchers in the 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). The previous studies showed that litter quality and quantity were the main factors to drive the process of litter decomposition (Keiluweit et al. 2015). Litter quality included litter C, N, P, Mn, Fe, Ca, Al, cellulose, hemi-cellulose and lignin (Aerts 1997; Berg & Mcclaugherty 2014; Keiluweit et al. 2015). Litter represents a major pathway for C cycling between vegetation and soil in terrestrial ecosystems, changes in aboveground litter quantity and quality could have important consequences for C cycling. Some researchers 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, soil total C and N contents were not sensitive to the process of litter decomposition, but soil organism was had been proved a sensitive indicator to the response of vegetation restoration (An et al. 2013; Huang et al. 2011). The quality of litter inputs

64 is the determinant on both genetic structure of soil microbial communities and their substrate use
65 patterns, which may have effects on soil microbial structure (Lamarche et al. 2007; Zhang et al.
66 2013). Thus, much more attention should be paid on soil sensitive indicators response to litter
67 decomposition with the increase of litter layer.

68 With the practice of Grain for Grain project in China since 1999, plant coverage, plant
69 biomass and litter layer were gradually enhanced on Loess Plateau (Deng et al. 2014). Soil quality
70 and soil carbon storage have been enhanced reported by many researchers (An et al. 2013; Cheng
71 et al. 2015; Deng et al. 2013). As litter decomposition also changed by litter quantity, little changes
72 for soil respiration by litter decomposition would have a great effect on global carbon recycle
73 (Bradford et al. 2016). Thus, well understanding litter quantity on soil system is necessary and of
74 great importance for global warming. These results also will provide suggestive guide for the
75 management of vegetation restoration for future in the Loess Plateau.

76 In this study, we analyzed soil community structure and diversity in an incubation experiment
77 with different litter quantities, including normal and double levels based on the data from annual
78 litter fall. The Illumina Hiseq sequencing was used to determine soil bacterial community
79 responding to litter decomposition. We hypothesized that (1) litter decomposition may enhance
80 the soil bacterial diversity and community composition, especially for the oligotrophic bacteria
81 and (2) this trend increased with the increase of litter quantity as more available nutrients from
82 litter decomposition. Our results could provide insight to better understanding the process of litter
83 decomposition and managing forest land with the fact of the accumulation of plant litter.

84 **2. Materials and Methods**

2.1 Site description

The field experiment was conducted at the Fuxian Observatory for Soil Erosion and Eco-environment that was established in 1989 on the eastern slope of the Ziwuling secondary Forest region (Tang et al. 1993). The land forms are characterized as low mountains and hills covered by loess with elevation ranging from 920 to 1683 m and a gully density of 4.5 $km \cdot km^{-2}$. The mean annual temperature ranges from 6 to 10℃, and mean annual precipitation is about 700 mm. Approximately 60% of the precipitation falls from July to September (Zheng et al. 2005). The soil type is Typic-Loessi Orthic Primosols according to Keys to Chinese Soil Taxonomy (3rd edition, 2001). As the largest natural secondary forest-covered region in the Loess Plateau, the Ziwuling Mountains play an important role in the control of soil erosion and climatic regulation in Northwest China. *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 would provide insight to 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 with the method descripted by Ukonmaanaho & Starr (Ukonmaanaho & Starr 2001). From two years' observations, the annual litter fall of *Quercus wutaishanica* was about 200 $g/m^2/yr$ (data not shown). This amount was the base of litter decomposition.

2.2 Soil and litter sampling

Soil samples from 0-20 cm were obtained during September 2015 when most leaf fallen. All the soil samples were sieved through a 2-mm screen, and removed the roots, stones, small animals

and other debris by hand. Soil organic carbon and total nitrogen contents were 18.26 g/kg and 1.60 g/kg, respectively. The mixed soils were used to conduct litter decomposition experiment in the laboratory. On the other hand, fresh litter was collected with the collector described above. To avoid damaging the litter structure, the leaves were air-dried for more than two weeks at room temperature until to a consistent weight.

2.3 Litter decomposition experiment

The nylon mesh bag technique was used to quantify the effects of soil chemical properties and soil microbial activities from litter decomposition. There were three treatments, including normal quantity (200 g/(m^2·yr)) litters, double quantity (400 g/(m^2·yr)) litters, and control (none litter) (Fig. 1). Litter bags (10 cm × 20 cm size) were constructed out of 1 mm nylon mesh. Firstly, we weighed 200 g soils placed in a 1 L plastic basin and then placed a litter bag (5 g, normal quantity; 10 g, double quantity) on the surface. Each treatment had three replicates. We also conducted a control experiment without litter bags. All the basins were incubated at 25 ℃ in a humid environment. Soil water content was adjusted by a weighting method every week. After 90 days, we collected soil sample layer below the litter bags to analyze soil properties and bacterial communities. After harvest, each soil sample was mixed and separated into two parts. One part was air-dried for the evaluation of the soil properties. The other part was frozen at -80 °C (like liquid nitrogen) for subsequent high-throughput pyrosequencing analysis.

2.4 The analysis of soil properties

Soil moisture was determined gravimetrically with fresh soils at 105 ℃ overnight, and the water content was expressed as a percentage of the dry weight. The fumigation-extraction method

was used to determine the soil microbial biomass carbon (MBC) and soil microbial nitrogen (MBN) (Vance et al. 1987). Soil dissolved carbon (DOC) and soil dissolved nitrogen (DON) were determined by the extraction of 0.5 mol/L K_2SO_4. The ratio of soil and solution was 4:1. Concentrations of soil total N (STN) were determined colorimetrically according to the Kjeldahl acid-digestion method (KDY-9830) after extraction with 0.02 mol/L sulfuric acid (Thomas et al. 1967). Soil organic carbon (SOC) was measured by a modified Mebius method (Ren et al. 2015). Briefly, 0.5 g soil sample was digested with 5 ml of 0.8 mol/L $K_2Cr_2O_7$ and 5 ml of concentrated H_2SO_4 at approximately 180 °C for 5 min, followed by titration of the digests with standardized 0.2 mol/L $FeSO_4$. Soil nitrate nitrogen (NO_3^--N) and soil ammonia nitrogen (NH_4^+-N) extracted by 1 mol/L KCl, and the extraction were measured by a Seal AutoAnalyzer3 (Zeng et al. 2016).

2.5 Soil NDA extraction and PCR amplification

Soil DNA was extracted from 0.5 g of soil sample with the method of CTAB. DNA concentration and purity was monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1 ng/μL using sterile water. The 16S rRNA V4 genes were amplified for each sample using primer sets of 515F/806R (Bergmann et al. 2011). All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). Mix same volume of 1X loading buffer (contained SYB green) with PCR products and operate electrophoresis on 2% agarose gel for detection. Samples with bright main strip between 400-450 bp were chosen for further experiments. PCR products was mixed in equidensity ratios. Then, mixture PCR products was purified with Qiagen Gel Extraction Kit (Qiagen, Germany).

2.6 Illumina Miseq sequencing

148 Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit
149 (Illumina, USA) following manufacturer's recommendations and index codes were added. The
150 library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent
151 Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina HiSeq 2500 platform
152 and 250 bp paired-end reads were generated. Raw sequence data in FASTQ format are accessible
153 from the NCBI SRA with the number of SRP107086.

154 **2.7 Statistical and bioinformatics analysis**

155 Sequences analysis were performed by Uparse software (Uparse v7.0.1001，http:/
156 /drive5.com/uparse/) (Edgar 2013). Sequences with ≥97% similarity were assigned to the same
157 OTUs (Stackebrandt & Goebel 1994). Taxonomy was assigned to each OTU via the Ribosomal
158 Database Project (RDP) classifier (Cole et al. 2009). Representative sequence for each OTU was
159 screened for further annotation. OTUs abundance information were normalized using a standard
160 of sequence number corresponding to the sample with the least sequences. Subsequent analysis
161 of alpha diversity and beta diversity were all performed basing on this output normalized data.
162 Alpha diversity is applied in analyzing complexity of species diversity for a sample through 6
163 indices, including Observed-species, Chao1, Shannon, Simpson, ACE, Good-coverage. All these
164 indices in our samples were calculated with QIIME (Version1.7.0) and displayed with R software
165 (Version 2.15.3). Beta diversity analysis was used to evaluate differences of samples in species
166 complexity, Beta diversity on both weighted and unweighted unifrac were calculated by QIIME
167 software (Version 1.7.0).

168 Principal Coordinate Analysis (PCoA) was performed to get principal coordinates and

169 visualize from complex, multidimensional data. A distance matrix of weighted unifrac among
170 samples obtained before was transformed to a new set of orthogonal axes, by which the maximum
171 variation factor is demonstrated by first principal coordinate, and the second maximum one by the
172 second principal coordinate, and so on. PCoA analysis was displayed by WGCNA package, stat
173 packages and ggplot2 package in R software (Version 2.15.3). The linear discriminant analysis
174 effect size (LEfSe) method was to determine the difference between normal and double litter
175 amount treatments (Segata et al. 2011). Several statistical analyses were performed separately on
176 the soil property datasets using the statistical package for the social sciences (SPSS version 20.0
177 for Windows), including one-way ANOVA, Student's t-test, and S-K-N multiple range comparison
178 (P=0.05). The relationships between soil bacterial composition and the environmental factors were
179 tested using Pearson relation analyses using SPSS 20.0 for Windows.

180 **3. Results**

181 **3.1 Soil chemical properties and microbial biomass response to litter decomposition**

182 Soil nitrogen fractions, carbon fractions and soil moisture were summarized in Fig. 2. Soil
183 moisture showed a significant reduction in normal treatment and an increase in double treatment.
184 No significant differences were observed among the treatments for soil NH_4-N, which ranged from
185 5.39 to 5.73 mg/kg. Litter addition significantly altered other soil available properties. MBN
186 content was significant higher in normal treatment, with the range from 43.50 to 124.14 mg/kg,
187 and showed the order of normal>double >control. DON showed an opposite trend with MBN, with
188 a highest one for control treatment. Soil nitrate nitrogen ranged from 21.98 to 27.90 mg/kg, and
189 there was no significant difference between normal and control treatments. Litter decomposition

significantly affected soil carbon fractions. Control treatment had the highest MBC and lowest DOC, and significantly differed from double treatment. With the increase of litter quantity, soil nitrate nitrogen, soil moisture, MBC, DOC and DON showed a significant reduction in the normal treatment, and a significant increase for MBN.

3.2 Soil bacterial community activity response to litter decomposition

Litter decomposition had no significant effects on soil bacterial diversity (Table 1), but litter quantity had significant effect on soil bacterial community structure. The relative abundance of bacterial community at phylum and class levels was showed in Fig. 3. The dominant groups across all the soil samples at the phylum level were Proteobacteria, Actinobacteria, Acidobacteria, Gemmatimonadetes, Bacteroidetes, Chloroflexi, Firmicutes, Verrucomicrobia, Planctomycetes and Nitrospirae. At the phuylum level, litter decomposition had no significant effect on soil Proteobacteria, Acidobacteria and Gemmatimonadetes, with the range from 37.65 to 41.68%, 18.50 to 20.00%, 4.99 to 5.02%, respectively. Actinobacteria, Bacteroidetes, Planctomycetes, Firmicutes and Nitrospirae in normal treatment were significant higher than double and control treatments (Fig. 3-A).

To explore the dynamics of major microbial taxa under different litter mount treatments, we found that Aphpa-, Beta-, Gamma-, and Delta-proteobacteria were the main members of Proteobacteria. Only Aphpa-proteobacteria showed no significant differences among different treatments, with the range from 15.50 to 17.82 %. With the increase of litter quantity, the relative abundance of Beta-, Gamma-, and Deltaproteobacteria showed decreased at normal treatment, and increased at the double treatment. Beta-, Gamma-, and Deltaproteobacteria occupied 5.75%,

211 6.00%, and 6.93% for normal treatment, which significantly differed from double and control
212 treatment (Fig 3-B). At the order level, Subgroup_6 and Subgroup_4 were the dominant taxa in
213 the Acidobacteria phylum, and showed no significant changes with the increase of litter quantity.
214 Rhizobiales was the dominant taxa of Aphpa-proteobacteria, with the range from 7.01 to 8.75%,
215 and showed similar variation with Aphpa-proteobacteria. Solirubrobacterales, Xanthomonadales,
216 Sphingobacteriales, Myxococcales and Gaiellales indicated significant differences among the
217 litter addition treatments (Fig. 4). All these differences were only detected between normal
218 treatment and double or control treatment. The cluster analysis and PCoA also indicted these
219 changes (Fig. 3 and Fig. 5). More specify, the profiles of bacterial communities at normal treatment
220 trend to group together and were separated from those at double and control treatments. T-test
221 showed that soil bacterial taxa were significantly differed between normal and double treatment,
222 including Proteobacteria (Xanthomonadales, Salinisphaerales, Legionellales, Chromatiales,
223 Syntrophobacterales, Sh765B-TzT-29, Myxococcales, SC-I-84, Sneathiellales, DB1-14 and
224 Caulobacterales), Planctomycetes (WD2101_soil_group, Phycisphaerales, CCM11a),
225 Actinobacteria (Micrococcales, Solirubrobacterales, Rubrobacterales and Acidimicrobiales) (Fig.
226 5). These results showed double and control had similar bacterial community.

227 LEfSe analyses were performed to identify the significance of different abundant taxa and
228 biological relevance of the species in each litter quantity treatment. By using the LEfSe, we found
229 that Bacteroidetes, Myxococcales and Deltaproteobacteria were primarily changed in high-litter
230 treatment (double). The green color indicated the significantly varied taxa in the normal treatment,
231 and these species could potentially be used as biomarkers in normal quantity treatment (Fig. 6).

232 Pearson analysis also showed that soil moisture, DON and MBN were main affecting factors with
233 significant relation with bacterial taxa (Table 2). DON was significantly related with the relative
234 abundance of Actinobacteria, Bacteroidetes, Verrucomicrobia, Verrucomicrobia, Firmicutes and
235 Nitrospirae, with the coefficient of -0.684, 0.812, 0.679, 0.669, -0.804 and 0.715, respectively. SM
236 and MBN had similar relation with bacterial community composition (Table 2). There were no
237 significant relations with the relative abundance of Acidobacteria, Gemmatimonadetes and
238 Chloroflexi, as the stable abundance among different treatments.

239 **4. Discussion**

240 Plant litter decomposition was a key process of soil element recycle (Berg & Mcclaugherty
241 2014). In this study, soil organic carbon, soil total nitrogen contents were not significantly changed
242 (Fig. 2). This was not consistent with other litter decomposition experiment. As this study was a
243 short-time experiment, litter decomposition had no significant effects on the accumulation of soil
244 total C and N. Generally, soil total C and N storage was a long-time process with different
245 machismos. But soil available nutrients like nitrite nitrogen, dissolved nitrogen were changed.
246 Litter decomposition altered soil available N fractions (. i.e., MBN, DON and NO_3-N), providing
247 N resources for the growth of microbial organisms (Cleveland & Townsend 2006; Wardle et al.
248 2004). MBC and DOC also differed from different treatment. These changes revealed that soil
249 available C and N nutrients were sensitive to litter decomposition, which could be as an indicator
250 of estimating and evaluating the effects of litter decomposition under global climate change, N
251 deposition, extreme drought and other environmental problems.

252 Litter decomposition altered bacterial community composition with a greater degree in

normal quantity treatment than double treatment soils, but not for bacterial diversity (Shannon and Ace indices). Short-term litter decomposition increased the relative abundance of Actinobacteria, Firmicutes, Thermoleophilia, and decreased the relative abundance of Deltaproteobacteria, Gammaproteobacteria, Betaproteobacteria and Sphingobacteriia, most likely as a result of avilable C and N input via litter deposition caused by soil or litter microorganism (Cleveland & Townsend 2006; Wardle et al. 2004). Soil copiotrophic Bacteroidetes, α-, β-, and γ-Proteobacteria were relatively more abundant in the control and double quantity litter treatment soils. Available nutrients released by litter stimulated microbial production of extracellular enzymes (Koyama et al. 2013), resulting in increased C and N availability, which also in turn altered bacterial community composition. Zhang et al (Zhang et al. 2016) also observed that soil Proteobacteria increased with the the years of succession in the Loess Plateau grassland, as the soil nutrients were enhanced. 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 soil may help explain why the increase in litter input altered soil bacterial community composition in the normal and control treatment.

Bacteria played an important role in the process of litter decomposition. Most of Alphaproteobacteria, Acidobacteria and Actinobacteria could 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 the litter addition, soil bacterial community composition had changed. These changes were indicated between control and normal

274 treatment. From the cluster tree analysis, double and control treatment had similar bacterial
275 community (Fig. 2). There results were consistent with the results of LEefSe analysis and taxa
276 abundance. Based on the results of LEefSe analysis indicted that Gaiellaes, Solirubrobacterales,
277 Thermoleophilia, Alphaproteobacteria significantly varied in normal treatment, and
278 Shphingobacteria, Myxococcales and Deltproteobacteria significantly changed in double
279 treatment, which suggested that litter addition had significant effects on certain bacterial species.
280 These changes were also found in other researchers. Soil available nutrients may be main reason
281 caused by these shifts. Zhong et al (Zhong et al. 2015) found that N addition caused the changes
282 of soil bacterial and fungal communities in the long term field experiment.

283 SOC was another main factor affecting soil bacterial community composition. Liu found that
284 Actinobacteria was significantly positively related with SOC, Deltaproteobacteria was
285 significantly negatively related with SOC (Liu et al. 2014). However, similar results were not
286 observed in this study, which was in accord with the result of Zhong et al (Zhong et al. 2015). We
287 also found that soil total N had no significant effect on soil community structure, but soil available
288 N was significantly related with soil bacterial community. Soil available N as the main resource
289 of soil bacterial growth, caused the variation of soil bacterial community structure. Zhang et al
290 (Zhang et al. 2016) reported that soil nitrate nitrogen content significantly related with soil
291 bacterial community along a natural succession. Yao et al (Yao et al. 2014) found that soil
292 ammonium nitrogen content played an important role in affecting soil bacterial community
293 compositions in grass land soil of China. Yuan et al (Yuan et al. 2014) also observed similar results
294 in the Tibetan Plateau soil. All the results confirmed that soil available N content was the main

295 factor to drive these changes in the soil bacterial communities.

296 **5. Conclusion**

297 These results suggested normal litter quantity could altered soil bacterial community not for
298 double quantity litter. Double litter quantity had no effects on soil microbial community. Beta-,
299 Gamma-, and Delta-proteobacteria showed significantly decreased at the normal quantity litter
300 addition, and subsequently increased at the double quantity litter addition. Bacterial communities
301 transitioned from Proteobacteria-dominant (Beta-, Gamma-, and Delta) to Actinobacteria-
302 dominant during the litter decomposition with normal quantity. Soil available nutrients and soil
303 copiotrophic bacterial communities were higher in control and double quantity of litter
304 decomposition. These results suggested litter addition affected soil bacterial structure, providing
305 guide to manage vegetation restoration with the increase of litter quantity.

306 **Additional Information and Declarations**

307 **Competing Interests**

308 The authors declare that they have no competing interests.

309 **Author Contributions**

310 Quanchao Zeng, Yang Liu and Shaoshan An conceived and designed the experiments, performed
311 the experiments, analyzed the data, prepared figures and tables, reviewed drafts of the paper

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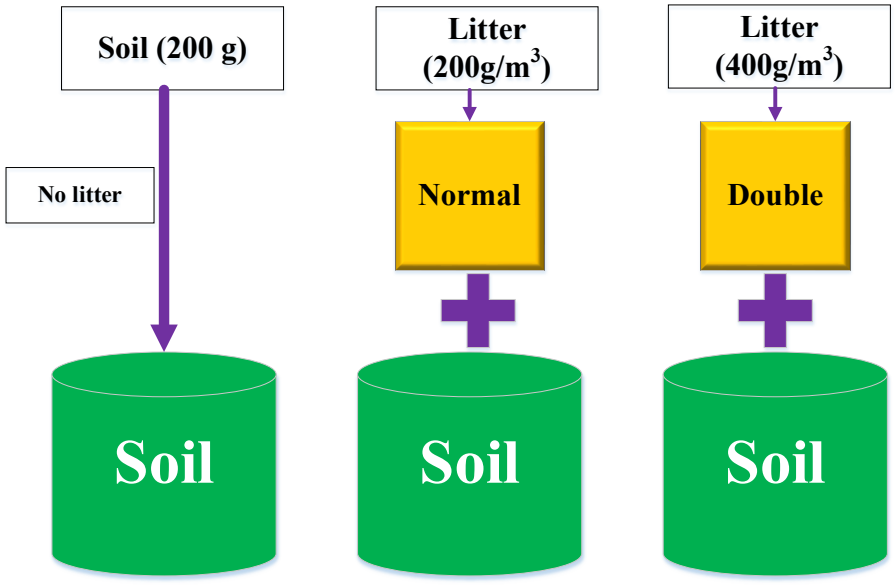
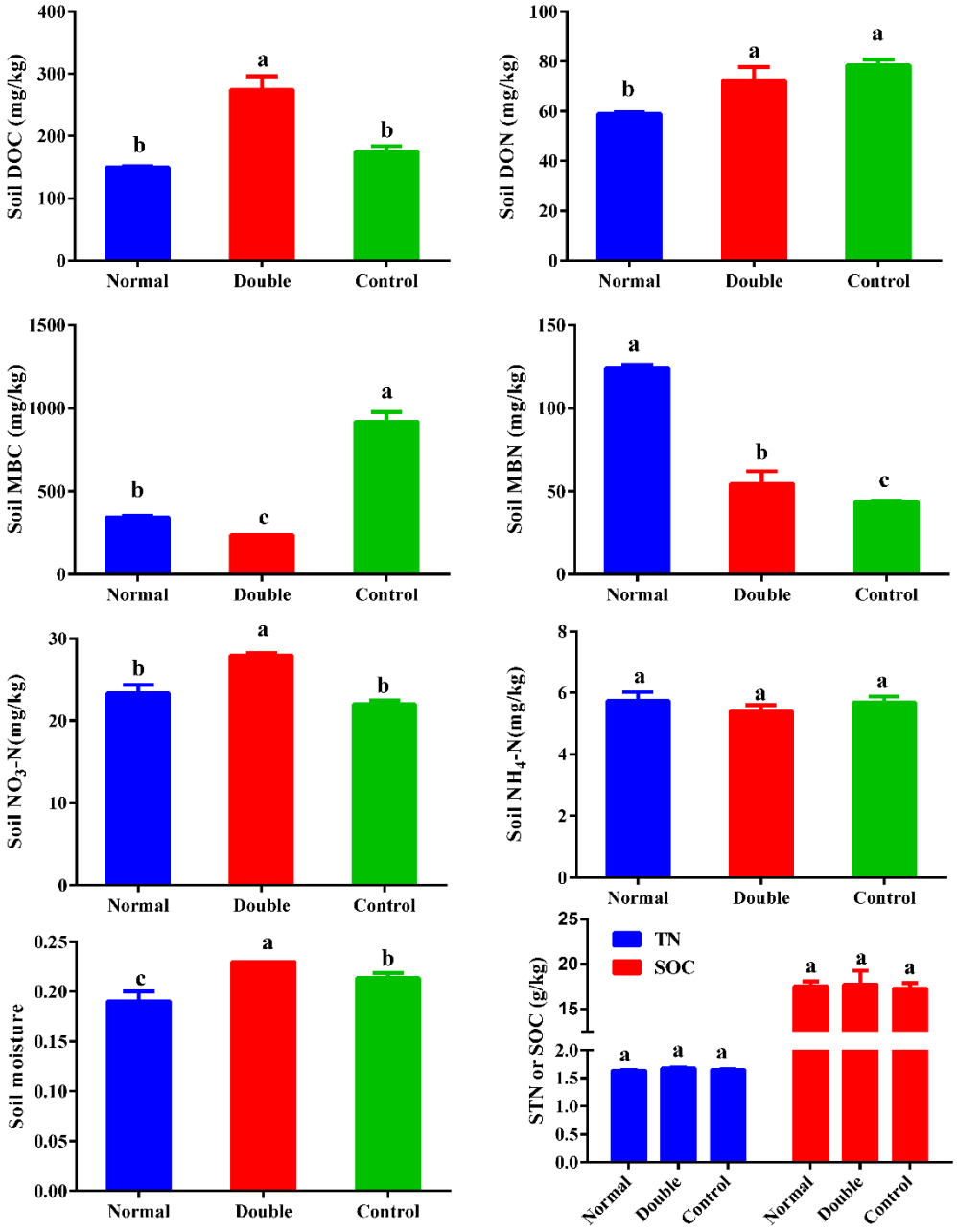


Fig. 1 The setup of litter decomposition experiment under different litter quantities.



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457 Fig. 2 Soil carbon and nitrogen fractions in the different treatments. Different lower case letter

458 indicated significant difference at the level of 0.05.

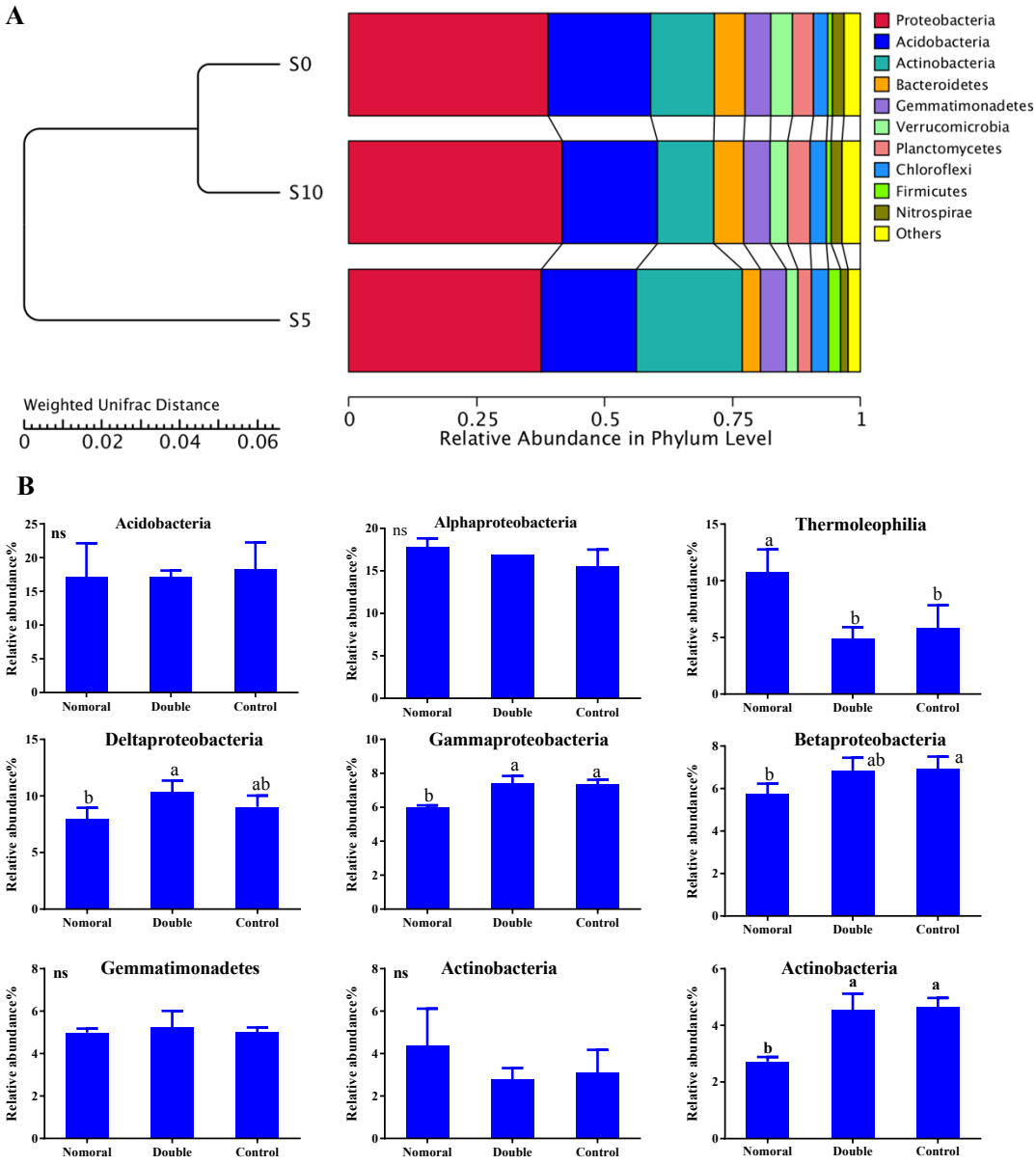


Fig .3 Soil bacterial communities under different litter quantity at the phylum level (A) and class level (B)

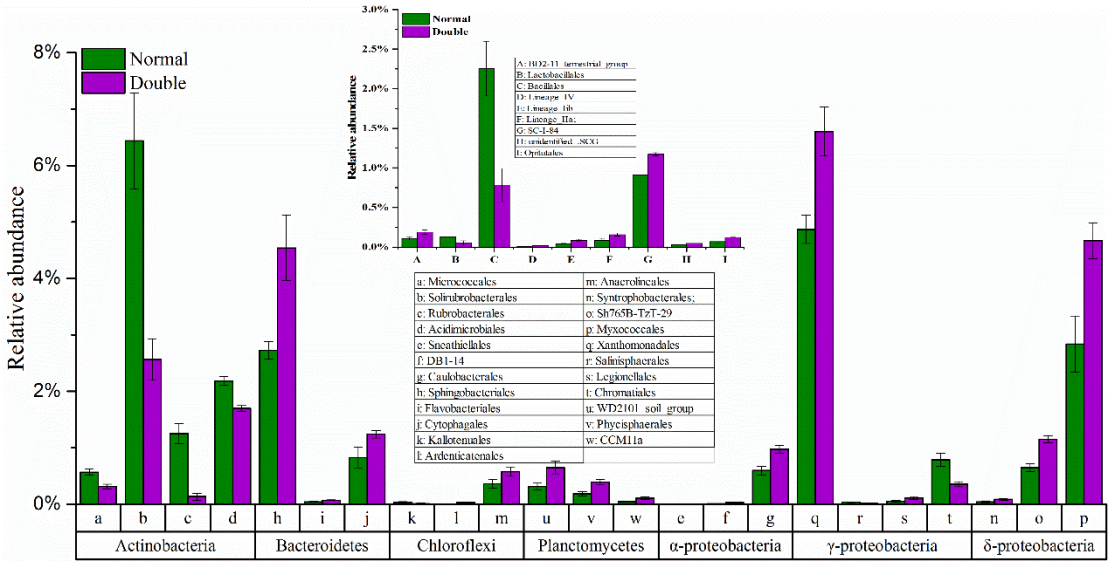
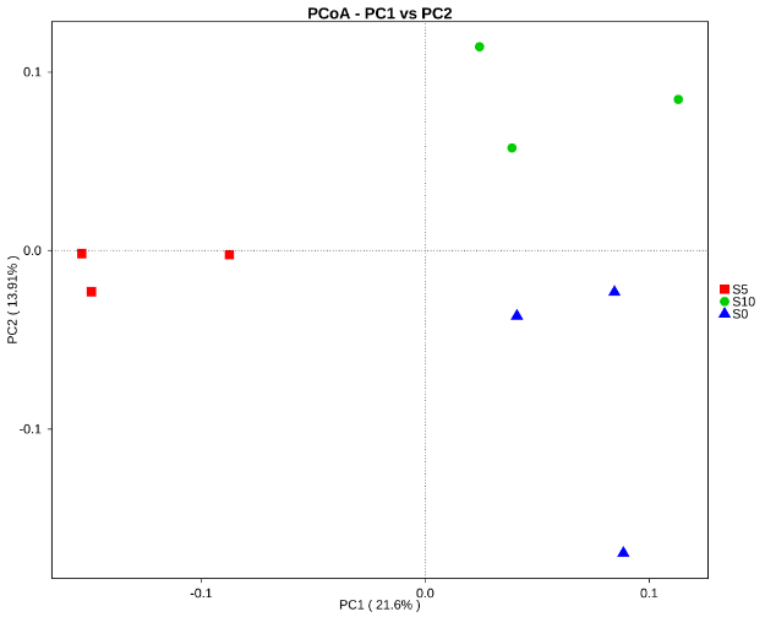


Fig. 4 The significantly different taxa between normal treatment and double treatment with T-test. The taxa showed in the figure were significant at the level of 0.05.



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479 Fig. 5 Principal coordinates analysis (PCoA) of soil bacterial community composition based on

480 Bray-Curtis distances

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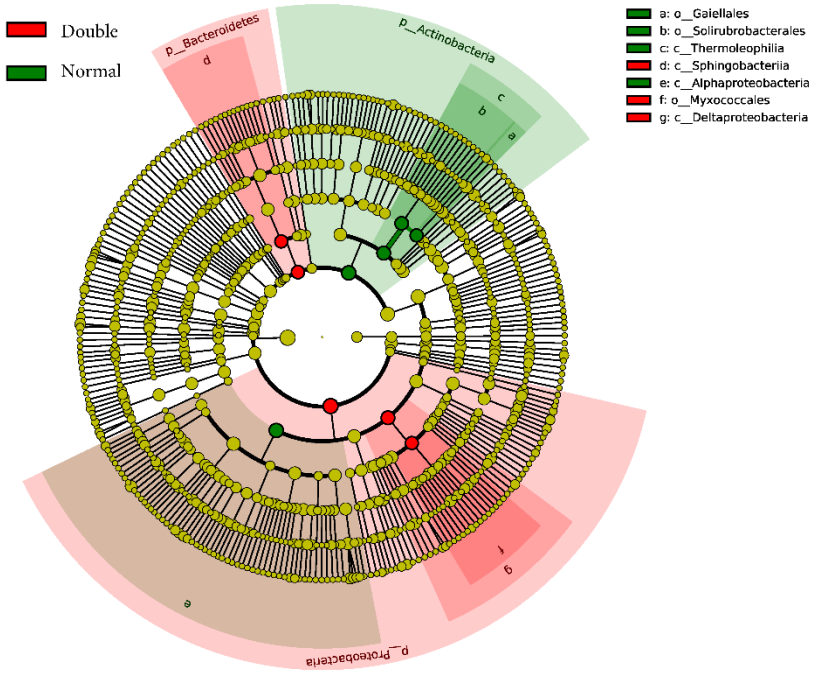

Fig. 6 A linear discriminant analysis effect size (LEsFe) method identifies the significantly different abundant taxa in bacteria under differ litter quantity treatment. Taxa with significantly different abundances among treatments are reprinted by color dots, and from the center outward, they represent the kingdom, phylum, class, order family and genus levels. The colored shadows represent trends of the significantly different taxa.

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

Treatment	Observed_species	Shannon	Simpson	Chao1	ACE	Goods_coverage	PD_whole_tree
Normal	3035±42	9.57±0.11	0.997±0	3512±2 72	3570±2 77	0.988±0	168.61±1.26
Double	2962±109	9.59±0.04	0.997±0	3258±1 70	3315±1 68	0.990±0	171.23±4.56
Control	2932±62	9.53±0.10	0.997±0	3244±7 3	3294±6 3	0.990±0	170.27±0.78

499 Note: All the indices were not significant under different treatments.

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516 Table 2 The relations between soil properties and 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
Acidobacteria	-0.080	0.118	0.21	-0.15	0.174	0.004	-0.204
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
Gemmatimonadetes	0.442	-0.153	-0.171	-0.066	0.174	0.249	-0.177
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
Chloroflexi	-0.028	-0.195	-0.527	0.289	-0.16	0.387	0.33
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

517 Note: DOC: dissolve organic carbon. DON: dissolve organic nitrogen. MBC: microbial biomass

518 carbon. MBN: microbial biomass nitrogen. SM: soil moisture. NO_3-N: nitrate nitrogen; NH_4-N:

519 ammonia nitrogen. * indicated significance at the level of 0.05, ** indicate significance at the

520 level of 0.01.

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