Keystone species affect the relationship between soil microbial diversity and ecosystem functioning under a subtropical land use change

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Abstract

Soil microbial diversity is a key control over soil element cycling and ecosystem functioning, but how the keystone species regulate the association between soil microbial diversity and ecosystem functioning (particularly, the whole-community and specialized species driving) under land use change remain unresolved. Here we identified the relationship of microbial diversity [e.g., soil phospholipid fatty acids (PLFAs) richness and composition, ammonia-oxidizing archaea and bacteria (AOA and AOB) communities] with both the broad (i.e. microbial basal respiration) and specialized function (i.e. nitrification rate) in the wood land, shrubland and adjacent cropland in subtropical China. The microbial richness was significantly positively related to the broad function, but negatively correlated with the keystone species across different land use types. The relationship of biodiversity with the broad ecosystem functioning varied with land use change, with stronger relationship in the afforested land compared to the cropland. In contrast to the broad function, land use change did not significantly affect the specialized function (i.e. nitrification rate), but the specialized function was positively related to the AOA richness in the cropland. Additionally, the specialized function was predominately driven by the keystone species composition in AOA and AOB communities and indirectly regulated by soil environmental factors (particularly, soil temperature) across land use change. Overall, our results provided direct experimental insight into the mechanisms underlying the role of the keystone species in regulating below-ground ecosystem functioning under land use change, more especially, our findings also revealed shift in the maintaining mechanisms of ecosystem function from the broad function (i.e. niche compensation effect) to the specialize function (i.e. identity effect).

Key words

Ammonia oxidizers, bacteria, broad functions, microbial PLFAs, specialized functions

Introduction

Soil microorganisms participate in almost all biogeochemical cycles and are extremely important for maintaining soil ecosystem functions (Bardgett & van der Putten 2014; Wagg *et al.* 2019; Zhou *et al.* 2020), whilst a large body of literature has documented that soil ecosystem functions can be threatened by land use change possibly due to loss of microbial biodiversity under land disturbance (Cardinale*et al.* 2012; Allan *et al.* 2015). For instance, previous studies have found that high microbial diversity is of stability via recruitment from the indigenous taxa pools, and hence land use change may lead to alpha diversity in decoupling of plant and microbial community (Allan *et al.* 2015; Zhou *et al.* 2020). While land use change, as a key component of global change, has occurred worldwide, particularly, recent land use change has induced dramatic alterations in vegetation type and microbial attributes (Cardinale*et al.* 2012; Cheng *et al.* 2013; Allan *et al.* 2015), and thus concurrently can affect belowground biodiversity and ecosystem functioning. However, the relationship between soil microbial biodiversity and ecosystem functions in response to land

use change remains largely uncertain due to soil microorganisms' huge diversity with the taxa ranging from hundreds to thousands per gram of soil (Torsvik & Øvreås 2002; Bardgett & van der Putten 2014).

Numerous studies conducted with plants and microbes support a consensus view that the relationship between microbial biodiversity and ecosystem functioning (i.e. BEF relationship) is the fundamental in addressing global environment changes (Tilman *et al.* 2014; Isbell *et al.* 2015). Some studies have generated that ecosystem functions decrease with the loss of biodiversity in the experiments and observation (Tilman *et al.* 2014; Meyer *et al.* 2018), as well as in the meta-analyses (Cardinale *et al.* 2012; Delgado-Baquerizo *et al.* 2016b; Zhou *et al.* 2020). Generally, the linear BEF relationship suggests that each species has a proportional effect on ecosystem function with no functional redundancy (Delgado-Baquerizo *et al.* 2016a; Trivedi *et al.* 2019a). Whereas, the logarithmic BEF relationship indicates that a small loss of biodiversity has a minimal or none impact on ecosystem due to the functional redundant (Louca *et al.* 2016; Trivedi *et al.*2019a).

Soil ecosystem functions such as the board (widely dispreads living microbe, e.g. decomposition) and specialized (i.e. ammoxidation) levels are critical for ecosystem energy gaining and nitrogen supplying processes (Bender *et al.* 2016; Manning *et al.* 2018; Zhou*et al.* 2020). And determining the BEF relationship at the broad and specialized level is of pivotal significance to estimate the impact of diversity losses induced by land use change on ecosystem functioning such as soil carbon sequestration or decomposition (Allan *et al.*2015; Delgado-Baquerizo *et al.* 2016a; van der Plas 2019). It has been argued that these two ecosystem functions are known as relatively independent microorganisms' communities (Srivastava & Vellend 2005; Delgado-Baquerizo *et al.* 2016a). For microbial keystone taxa usually drive community richness and function irrespective of their abundance (Banerjee *et al.* 2018), consequently, changes in keystone taxa can affect soil ecosystem function and reconstruct the BEF relationship. A big challenge is how to explicitly identify the relationship of the board and specialized functions with microorganisms in response to land use change. Although most of these interactions of microbes living network cannot directly observe, the network-based analytical approach is a useful tool to infer the microbial interaction and keystone species of the complex network (Deng *et al.* 2012; Weiss *et al.* 2016). Thus, the construction of a co-occurrence network enables our disentangling the BEF relationship under land use change.

In this study, we identified the BEF relationship under land use change in the Danjiangkou Reservoir area in central China. After the project of Middle Route of China's South-to-North Water transfer Project was constructed, large areas of cropland have been converted to shrubland and woodland since 1980s (Cheng *et al.* 2013). Our previous studies have demonstrated afforested lands enhanced soil quality, and microbial activities (Cheng *et al.* 2013; Feng *et al.*2018). Therefore, the variations in soil microbial communities and diversity are expected across over 30 years of land use change in our study site, which is useful to evaluate the relationship between microbial diversity for both broad (i.e. microbial basal respiration) and specialized function (i.e nitrification rate). Here, we hypothesize that: (i) the pattern of BEF relationship would vary with across land use change in the status that land use change would substantially alter soil microbial communities; (ii) considering for environmental changes across different land use types, the role of maintaining nitrification rate shifts between AOA and AOB possibly due to niche differentiation.

Methods and Materials

Study area and experimental design

The experimental site is located at Wulongchi Experiment Station (32°45'N, 111deg13'E; 280–400 m a.s.l) in the Danjiangkou Reservoir area of Hubei province, China. The climate in this region is characterized by the subtropical monsoon, with mean temperature 15.7 (monthly averages of 27.3 in July and 4.2 in January.), and mean precipitation 749.3 mm (70% – 80% occur between April and October) (Cheng *et al.*2013; Zhang *et al.* 2016). The soil is classified as yellow-brown soil in Chinese soil classification or Haplic Luvisols in the USDA Soil Taxonomy. More detail information was reported in previous articles (e.g., (Cheng *et al.* 2013; Zhang *et al.* 2016).

The experiment used a random block design, three land use types (woodland, shrubland, cropland) were chosen from three adjacent transects. Each block was approximately 3 hm^2 (600 m x 50 m) and the

distance between adjacent blocks was larger than 100m. Comprehensive surveys of soil and vegetation were conducted in winter (Dec. 2017) and summer (Jul. 2018) to detect the structure and seasonal dynamics of the microbial community and ecosystem functioning. All samples were taken at the same location to ensure the comparability (e.g., similar topography and soil types) of the soil sampling plots among the three land use types. Each sampling plot, six soil cores from 0 - 10 cm were taken using a 5 cm-diameter soil auger, and then thoroughly mixed and pooled as one complete soil sample after removing visible gravel and plant litter or detritus. Soil samples were immediately sieved through a 2.0 mm sieve and then separated into two parts: one stored in -20 for physical and chemical analysis, and the other one was stored at -80 for molecular analyses.

Soil physical and chemical analysis

Soil moisture was determined as the difference from fresh soil to constant weighted 105 for 24 h. Soil pH was measured with a digital pH meter from a soil water suspension (1:2.5 w : v). Soil organic C and N were measure with an element analyzer (Vario EL, Elementar Analysensysteme, Hanau, Germany) with the aliquots (approximately 10 g) dried soil samples treated with 10 mL 1N HCL for 24 h at room temperature to remove any soil carbonates. The ammonium (NH_4^+-N) and nitrate (NO_3^--N) were measured by a continuous flow analyzer (SAN++; Skalar, Breda, the Netherlands), as described by Yang *et al* . 2018.

Measurements of ecosystem broad function and specialized function

The basal microbial respiration (mSR) was selected as a broad function in this study, because it widely existed in almost all micro-organisms and had been considered as a proxy of total biological activity without environmental limitation (Campbell *et al.* 2003; Delgado-Baquerizo *et al.* 2016a). The mSR was measured by quantifying the carbon dioxide (CO_2) from the equivalent dry-weight fresh soil in ideal incubation environment. The details were described in previous studies (Qiao *et al.* 2014; Zhang *et al.* 2016).

The specialized functions (i.e. net nitrification rate, NR and potential nitrification rate, PNR) were measured, because they were a key microbial driven process in the N cycle and characterized by the oxidation of ammonia to nitrate via nitrite. Microbes holding the gene encoding ammonia monooxygenase (AMO) are the first and rate-limiting step of nitrification process (Hatzenpichler 2012; Trivedi *et al.*2019b; Jia *et al.* 2020). The NR and PNR were measured with the soil samples incubating in field and a standard environment (25 and 60% of the water saturation holding content) during a 14-day periods, respectively. The NR was calculated as the difference of NO_3^--N concentrations at the beginning and the end of the incubation periods.

Soil microbial community and structure

Soil microbial community structures were analyzed with PLFAs (phospholipid fatty acids) methods (Bossio & Scow 1998; Zhang *et al.* 2016), accounting for active microbes which were directly related to soil ecosystem functioning. Briefly, 8-g freeze-dried soil sample was added to 23 mL extraction solution containing chloroform: methanol: phosphate buffer (1: 2: 0.8 v/v/v). These extracted polar lipids were separated from neutral and glycolipids on a silica column (Cleanert Silica cartridge, 500 mg 6 mol L^{-1}, Agela Technologies Inc.). After mild alkaline methanolysis, polar lipids were converted to fatty acid methyl esters. The individual fatty acid methyl esters in the sample were analyzed by an Agilent 7890A gas chromatograph (Santa Clara, CA, USA) with MIDI peak identification software. The concentrations of each PLFAs were calculated based on their ratio to the 19:0 internal standard (5 mg mL^{-1}). Total extractable PLFAs were used as a proxy for soil microbial biomass.

Meanwhile, for each soil sample, total genomic DNA was extracted from 500 mg fresh soil using a MO BIO DNA extraction kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The purity and quality of the extracted DNA were examined with NanoDrop Spectrophotometer (NanoDropTechnologies Inc.Wilmington, DE, USA), and then diluted to 10 ng/μL and stored at -80 °C until use. The abundance of bacterial and archaeal *amoA* was determined on a real-time PCR detection system (Eco, illumine, USA) using primer sets Arch-amoAF/Arch-amoAR (Francis *et al.* 2005) for AOA and amoA-1F/amoA-2R (Rotthauwe *et al.* 1997) for AOB. The PCR products were purified using an AxyPrep DNA

Gel Extraction Kit (Axygen, USA). Prior to sequencing, the DNA concentration of each PCR product was measured by the QuantiFluor-ST blue fluorescence system (Promega, USA). Subsequently, purified amplicons were pooled in equimolar amounts and paired-end (PE) sequenced (2 x 300) on an Illumina MiSeq PE300 platform at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China (http://www.majorbio.com).

Raw sequences were processed and analyzed using QIIME pipeline (Caporaso*et al.* 2010). The quality control was conducted with removing the sequences those with a quality score < 20, containing ambiguous nucleotides, or not matching the primer and barcode. Chimeric sequences were checked and removed using the UCHIME algorithm (Edgar*et al.* 2011). Operational taxonomic units (OTUs) were identified with UPARSE pipeline (http://drive5. com/uparse/) at 85% similarity level (Pester *et al.* 2012). Taxonomy classification of*amoA* gene sequences was assigned using the ARB databases for AOA and AOB (Abell *et al.* 2012). An even number of sequences per sample were selected to correct for difference in sequencing efforts (10, 900 and 26, 190 for AOA and AOB, respectively). The raw sequences of archaeal and bacterial *amoA* genes were deposited into the NCBI Sequence Read Archive under accession numbers PRJNA637243, PRJNA637251, respectively.

Statistical analyses

Two-ways analyses of variance (ANOVAs) were conducted to figure out the main and interactive effects of afforestation and season on ecosystem functioning. To test whether significant relationships and covariations of ecosystem functioning with microbial diversity existed, we used the Pearson correlation test and scatter plots to examine and describe significant BEF relationships. To test whether the sensitivity of BEF relationship varied with different land use types, a standardized major axis regression was performed to examine the slope with the "smatr" package. Variance analyses of abundance of microbes and microbial functioning were performed to assess the effects of land use types and season dynamics. Principal coordinates analyses (PCoA) were used to evaluate the previous treatments effect on the Euclidean distance of soil microbial communities and Bray-Curtis distances of functioning genes with the "vegan" package. Given these distance matrices, we computed Mantel correlation between microbial communities (keystone species, non-keystone species and total communities) with environmental data with "vegan" package, and visualized these relationships with the "ggcor" package.

The co-occurrence patterns of microbial communities were conducted based on the correlation across different land use types. Spearman's correlation between any two OTUs was estimated, only the robust correlation with absolute value of correlation coefficients > 0.6 and FDR-corrected$P$ -value < 0.01 were used to form networks in "igraph" R package (Benjamini *et al.* 2006), and visualized network images with Gephi (*http://gephi.github.io/*). Moreover, 1, 000 Erdos–Renyi random networks with the same numbers of nodes and edges as the *corresponding* real networks were generated with "erdos.renyi.game" function in "igraph" package, and the mean value of each network metric was compared to real networks with Z-test (Hu *et al.* 2017). In each network each node represents one OTU, and same color nodes represent the same modules. The network and sub-network properties were calculated in the "igraph" package. The more detail topological features were estimated by a set of metrics and present in Table S1. The algorithm of fast greedy modularity optimization was applied to isolate modules. Keystone species were defined based on their roles in the network structure with the index (among and within module connectivity; Pi and Zi score). The node with either a high value was defined as keystone species (Zi >2.5 or Pi > 0.62) (Deng *et al.* 2012).

Structural equation modeling (SEM) was further used to evaluate the direct and indirect effect of environmental factor and microbial and keystone species richness on ecosystem functioning. Environmental and microbial communities were performed with principal component analyses. The first two components were chosen to represent the environments and community composition to eliminate potential collinearity. Models were conducted via the R package "lavaan". All statistical were performed in R and visualization with "ggplot2" and "ggpubr" packages (http://www.r-project.org/) unless otherwise indicated.

Results

3.1 Ecosystem functioning and microbial community richness

Both land use types and seasons significantly influenced ecosystem functioning including the mSR, NR and PNR (all $P < 0.05$; Fig. 1). Afforestation significantly promoted the mSR irrespective of season (F = 8.8, $P < 0.01$), with decrease in the order of woodland (3.03 ug/(g*day$^{)-1}$) > shrubland (2.36 ug/(g*day)$^{-1}$) > cropland (1.27 ug/(g*day$^{)-1}$). The NR did not significantly differ among different land use types irrespective of season, whereas woodland significantly inhibited the PNR compared to the shrubland and cropland (F = 9.5, $P < 0.05$) (Fig. 1). For seasonal pattern, the mSR was higher in summer than in winter in all land use types. However, the seasonal pattern of the NR and PNR varied with different land use types, with higher levels in summer than in winter in the cropland, and inconsistent trends in the afforested land (Fig. 1).

The microbial community diversity was based on the biomarker of PLFAs. The richness of microbial richness was significantly higher in summer than in winter with 73.4%, 44.4% and 53.4% higher level in the cropland, shrubland and woodland, respectively (Fig. 2a). There was no significant difference between land use types (F = 0.18, $P = 0.84$; Fig. 2a). Meanwhile, the 858, 591 and 1, 135, 240 high quality sequences of AOA and AOB were obtained from the 18 soil samples totally. After uniformed sequences (10,900 and 25,798) per sample, 1, 528 and 8,484 OTUs was identified of archaeal and bacterial communities, respectively. Land use types and seasons did not significantly interactively affect the alpha diversity of the microbial community and functional genes (Fig 2a, c, e). The alpha diversity indices of microbial communities of AOA and AOB including richness, Shannon index and chao1 index did not vary with different land use types, but the alpha diversity of microbial community was higher in summer compared to winter (Fig. 2 & S1). Furthermore, the ordination analysis revealed a less consistent pattern of the total microbial and *amoA* gene communities (Fig. 2b, d, f). The clustering effects of land use types (ANOSIM; R = 0.19, $P < 0.001$) and seasons (R = 0.63,$P < 0.001$) on the pattern of microbial communities were significant, whereas only the significant effect of afforestation were detected in the AOA (R = 0 .22, $P < 0.001$) and AOB (R = 0.25, $P < 0.001$) communities composition (Fig. 2b, d, f).

3.2 Biodiversity-functioning relationships across land use types

The positive correlations between microbial richness and ecosystem respiration were found across land use types (Fig. 3a). The AOA OTUs richness was positively correlated with the PNR in the cropland. Non-significant relationship was observed in the shrubland and woodland (Fig. 3c). There was not significant relationship between the AOB richness and PNR across land use types (Fig. 3e). More importantly, the keystone tax was observed to have a contribution to ecosystem functioning. The keystone PLFAs amounts were negatively associated with the mSR (Fig. 3b). Only statistically significant correlation was observed between the keystone taxon abundance and the PNR in the cropland, but the relationship showed the opposite trend (Fig. 3d). Furthermore, the slope of the relationship between microbial richness and the mSR in the cropland was statistically different with the woodland ($P < 0.001$) and shrubland ($P< 0.001$). By contrast, the scaling slopes of the relationship between keystone taxon and mSR were close ($P = 0.475$ across different land use types).

Meanwhile, for total microbes, the distance-corrected dissimilarities of keystone species or total community composition with those of ecosystem functioning and environmental factors were identified (Fig. 4). Overall, for total microbes, the total PLFAs nor the keystone species composition was the strongest correlated with the mSR. The total AOA composition and the keystone AOB species were significant correlated with specialized ecosystem functioning (i.e. PNR) (Fig. 4b, c).

3.3 Microbial andammoxidation genes community's co-occurrence patterns across land use types

In all land use types, the networks exhibited values of Modularity network, Clustering coefficient and Centralization degree were higher compared to their respective Erdos–Renyi random networks (Fig. 5; Table S2), suggesting all microbiome networks had small-world properties and modular structures. The overall topology indices of PLFAs communities did not significantly change across land use types possibly due to its low resolution. Networks had a greater number of negative correlations between nodes in the woodland of AOA (4.93) and the cropland of AOB (3.42). Meanwhile, the high network modality and average path

length were found in the woodland of AOA and cropland of AOB, which suggested a complex and stability of *amoA* gene community in response to land use types (Fig. 5; Table S3).

3.4 Keystone species and environmental factors influencing the ecosystem functioning

The SEM revealed that the PLFAs richness, PLFAs composition and substrate supplying factors (Bulk density, TC, TN, and C/N) were significantly directly associated with the mSR, and other environmental factors (pH, soil temperature and moisture) indirectly affected the mSR across land use types (Fig. 6a). With regards to specialized function (i.e. NR and PNR), the keystone AOA composition, and substrate supplying factors indirectly regulated the PNR, the environmental factors also dominated the NR (Fig. 6b). Whereas in the AOB community, we found the AOB keystone composition was closely related with the PNR, and the substrate supplying factor directly impacted the AOB community richness nor the AOB community composition, and indirectly impacted the NR (Fig. 6c).

Discussion

The relationship between microbial biodiversity and functioning under land use change

Our results provided direct experimental evidence that the association between soil microbial diversity and ecosystem functioning was primarily regulated by the keystone species under a subtropical land use change of China. As excepted, many studies have documented changes in the diversity of microbes and ecosystem functioning across land use types (de Vries & Shade 2013; Allan *et al.* 2015; Zhou *et al.*2020). A few studies have explored the biodiversity-functioning relationship varying in the natural assembled communities, which results may deviate from our familiar those in the experimental communities (van der Plas 2019). In natural ecosystems, biodiversity loss is random, and may be obscured by the abiotic factors (Turnbull*et al.* 2016; Veen *et al.* 2018). In this present study, our results emphasized that microbial richness was the principal factor in explaining the microbial respiration (mSR), and higher sensitivity of the mSR induced by changes in the microbial richness was observed in the afforested soils (Fig. 3; Fig. 4a). There were two possible explanations for these patterns. First, in phase with the positive linear diversity-ecosystem functioning relationship reported in experiments (Delgado-Baquerizo *et al.* 2016a; Rivett & Bell 2018), which indicated microbes contributed equally to the broad functioning (mSR). Second, it could be related to the microbial unique characteristics which might process much more rapid life cycle (e.g. hour to days) than in macro-organisms (e.g. plants, month to years) (Schimel & Schaeffer 2012; Delgado-Baquerizo *et al.*2016a). This in fact estimated a stable relationship when considering for relationship of microbial biodiversity-functioning across land use change. More especially, we found the negative relationship between the keystone species and mSR, suggesting the identify effect did not have a perceptible effect on the broad functioning, which occupied much resource than others to some extent (Trivedi *et al.* 2017b; Chen *et al.* 2020).

In contrast, our results showed that the biodiversity-functioning relationship of specialized functioning varied with land use types (Fig. 3d, e), possibly due to the heterogeneity across land use types. This also revealed the existence of some degree of functional redundancy in the *amoA* communities in afforested soils. It has been suggested that cultivation applications may act as an environmental filter selecting for those *Nitrososphaera* (affiliate to AOA), which are sensitive to soil organic N supply (Zhou *et al.* 2015; Gao*et al.* 2018).

Factors indirectly and directly regulate ecosystem functions

We also explored the direct and indirect effects of environmental factors on ecosystem functions under land use change (Fig. 6), which are pivotal for better understanding the underlying mechanisms of diversity-ecosystem functioning relationship under varying environments (Brose & Hillebrand 2016; Peters *et al.* 2019; Wang *et al.*2019). We found that the substrate supplying factors (e.g. TC, TN, and C/N) were directly associated with the mSR (Fig. 6a), highlighting the evidence that substrate quality dominated ecosystem functions (Nie*et al.* 2013; Zhang *et al.*2016). It has been well established that increased soil C and N supply could stimulate microbial activities as they were sources of nutrients and energy to microorganisms (Kaiser *et al.* 2016; Yang *et al.* 2018). Although environmental factors, especially soil pH has been considered as best predictor of microbial community composition (Fierer 2017; Yang*et al.* 2018) and ecosystem functioning

(Delgado-Baquerizo*et al.* 2017; Zhou *et al.* 2020), here it was evidenced that the indirect environmental factors predominately controlled over ecosystem broad function by shifts in microbial community richness (Figure 6). The possible explanation for the indirect effect was that environmental factors span a narrow range, and did not rapidly respond to land use change (Fierer 2017). Thus, the positive relationship between the substrate supplying and microbial broad functions suggested that the microbial respiration was probably both energy- and nutrient- limited across land use types in our study site (Trivedi *et al.*2016; Zhang *et al.* 2016).

By comparing ammonium availability in yellow-brown soil of our study site (0.8 mg/Kg, low level) to black soil (1.89 mg/Kg) of other studies e.g. (Liu *et al.* 2018), we inferred the PNR was maintained by substrate supplying factor irrespective of alterations in the microbial communities. However, in our study, the NR was directly related with environmental factors (especially, soil temperature), and the PNR was directly regulated by the AOA and AOB keystone species community (Fig. 6b, c). This result accorded with a previous study that found soil temperature was the important driver of global soil nitrification rate (Li *et al.* 2020). Some studies have observed niche differentiation between the AOA and AOB communities across different land use types (Dai *et al.* 2018; Hink *et al.* 2018; Liu*et al.* 2018). The inconsistency between our results and previous hypothesis indicated that land use changes in alkaline soil did not significantly affect the AOA and AOB community diversity (Fig. 2). This founding could be explained by a previous review with neutral pH and low nitrogen availability (Shen *et al.* 2012).

General function and specific function with community-level diversity

Our results unequivocally showed that microbial community diversity enhanced ecosystem broad functioning, and had a proportional effect with no functioning redundancy among land use types (Fig. 3a). These observations are consistent with previous reports of positive effect of microbial richness on ecosystem functioning (Bell *et al.* 2005; Delgado-Baquerizo *et al.* 2016b). However, these previous studies did not explicitly check for the relationship of the specialized microbes' richness with their function. In this present study, the empirical evidence demonstrated that any loss in microbial diversity could reduce multifunctionality, but the specialized microbes did not follow the significant correlation with its functions in afforested lands (Fig. 3c, e).

The different patterns between the broad and specialized BEF relationship could be explained by the below reasons. Generally, microbial respiration is an activity of active microbes' consumption soil carbon and important energy acquisition pathway, while the existing of specialized microbes may rely on environmental context (Delgado-Baquerizo *et al.* 2016a; Trivedi *et al.* 2019a). For example, previous studies reported that the richness of*Nitrososphaera (* affiliate to AOA*)* were drastically increased and the obligate N fixers were detrained from species pools under nitrogen fertilization scenery, with no significant changes of*Nitrosospira* (affiliate to AOB) (Shen *et al.* 2012; Gao*et al.* 2018). Notably, we found that the effect of microbial community on the specialized function was stronger compared to the abiotic factors in the croplands (Table. S1), in this case that there were high energy and nitrogen deficit for soil specialized microbes under low nutrient status (Table S1; (Delgado-Baquerizo *et al.*2016a; Feng *et al.* 2018). Another reason could be low resolution of the broad microbes. Although the active microbes can be detected by the PLFAs, the difference in the phospholipid markers was not enough to distinguish the probability of community redundancy, thus a small change in the richness of phospholipid markers could lead to changes in the broad functioning (Zhang *et al.* 2016; Veum*et al.* 2019). Additionally, we found different patterns between the specialized microbes (AOA and AOB), namely, the AOA communities did not exhibit direct and significant relationship with the NR (Fig. 6b, c). As noted previously, low rates of ammonium supply and alkaline soil in our study site could be a benefit for the AOB keystone species (*Nitrosospira* , Table S4) (Ke *et al.* 2013; Gao *et al.* 2018).

General function and specific function associated with keystone species diversity

The keystone species have been proven to exert a predominant ecological function in microbial functions (Banerjee *et al.* 2016; Trivedi*et al.* 2017b; Banerjee *et al.* 2018). The keystone species showed significant negative correlations with the broad functions (Fig. 4; Fig 6b, c), which could be expected that the broad

functions were maintained by more free-living populations (Hooper *et al.* 2005; Fan *et al.* 2018). And the higher proportion of negative relationship of the PLFAs network indicated the negative feedback primarily dominated microbe communities with less dynamic structure and greater ecological stability (Fig. S3). In addition, the maintains of community richness was a foundation of the broad function irrespective of land use types (Fig. 4). To some extent, our studies demonstrated the broad functions were determined by community richness and irrespective of environmental changes (Isbell *et al.* 2015; Delgado-Baquerizo*et al.* 2016b).

Inconsistent with the results observed for broad function, we found a lack of relationship of between keystone species and specialized functions except the AOA community in the cropland (Fig. 3d). Meanwhile, the bacterial network hubs, the keystone OTUs composition belonged to *Nitrosospira* was slightly related to the PNR (Fig. 4c). The present results demonstrated that losses of some specific groups of AOB could collapse the metabolic routes at specific environments (Fig. 4c) (Liu *et al.* 2019). This finding was inconsistent other observations with no context-dependence (Delgado-Baquerizo *et al.* 2016a; Trivedi *et al.* 2019a), which results showed little functional redundancy in the relationship between the functional genes diversity and specialized ecosystem functions from independent sites. By comparing other studies (Trivedi*et al.* 2017a; Banerjee *et al.* 2019) with natural assembled communities in our study, the keystone species composition under the dilution-to-extinction approach may not fundamentally change, which has a disproportionate effect on ecosystem functions (Berry & Widder 2014; Trivedi *et al.* 2019a). According to the traditional paradigms (niche complementarity and sampling effects) (Tilman *et al.* 2001), some studies documented that the increased functional rates observed were ascribed to the presence of particular species, rather than to an increase in species diversity (Strong *et al.* 2015; Daam *et al.* 2019). Consequently, in low substrate status, the lack of biodiversity-functioning relationship of the specialized function could be related to the community composition of keystone species (Strong *et al.* 2015; Daam *et al.* 2019).

In conclusion, the BEF relationship showed different patterns in the broad function and specialized function. The broad function was maintained primarily by the community-level diversity, although the keystone species played a large role in the broad function, but the weak relationship indicated diversity effect (niche compensation) could dominate this process and community richness recruitment from other free-living species. In contrast, the relationship between the specialized function and keystone species composition, nor the*amoA* richness was observed in the afforested soils. These results suggest that the keystone species can maintain ecosystem function through identity effect. More importantly, we found that land use change did not alter the robust biodiversity-functioning relationship, but revealed more sensitivity of diversity loss in the afforested soils. However, land use change directly regulated the NR by environmental factors (particularly, soil temperature), and affected the PNR by regulating keystone species. Collectively, unlikely the classical positive but decelerating biodiversity-functioning relationship (Tilman*et al.* 2014; Delgado-Baquerizo *et al.* 2016a), our finding provided direct evidence the positive relationship only existed in the broad functioning, but the specialized functioning depended on land use scenario. These results further reveal the different underlying mechanisms in maintaining the relationship of the broad and specialized function with the microbial diversity, highlighting the important role of keystone species in corresponding specialized soil functions (e.g. nitrate availability) to harness their potential for predicting modelling of biochemistry cycle under global land use change.

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