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

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Abstract

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

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

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

1. Introduction

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

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

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

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

Litter quality is a broad term that includes chemical variables such as energy source (C or lignin contents), and nutrients content (e.g. N and P) and their ratios (C/N, C/P, N/P, and lignin/N) (Cadisch and Giller, 1997). It can affect not only the diversity of the microbial community, but also the relative abundance of specific taxa that are involved 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.

2. Materials and methods

2.1 Litter and soil collection

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

The long-term N-addition experiment was established in September 2008. There were nine of N-addition levels (0, 1, 2, 3, 5, 10, 15, 20, 50 g N m^{-2} yr^{-1}) applied at two frequencies (2 times and 12 times per year). For the present study, seven N-addition levels (0, 2, 5, 10, 15, 20, 50 g N m^{-2} yr^{-1}, 2 times per year) were selected. Hereafter, the N-addition treatments will be denoted as: N_0, N_2, N_5, N_{10}, N_{15}, N_{20}, and N_{50}. 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 × 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-

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

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

2.2 Microcosm experiment

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

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

2.4 DNA isolation, amplification and illumina Miseq sequencing

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

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

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

2.5 Processing of molecular data

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

2.6 Statistical analysis

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

3. Results

3.1 Effect of N-addition on litter quality

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

3.2 Bacterial and fungal alpha-diversity

Neither N-addition rates nor litter type significantly affected bacterial alpha-diversity (Fig. 2). Higher number of fungal OTUs, Chao1 estimator and diversity (H′) were observed in soil with mixed-species litter than in soil with single-species litter, whereas the Shannon evenness index was higher in soil with single-species litter (Fig. 2). N-addition significantly affected fungal diversity. For mixed-species litter, the Shannon diversity of the fungal community slightly increased with increasing N-addition rates and the number of fungal OTUs was positively correlated with the litter N/P ratio, and negatively correlated with the C/N and Lignin/N ratios. The fungal Chao1 estimator was negatively correlated with the Lignin/N ratio for mixed-species litter treatments. For single-species litter, the number of fungal OTUs and the Chao1 estimator were 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*

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

4. Discussion

4.1 Litter quality changed fungal diversity but not bacterial diversity

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

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

4.2 Effect of litter quality on bacterial and fungal community composition

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

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

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

5. Conclusions

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

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

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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 %	*P*	Explained %	*P*	Explained %	*P*	Explained %	*P*
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	*P*	F	*P*	F	*P*
Bacteria	Order	*Nitrosomonadales*	21.29	<0.001	3.06	0.02	0.79	0.58
		Rhodospirillales	7.76	0.01	3.56	0.01	1.03	0.43
	Family	*Nitrosomonadaceae*	21.29	<0.001	3.06	0.02	0.79	0.58
		Oxalobacteraceae	3.48	0.08	4.58	<0.01	0.98	0.46
		Rhodospirillaceae	5.55	0.03	3.35	0.02	1.55	0.21
Fungi	Order	*Pleosporales*	14.33	<0.01	4.27	<0.01	0.53	0.78
	Family	*Pleosporaceae*	49.77	<0.001	2.73	0.04	0.73	0.63
		Hypocreaceae	8.19	<0.01	5.79	<0.01	6.54	<0.001
		Bionectriaceae	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. R^2 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.

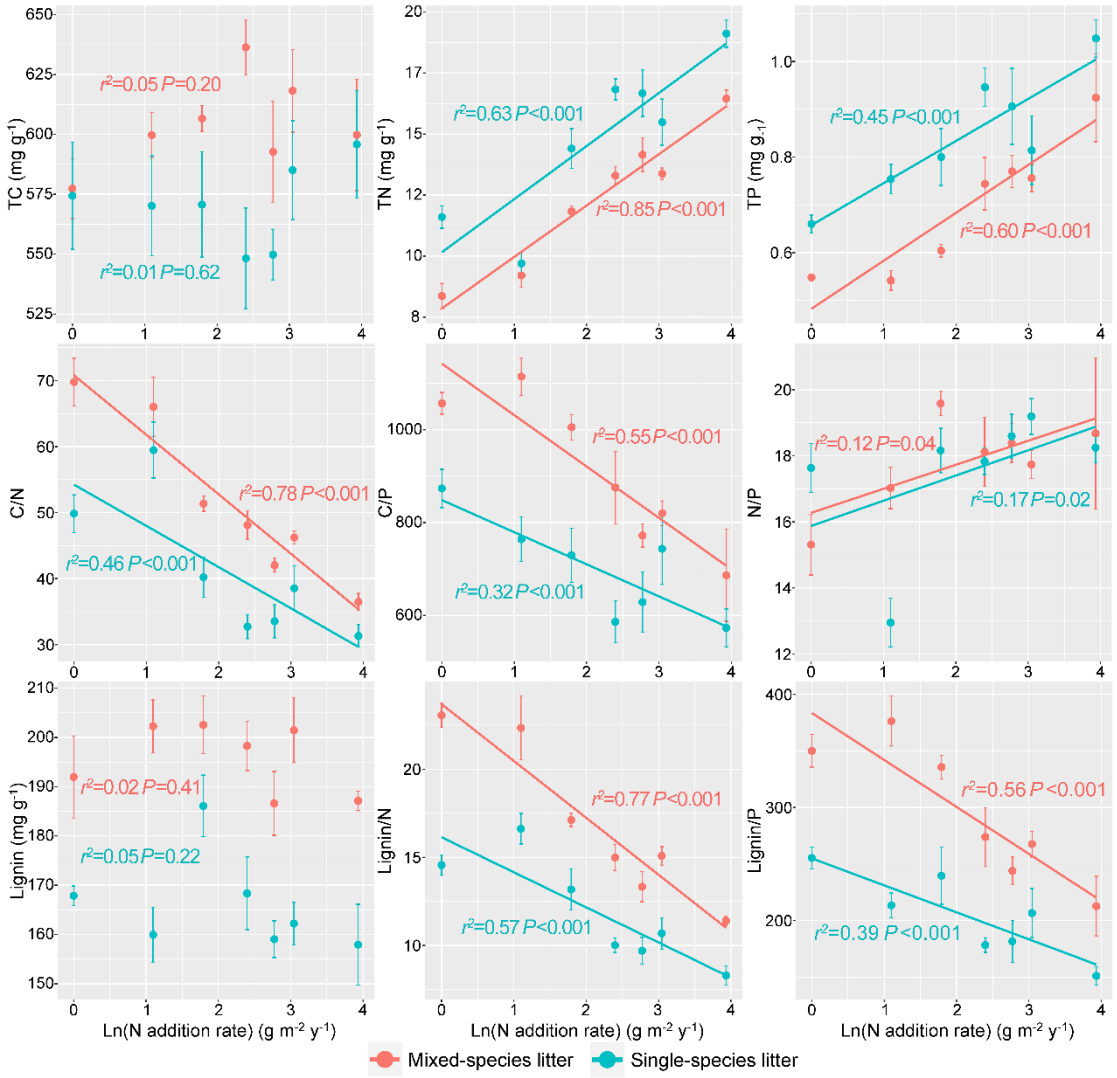
692 **Fig. 3** Nonmetric multidimensional scaling (NMDS) ordination of the bacterial and
693 fungal community composition. Communities are compared using Bray-Curtis distance
694 similarities based on the abundance of OTUs.

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

703



704

705 Fig.1

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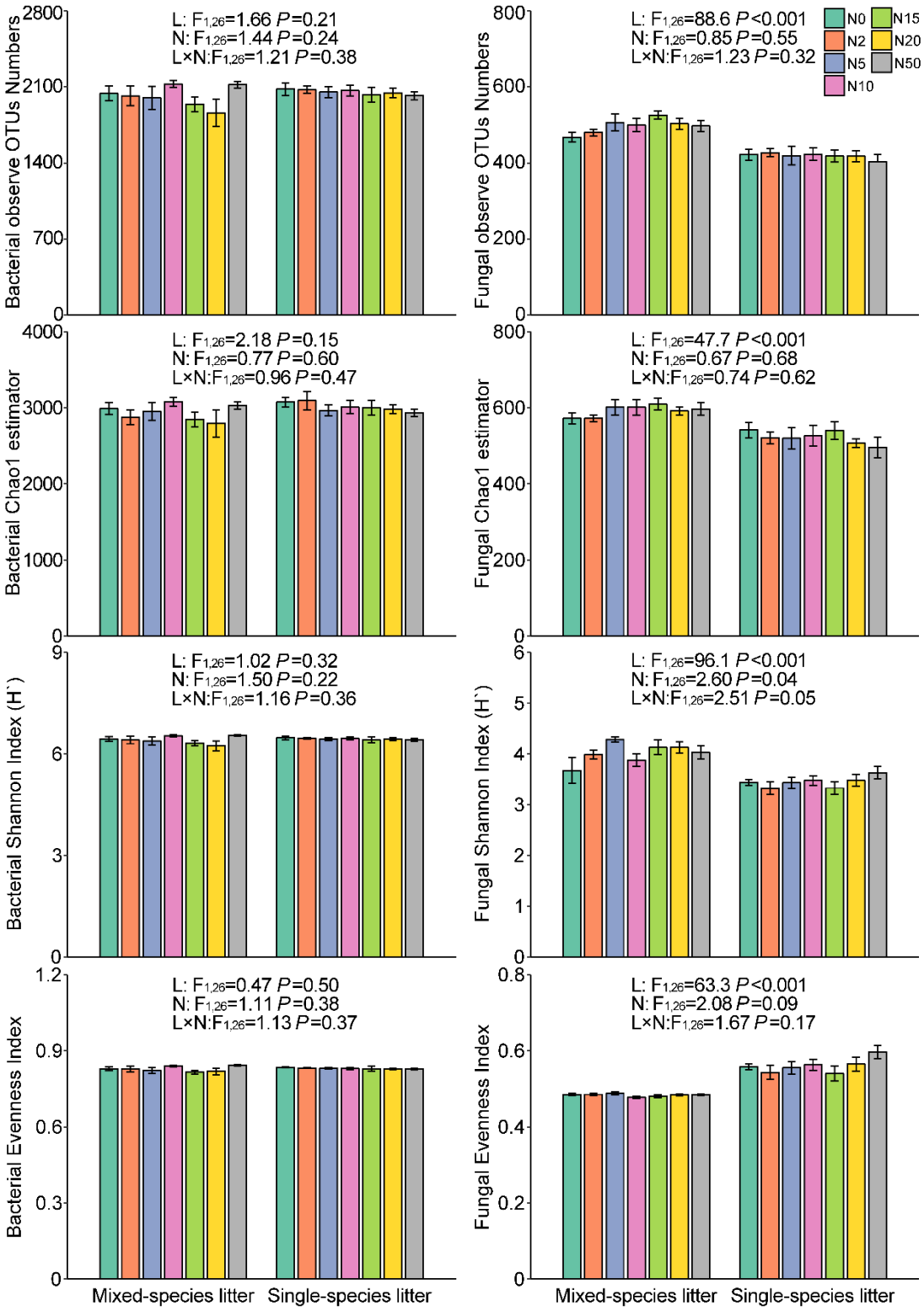

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708 Fig.2

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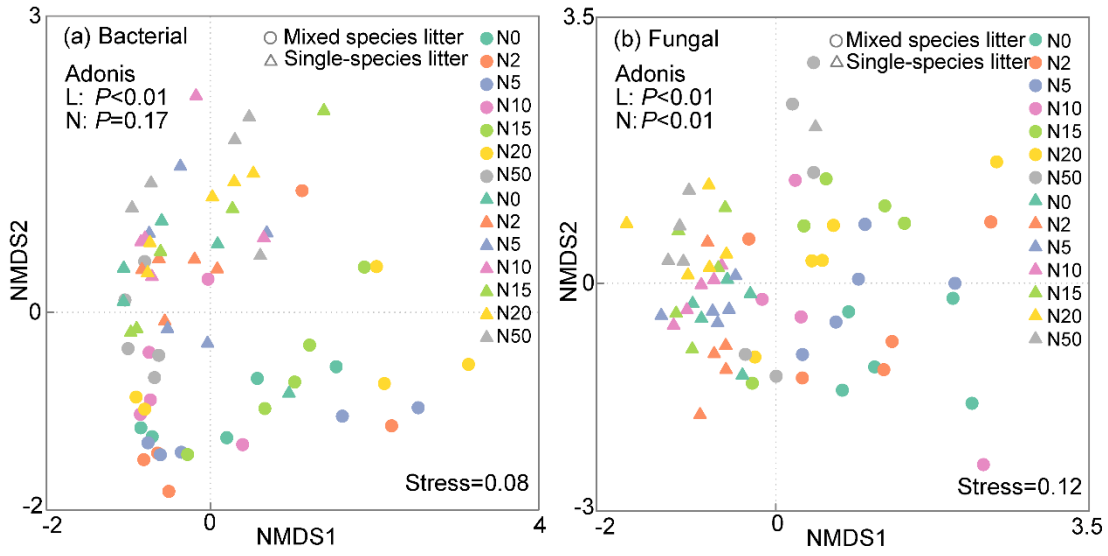

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711 Fig.3

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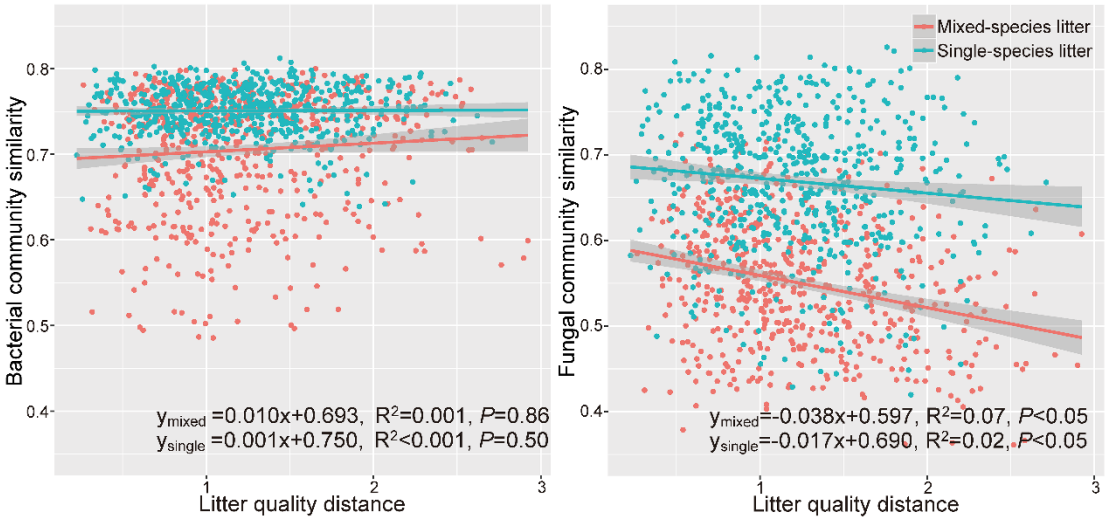

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714 Fig.4

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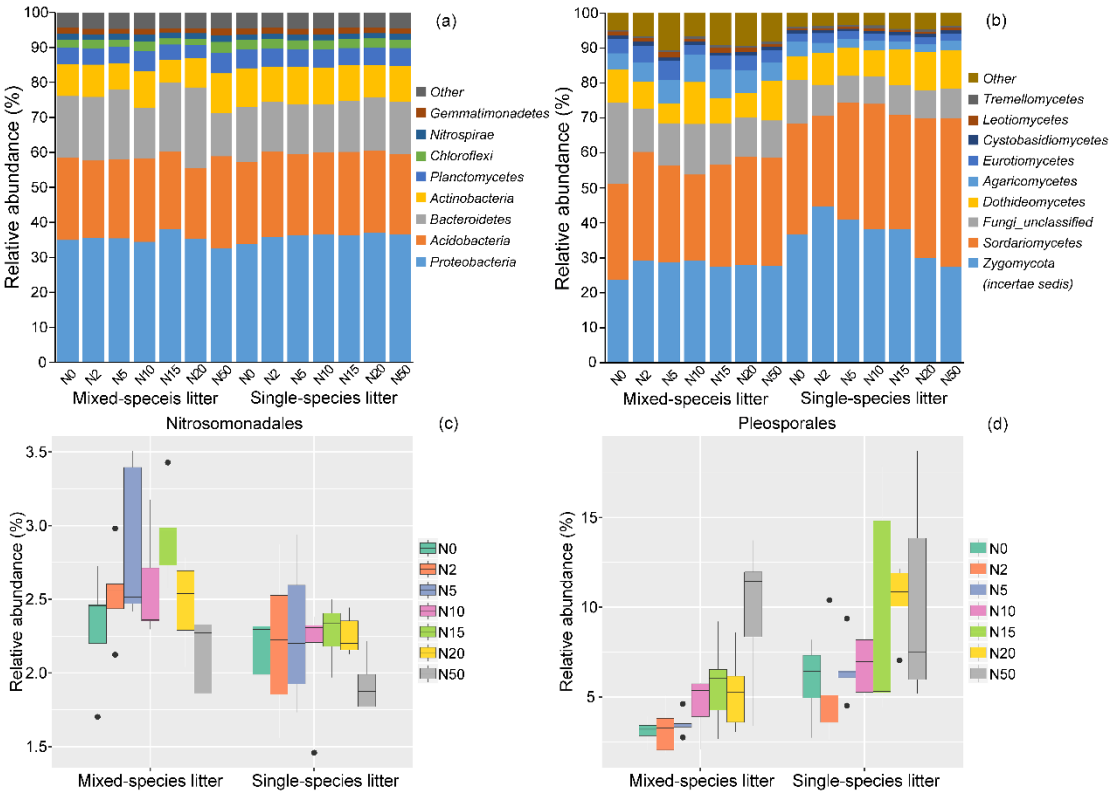
(a)
Relative abundance (%)
Other
Gemmatimonadetes
Nitrospirae
Chloroflexi
Planctomycetes
Actinobacteria
Bacteroidetes
Acidobacteria
Proteobacteria
N0 N2 N5 N10 N15 N20 N50 N0 N2 N5 N10 N15 N20 N50
Mixed-speceis litter
Single-species litter
(b)
Relative abundance (%)
Other
Tremellomycetes
Leotiomycetes
Cystobasidiomycetes
Eurotiomycetes
Agaricomycetes
Dothideomycetes
Fungi_unclassified
Sordariomycetes
Zygomycota
(incertae sedis)
N0 N2 N5 N10 N15 N20 N50 N0 N2 N5 N10 N15 N20 N50
Mixed-speceis litter
Single-species litter
Nitrosomonadales
(c)
Relative abundance (%)
N0
N2
N5
N10
N15
N20
N50
Mixed-species litter
Single-species litter
Pleosporales
(d)
Relative abundance (%)
N0
N2
N5
N10
N15
N20
N50
Mixed-species litter
Single-species litter

716

717 Fig.5