

1 Type of contribution: Regular paper

2 Date of preparation: August 25, 2018

3 Number of text pages: 36

4 Number of tables: 2

5 Number of figures: 5

6 **Changes in litter quality induced by N deposition alter soil microbial**
7 **communities**

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21

22 **Abstract**

23 Soil microbial community composition and litter quality are important drivers of litter
24 decomposition, but how litter quality influences the soil microbial composition largely
25 remains unknown. We conducted a microcosm experiment to examine the effects of
26 changes in litter quality induced by long-term N deposition on soil microbial
27 community composition. Mixed-species litter and single-species litter were collected
28 from a field experiment with replicate plots exposed to long-term N-addition in a
29 semiarid grassland in northern China. The litters were decomposed in a standard live
30 soil after which the composition of the microbial community was determined by
31 Illumina MiSeq Sequencing. Changes in litter stoichiometry induced by N-addition
32 increased the diversity of the fungal community. The alpha-diversity of the fungal
33 community was more sensitive to the type of litter (mixed- or single-species) than to
34 the N-addition effects, with higher abundance of fungal OTUs and Shannon-diversity
35 observed in soil with mixed-species litter. Moreover, the relative abundance of
36 saprophytic fungi increased with increasing N-addition rates, which suggests that fungi
37 play an important role in the initial stages of the decomposition process. Litter type and
38 N addition did not significantly change the diversity of bacterial community. The
39 relative abundance of ammonia-oxidizing bacteria was lower in high N-addition
40 treatments than in those with lower N input, indicating that changes in litter
41 stoichiometry could change ecosystem functioning via its effects on bacteria. Our

42 results presented robust evidence for the plant-mediated pathways through which N-
43 deposition affects the soil microbial community and biogeochemical cycling.

44 *Keywords:* N addition; Litter quality; Decomposition; Soil microbial community;
45 Illumina MiSeq Sequencing; Diversity.

46

47 **1. Introduction**

48 Plant litter inputs are important resources for soil organisms in terrestrial ecosystems.
49 Litter decomposition depends on litter quality and the composition of the decomposer
50 community (Hättenschwiler et al., 2005; van der Heijden, et al., 2008; Bardgett and
51 Wardle 2010). Litter chemical properties, particularly lignin and N-contents are
52 important controllers of litter decomposition that may explain up to 70% of the variation
53 in decomposition rates (Aerts, 1997; Zhang et al., 2008). Changes in soil microbial
54 community composition can also alter decomposition processes (de Boer et al., 2005;
55 van der Wal et al., 2013). For instance, only some specific taxa of fungi and bacteria
56 can decay lignin (Bugg et al., 2011; van der Wal et al., 2013; Brown and Chang, 2014)
57 and the abundance of these microbes in soils varies greatly at local scales (Leite et al.,
58 2017).

59 The number of fungal and bacterial species (richness) and their relative abundance
60 (evenness) are important characteristics of the soil microbial community (Nannipieri et
61 al., 2003; van der Wal et al., 2013). Several authors have argued that microbial diversity
62 is positively related to decomposition processes (e.g. Naeem et al., 2000; McGuire et

63 al., 2010). Increased microbial diversity generally leads to a more efficient use of
64 organic substrates because of greater functional exploitation (Loreau, 2001;
65 Hättenschwiler et al., 2011). Competitive interactions between species within a
66 community can also lead to negative relationships between microbial diversity and litter
67 decomposition (Fukami et al., 2010; Nielsen et al., 2010; Song et al., 2012;). How the
68 diversity of the soil decomposer community responds to variation in litter quality within
69 one grassland soil is less well understood (Zak et al., 2003; Strickland et al., 2009).

70 Litter mixtures may have different nutrient contents depending on the composition
71 of plant species making up the mixture and may provide different substrate qualities or
72 chemical compounds to the microbial community decomposing this litter (Hooper et
73 al., 2000; Pei et al., 2017). Hence, a higher diversity in litter characteristics,
74 representing more diverse substrates for decomposition, may lead to higher microbial
75 diversity than litter from a single plant species (Hu et al., 2006; Szanser et al., 2011).
76 Shihan et al. (2017) found that enhanced litter species richness increased the catabolic
77 diversity of the soil microbial community. How the diversity of the soil decomposer
78 community responds to the changes of litter composition is less well understood.

79 Litter quality is a broad term that includes chemical variables such as energy source
80 (C or lignin contents), and nutrients content (e.g. N and P) and their ratios (C/N, C/P,
81 N/P, and lignin/N) (Cadisch and Giller, 1997). It can affect not only the diversity of the
82 microbial community, but also the relative abundance of specific taxa that are involved
83 in the decomposition process. Bacteria and fungi in the soil differ greatly in growth

84 strategies, competitiveness and in how they use resources, and hence the quality of litter
85 can influence soil microbial community composition (Schneider et al., 2012; Kaiser et
86 al., 2014). Fungi have lower nutrient requirements than bacteria and exhibit a high
87 carbon-use efficiency on poor-quality substrates (Six et al., 2006; Keiblinger et al.,
88 2010), with the capacity to degrade more recalcitrant substrates such as lignin (McGuire
89 et al., 2010). Most enzymes that can degrade recalcitrant C-substrates are secreted by
90 fungi such as the ones that belong to saprobes (Schneider et al., 2012). As a result, the
91 relative abundance of fungi may decrease with increases of litter quality, especially for
92 saprobes fungi. Bacteria often have fast growth and turnover rates, contain higher
93 amounts of N, P and organic compounds and favor low substrate C/N ratios (Güsewell
94 and Gessner, 2009; Kaiser et al., 2014). Increased quality of resources would increase
95 substrate availability to the microbial community, increase rates of N transformations
96 by higher available organic N stocks for N mineralization, and coincide with an increase
97 in the relative abundance of microbial functional groups that cycle N (Wieder et al.,
98 2013). Thus, increases in the quality of resources can change bacterial composition e.g.
99 via increasing the relative abundance of bacteria related to N-cycling. Although
100 previous studies have shown that soil microbial communities can shift in response to
101 resource quality, how specific microbial taxa or lineages responses to decomposition of
102 different types of litter is not clear.

103 With the rapid increase of fossil fuel combustion and agricultural practices, human
104 activities have dramatically increased the deposition of reactive N (Galloway et al.,

105 2008; Gruber and Galloway, 2008). Since most of the terrestrial ecosystems are N
106 limited, N-enrichment resulting from N-deposition has greatly changed ecosystem
107 processes, structure, and functioning (Elser et al., 2007; Pierik et al., 2011). N-
108 deposition could reduce plant species richness and alter community composition in
109 grasslands (Bai et al., 2010; Bobbink et al., 2010; Pierik et al., 2011). Furthermore, N-
110 deposition would enhance litter quality by increasing litter N concentration and
111 decreasing C/N ratios (Henry et al., 2005; Han et al., 2014). Therefore, N-deposition
112 can influence decomposition processes through altering both litter composition and
113 intra-specific chemical quality (Hobbie, 2005; Knorr et al., 2005).

114 To evaluate how changes in litter quality following N-deposition would influence
115 soil microbial communities, we conducted a microcosm experiment. Mixed-species and
116 single-species litters were collected from a long-term experiment with different N-
117 addition levels in a semiarid grassland in northern China. After four months of
118 decomposition on a standard soil in microcosms, soil samples were collected, and the
119 bacterial and fungal community was analyzed by sequencing. Specifically, we address
120 the following hypotheses: (i) decomposer communities exposed to mixed-species litter
121 will exhibit higher diversity than those exposed to single-species litter; (ii) higher litter
122 quality following long-term N deposition will increase microbial community diversity
123 (higher richness or evenness); (iii) increased litter quality will change the composition
124 of the microbial community, with a decrease in the relative abundance of saprophytic
125 fungi, and an increase in the relative abundance of bacteria related to N-cycling.

126 **2. Materials and methods**

127 *2.1 Litter and soil collection*

128 Litter and soil samples were collected from a long-term N-addition experiment
129 conducted in a natural steppe ecosystem near the Inner Mongolia Grassland Ecosystem
130 Research Station (IMGERS, 116°14'E, 43°13'N) of the Chinese Academy of Sciences.
131 The mean annual temperature was 0.9 °C, with mean monthly temperatures ranging
132 from -21.4 °C in January to 19.7 °C in July (mean temperature of May to September is
133 around 16°C). The mean annual precipitation is 355.3 mm, with 60%-80% falling
134 during the growing season (May to August). According to the FAO (Food and
135 Agriculture Organization of the United Nations) classification system, the soil is
136 classified as Haplic Calcisol. The perennial rhizomatous grass *Leymus chinensis* (Trin.)
137 Tzvel. and the perennial bunchgrass *Stipa grandis* P. Smirn account for more than 60%
138 of the total aboveground biomass in the plant community. More information about the
139 plant species in this experiment can be found in Zhang et al. (2014b) and Zhang et al.
140 (2017). This area had received no fertilizer before the experiment started and ambient
141 total N-deposition is less than 1.5g N m⁻² yr⁻¹ (Lue and Tian, 2007).

142 The long-term N-addition experiment was established in September 2008. There
143 were nine of N-addition levels (0, 1, 2, 3, 5, 10, 15, 20, 50 g N m⁻² yr⁻¹) applied at two
144 frequencies (2 times and 12 times per year). For the present study, seven N-addition
145 levels (0, 2, 5, 10, 15, 20, 50 g N m⁻² yr⁻¹, 2 times per year) were selected. Hereafter,
146 the N-addition treatments will be denoted as: N₀, N₂, N₅, N₁₀, N₁₅, N₂₀, and N₅₀. To

147 mirror the seasonal pattern of natural N-deposition, in June, NH_4NO_3 was mixed with
148 purified water (9.0 L per plot; the N_0 treatment received only purified water) and
149 sprinkled evenly using a sprayer to each plot to simulate wet deposition. In November,
150 NH_4NO_3 was mixed with clean sand (0.5 kg sand per plot; the N_0 treatment received
151 only sand) and broadcasted evenly by hand to simulate dry deposition. The experiment
152 was designed according to a randomized block design with 10 replicate blocks; each
153 block was 45×70 m. The blocks were separated by 2-m walkways. There were nine
154 plots treated with nine N-addition levels in each block. Each plot measured 8×8 m,
155 and plots were separated by 1m walkways. We randomly selected five blocks for litter
156 collection (7 N treatments \times 5 replicate blocks = 35 plots).

157 At the end of September 2014, when most of the aboveground plants material had
158 senesced, we collected litter from each treated plot and soil with no N-additions (as
159 standard soil) from outside the plots but within the fence. Litter was collected in two
160 ways, mixed species litter and litter from one dominant species (*L. chinensis*), hereafter
161 called ‘mixed-species litter’ and ‘single-species litter’. To obtain a representative and
162 homogenous litter sample for the mixed-species litter treatment in each plot, litter was
163 sampled by clipping 2 cm above soil surface in three randomly quadrats (15×15 cm).
164 The three quadrats were separated by at least 50 cm and the samples collected from one
165 plot were merged and homogenized. Senesced plant material of *L. chinensis* was
166 clipped at 2 cm above soil surface and collected throughout the plot. Since soil biotic
167 and abiotic characteristics in the experiment plots were significantly affected by N-

168 addition treatments (Zhang et al., 2014a). Standard soil was collected from an area
169 inside the fence of the experimental area but outside the experimental plots to eliminate
170 the effect of N addition. Therefore, the standard soil had not received additional N.
171 After removal of the litter layer, thirty $10 \times 10 \times 10$ cm soil blocks (at least 50 cm apart)
172 were dug out using a spade and then placed individually in plastic bags.

173 Litter and soil were transported to the laboratory within 3 days after collection. In
174 the laboratory, the litter samples were divided into two parts. One part was oven-dried
175 at 60 °C for 48 h to constant weight and then clipped into fragments of 1 cm in length
176 for the decomposition experiment. The other part was oven-dried at 40 °C for 48h for
177 chemical analysis (See below). All soil blocks were passed through a 5-mm sieve and
178 then homogenized thoroughly to one composite sample.

179 *2.2 Microcosm experiment*

180 We tested how the addition of the two types of litter from seven N-addition treatments
181 influenced the composition of the soil microbial community in a microcosm experiment.
182 To eliminate spatial variation in microclimate and soil heterogeneity, we used a
183 microcosm approach. We constructed 70 microcosms (7 N treatments \times 5 replicate
184 blocks \times 2 litter types) in plastic containers (10 cm diameter, 10 cm height). The
185 containers were filled with 400g soil ensuring a bulk density of 1 g cm^{-3} to resemble
186 the situation in the field. The depth of the soil layer in each container was 6 cm. Soil
187 water content was determined by oven-drying subsamples and soils were adjusted to
188 20% soil moisture with distilled water. Microcosms were incubated at constant

189 temperature conditions (10 °C at night and 20 °C at daytime) in the laboratory to
190 simulate the temperature of May to September in field (mean temperature around 16
191 °C) when microbial activity is strongest, and most of the decomposition is occurring.
192 After ten days, 4.0 g litter (1 cm in length) was evenly mixed into the 1 cm surface layer
193 of the standard live soil. A non-transparent perforated plastic film was used to cover
194 each microcosm to reduce light availability and water loss. Soil moisture in each
195 microcosm was maintained by weighing and adding distilled water once every three
196 days. After 120 days, the 1 cm surface layer of the soil was collected, passed through a
197 2-mm sieve to remove large pieces of litter, but smaller pieces of partly decomposed
198 litter went through the mesh of the sieve and hence this litter was included during DNA
199 extraction. Then the soil was stored at -80 °C to be used later for DNA extraction (See
200 below). Since the litter was partly decomposed and soil and humus material attached to
201 the litter could not be removed, we did not measure litter quality at the end of the
202 experiment.

203 *2.3 Chemical analyses of litter samples*

204 Litter samples were ground and passed through a 0.25 mm sieve for chemical analyses.
205 Total C (TC) was determined using an elemental analyzer (Jena Corporation, Germany).
206 Total N (TN) was measured using the modified Kjeldahl method (ISO, 1995). Total P
207 (TP) was measured colorimetrically after reaction with molybdenum blue. Lignin
208 content was fractionated into acid insoluble material and acid soluble material by
209 sulfuric acid hydrolysis. The acid insoluble material was determined by Muffle furnace

210 (Ney Vulcan, USA). The acid soluble material was measured by UV-Vis spectroscopy
211 TU-1901 (Purkinje General Instrument Ltd., China). Litter C/N, C/P, N/P, Lignin/N
212 and Lignin/P were calculated using TC, TN, TP and lignin dataset.

213 *2.4 DNA isolation, amplification and illumina Miseq sequencing*

214 Microbial genomic DNA was extracted from 0.5g soil using the FastDNA SPIN Kit for
215 Soil (MP Biomedicals LLC. Solon, OH, USA) according to the manufacturer's
216 instructions. The quality and concentration of the extracted DNA was quantified based
217 on 260/280 nm and 260/230 nm absorbance ratios measured by NanoDrop ND-2000
218 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). To
219 determine the soil bacterial and fungal community composition and diversity, an
220 amplicon survey of the 16S and ITS rRNA was implemented. The V4 hypervariable
221 regions of 16S rRNA gene was amplified using the 515F (5'-
222 GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3')
223 primer set. The primers ITS1F (5'-GTGCCAGCMGCCGCGG-3') and 2043R (5'-
224 GCTGCGTTCTTCATCGATGC-3') were used to amplify the ITS1 region of the
225 fungal rRNA. Both primers were tagged with an adaptor, a pad, and a linker, and a
226 unique barcode sequence to each sample.

227 Each sample was amplified in triplicate using a Gene Amp PCR-System 9700
228 (Applied Biosystems, Foster City, CA, USA) in a 20 µl reaction system containing 4 µl
229 5×FastPfu Buffer, 0.5 unit of TransStart FastPfu DNA Polymerase (TransGen Biotech
230 Co. Ltd. Beijing, China), 2 µl of 2.5 mM dNTPs, 0.8 µl of 5 µM forward and reverse

231 primer, and 10 ng template DNA. 16S rRNA thermal cycling conditions were as follows:
232 an initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturation at 95 °
233 C for 30 s, 55 ° C for 30 s, 72 ° C for 45 s, and a final extension at 72 ° C for 10 min.
234 ITS rRNA thermal cycling were performed in the same way, except that 32 cycles of
235 denaturation were used. After amplification, 3 µl of the PCR product was used for
236 agarose gel (2%) detection. The triplicate PCR reactions for each sample were
237 combined and quantified with PicoGreen. Then equimolar amounts of the PCR product
238 were pooled together and purified with an AxyPrepDNA gel extraction kit (Axygen
239 Scientific, Union City, CA, USA). Sequencing was performed on 300PE Miseq
240 sequencer at the Majorbio Bio-Pharm Technology Co. Ltd. Shanghai, China.

241 *2.5 Processing of molecular data*

242 The sequences were trimmed, merged and assigned in QIIME v 1.7.0. Low quality
243 sequences (< 200bp in length with an average quality score < 20) were removed. After
244 removing the chimera, high quality sequences were clustered into Operational
245 Taxonomic Units (OTUs) at a 97% similarity. A representative sequence was aligned
246 using the Python Nearest Alignment Space Termination (PyNAST) against sequence
247 within the Sliver database for bacteria and Unite database for fungi. To correct for
248 sampling effects on diversity, sequence numbers of each sample were rarified to the
249 sample with the lowest number of reads (15000 reads for bacteria and 20000 reads for
250 fungi).

251 *2.6 Statistical analysis*

252 Four alpha-diversity indices, the number of OTUs, the Chao1 estimator of richness,
253 Shannon diversity, and Shannon evenness, were calculated in QIIME (Caporaso et al.,
254 2010). Significant differences among N-addition levels were tested separately for each
255 litter type by one-way analysis of variance (ANOVA) followed by a Tukey HSD test.
256 The relationships between alpha-diversity indices and litter quality parameters were
257 tested with a Spearman correlation analysis using SPSS 19.0. Bacterial and fungal
258 community composition was visualized by non-metric multidimensional scaling
259 (NMDS) plots based on the Bray Curtis similarity matrix. Adonis analysis was used to
260 determine whether bacterial and fungal community structure were significantly
261 influenced by N-addition level and litter type. The significance of the relationship
262 between community similarity and litter quality dissimilarity (Euclidean distance based
263 on nine litter quality parameters) for each litter type was analyzed using a Mantel test
264 (Spearman's rank correlation). The Bray-Curtis similarity between samples, based on
265 OTUs, was used to compare the bacterial and fungal community similarity (1-Bray-
266 Curtis distance). A distance-based RDA (db-RDA) was used to examine the effect of
267 each litter quality parameter on the bacterial and fungal community (based on OTUs
268 composition) in CANOCO Version 5.0 (Plant Research International, Wageningen,
269 The Netherlands). Two-way ANOVA was used to test the effects of litter type (mixed-
270 and single-species), N-addition level and their interaction on bacterial and fungal
271 phylogenetic taxa (Phylum, Class, Order and Family), litter quality and alpha diversity.

272 **3. Results**

273 3.1 *Effect of N-addition on litter quality*

274 Both litter type and N-addition rate significantly affected litter quality (Table S1 and
275 Fig. 1). The concentrations of total C and lignin, and the ratios of C/N, C/P, Lignin/N,
276 Lignin/P were higher in mixed-species litter than in single-species litter, whereas the
277 concentrations of total N and P were higher in single-species litter than in mixed-species
278 litter. For both single-species and mixed-species litters, the concentration of total N, P
279 and the ratio of N/P increased, and the ratios of C/N, C/P, Lignin/N and Lignin/P
280 decreased with increasing N-addition rates. There was no significant variation of total
281 C and lignin across different N-addition rates (Fig. 1).

282 3.2 *Bacterial and fungal alpha-diversity*

283 Neither N-addition rates nor litter type significantly affected bacterial alpha-diversity
284 (Fig. 2). Higher number of fungal OTUs, Chao1 estimator and diversity (H') were
285 observed in soil with mixed-species litter than in soil with single-species litter, whereas
286 the Shannon evenness index was higher in soil with single-species litter (Fig. 2). N-
287 addition significantly affected fungal diversity. For mixed-species litter, the Shannon
288 diversity of the fungal community slightly increased with increasing N-addition rates
289 and the number of fungal OTUs was positively correlated with the litter N/P ratio, and
290 negatively correlated with the C/N and Lignin/N ratios. The fungal Chao1 estimator
291 was negatively correlated with the Lignin/N ratio for mixed-species litter treatments.
292 For single-species litter, the number of fungal OTUs and the Chao1 estimator were
293 negatively correlated with the total C content of the litter, and the Shannon diversity

294 and evenness indices were negatively correlated with the C/N ratio (Table S2). For the
295 bacterial community, we only detected a significant negative correlation between the
296 Shannon evenness index and the total P of the litter in soil with single-species litter
297 (Table S2).

298 *3.3 Bacterial and fungal composition*

299 The NMDS plot revealed a clear separation between samples collected from mixed-
300 species litter and single-species litter treatments (Fig. 3). The distribution of bacterial
301 communities was not clearly separated by N-addition rates (Fig. 3 a). Results from
302 Adonis analysis also showed that soil bacterial community composition was
303 significantly influenced by litter type ($F=4.37$, $P<0.01$), but not by N-addition rate
304 ($F=1.60$, $P=0.17$).

305 For fungal communities, the samples from the two types of litter were clearly
306 separated by the first axis of the NMDS plot, while the second axis differentiated the
307 samples with litter from low N-addition levels (N_0 to N_{10}) and higher N-addition levels
308 (N_{15} to N_{50}) (Fig. 3b). Results from Adonis analysis showed that the fungal community
309 composition was significantly affected by N-addition rate ($F=1.60$, $P<0.01$) and litter
310 type ($F=15.89$, $P<0.01$).

311 There was no relationship between similarity of bacterial communities and similarity
312 in litter quality (Fig. 4a). The similarity of the fungal communities was negatively
313 correlated with litter quality distance (Fig. 4b). Distance-based RDA analysis based on
314 OTUs composition indicated that bacterial communities in both litter types were not

315 related to the litter characteristics. In contrast, fungal communities for both types of
316 litter were significantly correlated with total N and total P, and the ratios of C/N, C/P
317 and N/P of the litter (Table 1).

318 Across all the treatments, the dominant bacterial phyla with relative abundance > 1%
319 were: *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, *Planctomycetes*,
320 *Chloroflexi*, *Nitrospirae*, and *Gemmatimonadetes* (Fig. 5a). The dominant fungi classes
321 (relative abundance > 1%) were: *Zygomycota* (incertae sedis), *Sordariomycetes*,
322 *Dothideomycetes*, *Agaricomycetes* and *Eurotiomycetes* (Fig 5b). None of the relative
323 abundances of phyla or classes taxa were affected by N-addition level for both bacteria
324 and fungi. At the order level, the relative abundance bacteria *Nitrosomonadales*,
325 *Rhodospirillales* and fungi *Pleosporales* were significantly affected by N-addition level
326 (Table 2). The relative abundance of *Nitrosomonadales* was higher in the mixed-species
327 litter treatments than in the single-species litter treatments (Fig. 5c). Conversely, the
328 relative abundance of *Pleosporales* was lower in mixed-species litter and increased with
329 increasing N-addition levels (Fig. 5d). The bacterial families *Nitrosomonadaceae*,
330 *Oxalobacteraceae*, *Rhodospirillaceae* and fungal families *Pleosporaceae*,
331 *Hypocreaceae*, *Bionectriaceae* were significantly affected by N-addition (Table 2).
332 Pearson's coefficients revealed a significant correlation between those taxa and litter
333 quality properties (Fig S1). The relative abundance of order *Nitrosomonadales* and its
334 family *Nitrosomonadaceae* were negatively related to the litter N and P concentrations,
335 and positively correlated with litter C/N, C/P ratios and lignin content. *Rhodospirillales*

336 and *Rhodospirillaceae* were most closely related to litter lignin content. In contrast, the
337 fungal order *Pleosporales* and family *Pleosporaceae* were positively related to litter N
338 and P concentrations. Family *Bionectriaceae* was negatively related to litter lignin
339 content (Fig S1).

340 **4. Discussion**

341 *4.1 Litter quality changed fungal diversity but not bacterial diversity*

342 In agreement with our first hypothesis, our results showed that fungal diversity was
343 higher in soils with mixed-species litter than in soil with single-species litter. Mixed-
344 species litter can improve the substrate heterogeneity and therefore create a more
345 complex and beneficial environment for decomposers (García-Palacios et al., 2013).
346 Further, litter species traits can physically change the litter surface area in diverse
347 mixtures and increase the number and diversity of microhabitats (Hector et al., 2000;
348 Pei et al., 2017). Our study emphasizes the importance of litter species characteristics
349 for fungal communities. A recent study in the same Long-term N addition experiment
350 found that the litter quality of seven dominant species was consistently increased with
351 N-addition rates (Hou et al., 2018). This suggests that the effects of N-addition via
352 changing litter quality on the soil microbial community, that we observed in our single-
353 species treatment, which unfortunately due to practical reasons could only be done with
354 one plant species, may be extrapolated to other grassland species. However, further
355 studies are needed to confirm the generality of litter changes via N-addition effects on
356 soil microbial communities. Theoretically, fungi are more strongly influenced by

357 substrate quality than substrate heterogeneity (Cadisch and Giller, 1997). In this study,
358 N-addition slightly increased the fungal Shannon index, only in the mixed-species litter
359 treatments. Our results show that fungal alpha-diversity appears to be more sensitive to
360 the litter heterogeneity by mixing species than to the litter quality changed by N-
361 addition.

362 Fungal community similarity (beta-diversity) was significantly correlated with litter
363 quality distance in both litter type treatments, and the sampling points in the fungal
364 ordination plot, clearly separated between the low and high levels of N-addition in both
365 litter treatments. This confirms that heterogeneity in litter quality alters the composition
366 of the fungal community. Fungal beta-diversity, generally, is strongly related to the less
367 abundant operational taxonomic units (OTUs) in the community (Carvalho et al., 2016).
368 Therefore, changes in litter quality induced by long-term N-addition may have a greater
369 impact on rare than on dominant species in the fungal community. Our results show
370 that changes in litter quality can evoke bottom-up effects in the fungal community.
371 Fungi are key players during litter decomposition due to their ability to degrade
372 recalcitrant compounds such as lignin and cellulose with extracellular enzymes
373 (Schneider et al., 2012). Further studies should examine how N-driven changes in
374 fungal community composition influence ecosystem processes.

375 We found that bacterial diversity did not change in response to variation of litter
376 quality and litter species composition. How bacteria respond to litter quality is poorly
377 understood, although some studies have shown a relationship with changes in litter

378 quality during decomposition (Cadisch and Giller, 1997; Dilly et al., 2004). It is
379 generally considered that bacteria are more sensitive than fungi to alteration of nutrient
380 availability because they have much shorter turnover times than fungi and react faster
381 to changes in soil nutrients (Yin et al., 2010). In our study, the relatively short duration
382 of litter decomposition may have limited effects on soil nutrients. Sun et al. (2015)
383 found that incorporation of wheat straw into soil did not change the composition of
384 bacterial communities. Furthermore, bacterial populations are largely regulated by
385 predation, and this implies that the nature of resources that are available will less likely
386 affect bacterial communities (Wardle et al., 1995; Cadisch and Giller, 1997). Although
387 bacterial community composition did not change significantly during the initial stages
388 of decomposition, in the longer term, litter quality may still influence bacterial
389 communities (Keiser et al., 2011; Kaiser et al., 2014). Decomposer communities can be
390 specialized to break down the locally available plant litter, leading to a ‘home-field
391 advantage (HFA)’ effect (Ayres et al., 2009; Keiser et al., 2013). Recently we showed
392 that long-term N-deposition in grasslands influenced litter HFA effects and that these
393 effects differed between single-species and mixed-species litters (Li et al., 2017). The
394 substantial changes in fungal communities but not in bacterial communities, as found
395 in this study, imply that fungi may play important roles in these HFA effects during the
396 initial stage of decomposition.

397 *4.2 Effect of litter quality on bacterial and fungal community composition*

398 There are many mechanisms for the responses of microbial decomposer to the large
399 complexity and variability of substrates quality (Fanin et al., 2013; Mooshammer et al.,
400 2014), such as adjust extracellular enzymes production (Sinsabaugh et al., 2008;
401 Moorhead et al., 2012) and element use efficiencies (Manzoni et al., 2012; Kaiser et al.,
402 2014). In addition, hyphae of saprophytic fungi have been shown to often extend
403 beyond the resource that they decompose (Strickland and Rousk, 2010) and to mediate
404 nutrient import from poor-nutrient patches to rich-nutrient patches (Chigineva et al.,
405 2011). Saprophytic fungi thereby can facilitate decomposition by supporting bacterial
406 decomposer communities at site where elements are lack. We observed a significant
407 increase in the relative abundance of *Pleosporales*, the largest order in the fungal class
408 *Dothideomycetes*. Most of these species are saprobes and live on decaying plant
409 material (Zhang et al., 2009). The increase in saprophytic fungi supports the important
410 role of fungi in decomposition processes, especially in the decomposition of high-
411 quality litter.

412 Bacteria belonging to the family *Nitrosomonadaceae* were negatively correlated with
413 litter N contents. Members of the genus *Nitrosomonas* oxidize ammonia to nitrite, a
414 process known as nitrification. The majority of the N-demand in an ecosystem is met
415 via internal N recycling through litter decomposition, mineralization, and assimilation
416 (Likens, 2013). Increased litter N content inputs would increase soil N pool and
417 associated rates of soil N transformations and availability (Wieder et al., 2013). Thus,
418 increasing N availability via litter inputs could also increase the abundance of

419 ammonia-oxidizing bacteria, and accelerate NO_3^- production. Unexpectedly, our results
420 did not support our third hypothesis. Wieder et al. (2013) reported that doubling leaf
421 litter inputs into the soil decreased the gross nitrification rates, and the relative
422 abundance of ammonia-oxidizing microorganisms. Augmenting litter N-inputs to soil
423 exacerbated the stoichiometric imbalance between microbes and their resources
424 (Mooshammer et al., 2014). Furthermore, ammonia-oxidizing bacteria are generally
425 worse competitors for ammonia than heterotrophic microbes (Gerards et al., 1998). This
426 may explain why the relative abundance of *Nitrosomonadaceae* was lower in high N-
427 addition treatments. Ammonia-oxidizing bacteria play an essential role in nitrogen
428 transformation and related processes during litter decomposition (Carey et al., 2016).
429 The decreased relative abundance of *Nitrosomonadaceae* suggests that the
430 biogeochemical consequences of N-deposition in grasslands may be influenced by the
431 quality of the litter that is produced and its effect on bacterial-mediated ecosystem
432 functions.

433 **5. Conclusions**

434 Our study shows that changes in litter quality following N-deposition alters soil fungal
435 community diversity in our grassland soils but that it had no significant impacts on soil
436 bacterial diversity. Our results, therefore, suggest that changes in litter quality and
437 species composition can drive specialization in fungal communities, at least at the initial
438 stage of decomposition. Increased saprophytic fungi and decreased ammonia-oxidizing
439 bacteria with the enhancement of litter quality suggest that N-deposition via its effects

440 on plant composition and nutritional quality, can also impact ecosystem functions such
441 as decomposition through litter quality mediated changes in the microbial community.
442 This may have a profound influence on the biogeochemical cycling in terrestrial
443 ecosystems.

444

445 **Acknowledgements**

446 This research was supported by Strategic Priority Research Program of the Chinese
447 Academy of Sciences (XDB15010402), the National Natural Science Foundation of
448 China (31570519, 3143000565, and 31400370), the Chinese Academy of Sciences
449 Visiting Professorship Program for Senior International Scientists (2017VCA0004)
450 and Youth Innovation Promotion Association CAS (2014174).

451

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677 **Table. 1** The influence of litter chemical characters on bacterial and fungal community
 678 determined by distance-based redundancy analysis (Bray-Curtis distance).

Litter	Bacterial community				Fungal community			
	Mixed-species litter		Single-species litter		Mixed-species litter		Single-species litter	
	Explained %	<i>P</i>	Explained %	<i>P</i>	Explained %	<i>P</i>	Explained %	<i>P</i>
TC	2.3	0.41	4.1	0.23	1.4	0.80	4.0	0.40
TN	4.7	0.16	5.0	0.17	16.1	<0.01	16.8	<0.01
TP	3.1	0.31	5.8	0.13	9.0	0.02	12.0	<0.01
C:N	4.4	0.23	5.0	0.17	16.1	<0.01	17.4	<0.01
C:P	2.9	0.38	5.5	0.13	9.9	<0.01	11.7	<0.01
N:P	2.1	0.47	0.4	0.95	9.4	0.01	10.1	0.01
Lignin	2.1	0.49	2.0	0.54	7.2	0.06	2.9	0.40
Lignin:N	2.4	0.43	1.9	0.64	8.2	0.04	7.2	0.05
Lignin:P	2.0	0.48	3.0	0.36	3.6	0.34	3.4	0.32
Total	23.3	0.62	25.3	0.54	39.7	0.02	37.5	0.03

679

680 **Table. 2** Two-way ANOVAs (F- and *P* values) of the effect of litter type (L), nitrogen
 681 addition (N) on bacterial and fungal order and family. Only those with significant N
 682 effects are presented.

Taxa			L		N		L×N	
			F	<i>P</i>	F	<i>P</i>	F	<i>P</i>
Bacteria	Order	<i>Nitrosomonadales</i>	21.29	<0.001	3.06	0.02	0.79	0.58
		<i>Rhodospirillales</i>	7.76	0.01	3.56	0.01	1.03	0.43
	Family	<i>Nitrosomonadaceae</i>	21.29	<0.001	3.06	0.02	0.79	0.58
		<i>Oxalobacteraceae</i>	3.48	0.08	4.58	<0.01	0.98	0.46
		<i>Rhodospirillaceae</i>	5.55	0.03	3.35	0.02	1.55	0.21
Fungi	Order	<i>Pleosporales</i>	14.33	<0.01	4.27	<0.01	0.53	0.78
	Family	<i>Pleosporaceae</i>	49.77	<0.001	2.73	0.04	0.73	0.63
		<i>Hypocreaceae</i>	8.19	<0.01	5.79	<0.01	6.54	<0.001
		<i>Bionectriaceae</i>	20.26	<0.001	6.89	<0.001	1.37	0.27

683

684 **Figure legend**

685 **Fig. 1** Effects of nitrogen addition (0, 2, 5, 10, 15, 20, 50 g N m⁻² year⁻¹) and litter type
686 (Mix, mixed-species litter; Mono, single-species litter) on litter stoichiometry. Data are
687 shown as mean ± 1 SE. R² and P values are from a Pearson correlation analysis.

688 **Fig. 2** Effects of nitrogen addition and litter type (Mix, mixed-species litter; Mono,
689 single-species litter) on bacterial and fungal alpha-diversity. Error bars indicate ± 1 SE.
690 P- values from a two-way ANOVA on the effects of the litter type (L) and nitrogen (N)
691 are also presented.

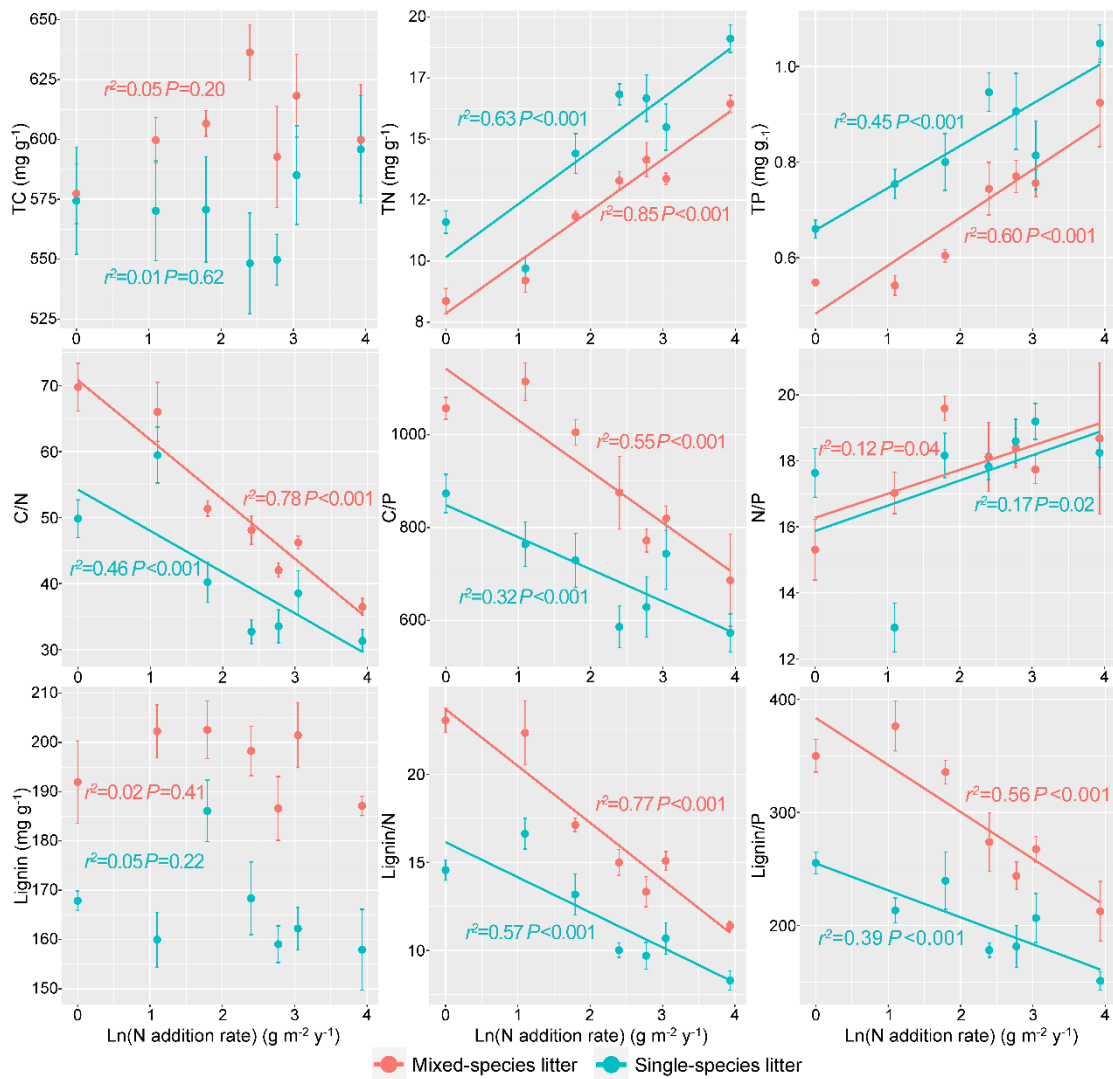
692 **Fig. 3** Nonmetric multidimensional scaling (NMDS) ordination of the bacterial and
693 fungal community composition. Communities are compared using Bray-Curtis distance
694 similarities based on the abundance of OTUs.

695 **Fig. 4** Correlation between bacterial and fungal community similarity (Bray Curtis) and
696 litter quality similarity (Euclidean distance).

697 **Fig. 5** Mean relative abundance of dominant (a) bacterial phyla across different nitrogen
698 additions and (b) fungal classes for the two litter types and the different nitrogen
699 addition levels. Lower pannels present box charts showing the relative abundance of
700 the order (c) *Nitrosomonadales* and (d) *Pleosporales* for the two litter types and the
701 different nitrogen addition treatments.

702

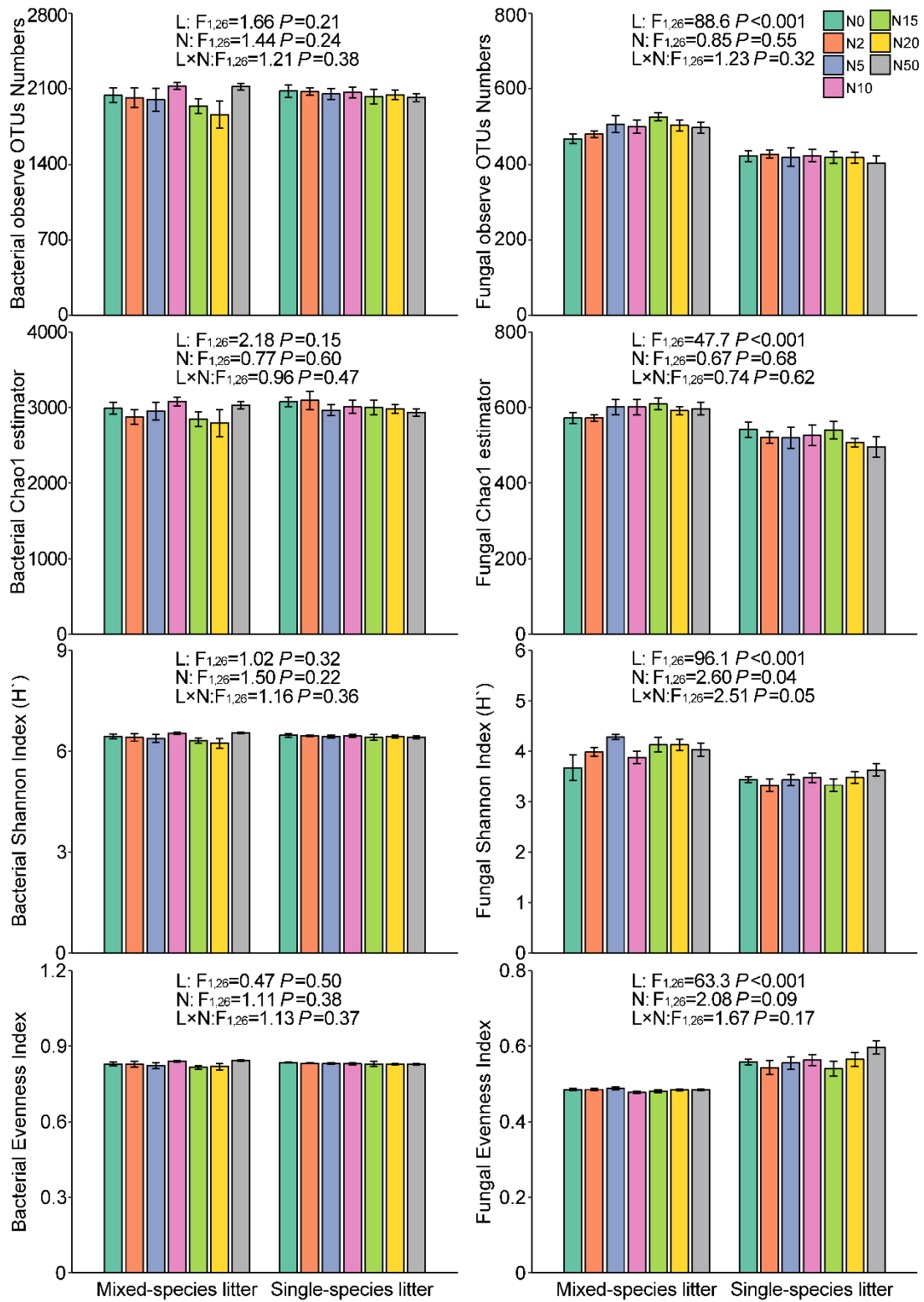
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705 Fig.1

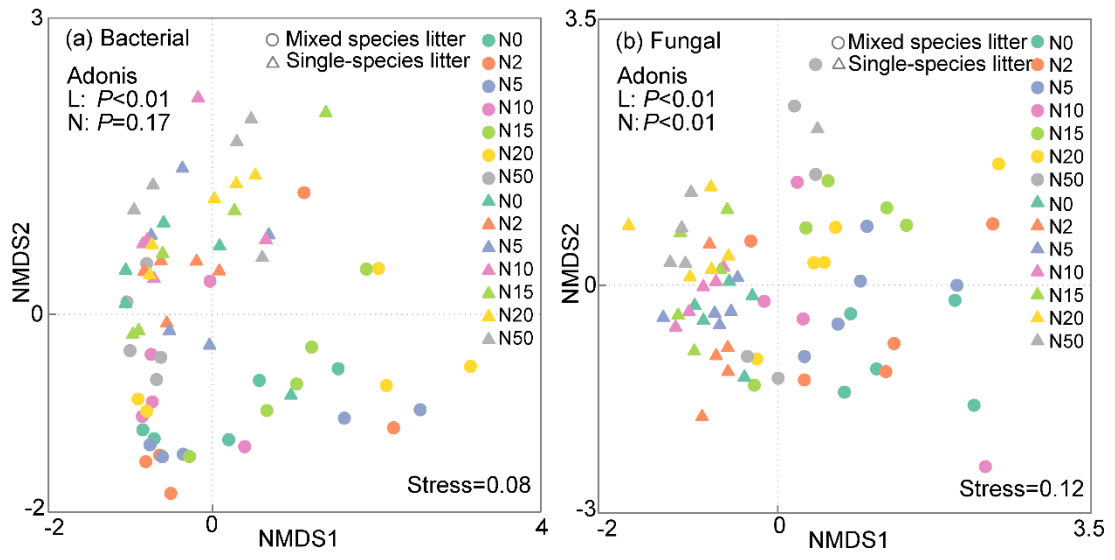
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708 Fig.2

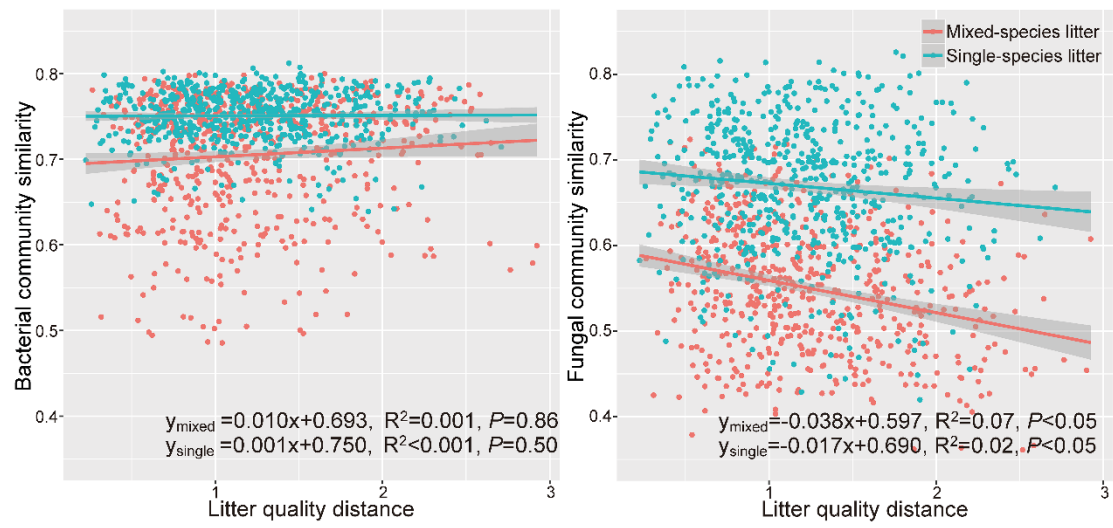
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710

711 Fig.3

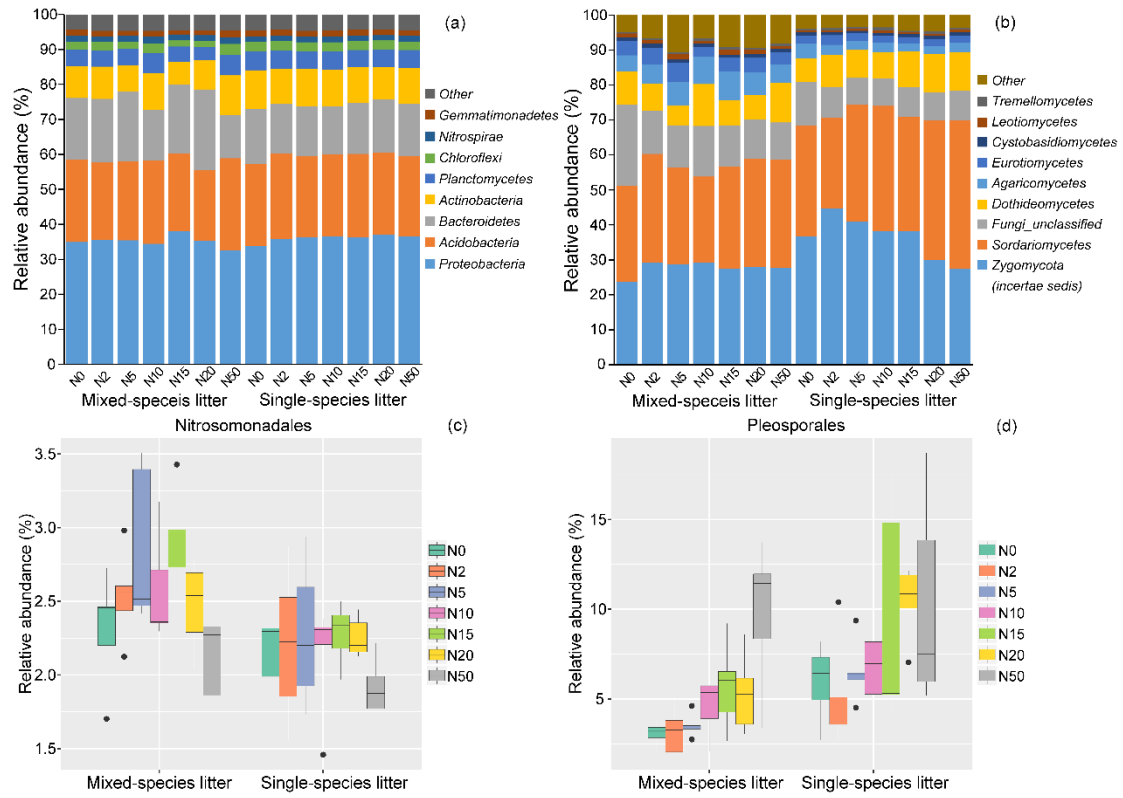
712



713

714 Fig.4

715



716

717 Fig.5