

# Afforestation effects on soil microbiomes modulated by changing soil physicochemical properties in temperate grassland

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**Short title:** Afforestation effect on soil microbes

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## Conflict of Interest

The authors declare that they have no conflict of interest.

## Abstract

Grassland afforestation dramatically affects the abiotic, biotic, and ecofunction properties of the original ecosystems. Interference from afforestation might disrupt the stasis of soil physicochemical properties and the dynamic balance of microbiota, although some studies have suggested low sensitivity of soil properties and a small response of bacterial community to afforestation. However, this “small response” is probably due to the confounding effects of the generalist habitat and rare microbes. In this study, soil physicochemical and prokaryotic properties in a 30-year-old Mongolia pine (*Pinus sylvestris* var. *mongolica* Litv.) afforested region and adjacent grassland in Inner Mongolia were classified and quantified. Our results indicate that the high richness of rare microbes accounts for the alpha-diversity of the soil microbiome, whereas generalist (core microbiota) and habitat-specialist microbes present in few numbers but high abundance govern the beta-diversity of the grassland and afforested land microbiomes. Afforestation has changed the soil physicochemical properties, thus indirectly affecting the soil microbial composition rather than richness. The contents of soil P, Ca<sup>2+</sup>, and Fe account for the ecofunctional changes in soil microbiomes due to grassland afforestation. We conclude that grassland afforestation has changed the physicochemical properties and composition of the soil and ecofunctions of the soil bacterial community and that these effects of afforestation on the microbiome have been modulated by changes in soil physicochemical properties.

**Keywords:** grassland afforestation; microbial composition; microbial ecofunction; soil physicochemical properties

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## Introduction

After a decline in forest coverage in China to only 8.6% due to war, urban construction, and reclamation, efforts by the Chinese government in the last 30 years to promote afforestation have increased forest coverage to nearly 20% (State Forestry Administration of China 2011). However, these plantations are not exclusively located at the original sites of deforestation. Consequently, these deforestation and afforestation events have greatly changed the landscape and ecosystems of China (Ahrends et al. 2017). Mongolia pine (*Pinus sylvestris* var. *mongolica* Litv.), an endemic tree in Inner Mongolia, is one of the main forestation species in temperate regions of China. Scots pine (*P. sylvestris* L.), a relative of Mongolia pine that is widespread from Western Europe to Eastern Siberia, is also an important tree in forestry (Krakau et al. 2013). Studies of Mongolia pine are scarce, but several studies have revealed that Scots pine is genetically and physiologically sensitive to environmental pollution (Chudzińska et al. 2014), geographic weather variation (Oleksyn et al. 2003) and climate change (Hurme et al. 1997; Savolainen et al. 2004). Phenological variation in response to climatic adaptation has also been suggested to be linked to quantitative trait loci (Hurme et al. 2000). These studies have demonstrated that Scots pine and, by reasonable extension, Mongolia pine have broad, plastic adaptability in response to environmental heterogeneity, supporting the wide use of these trees in afforestation.

Grassland afforestation is an artificial and direct change in vegetation that alters both the above- and underground ecosystems, including abiotic changes [e.g., land surface temperature (Li et al. 2016; Peng et al. 2014), hydrological connectivity, litterfall and litter decomposition (Khamzina et al. 2016; Lafleur et al. 2015), and soil physicochemical properties (Chen et al. 2008; Lafleur et al. 2015; Wang et al. 2016; Zheng et al. 2017)], biotic changes [e.g., flora (Ma et al. 2013) and fauna compositions (Márquez et al. 2015; Pedley et al. 2014) and soil microbiota (Gunina et al. 2017; Šnajdr et al. 2013; Xiao et al. 2017)], and ecofunctional changes [e.g., plant-soil and plant-microbe interactions (Lu et al. 2017; Ren et al. 2016) and functional diversity of communities (Cibils et al.

2015)]. Land use is a significant determinant of runoff and soil redistribution processes (Arnáez et al. 2015), and biotic responses could be sensitive to compositional changes in both species and ecofunctions (Xiao et al. 2017). Therefore, changes in microbial composition and function in soil can be quantified as indicators to monitor microenvironmental changes due to afforestation or reforestation. |

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The afforestation process may alter the original environment. Although the environmental microbial composition may be sensitive to environmental variation, the effects of these microbial compositional changes on ecofunction remain unclear. A comprehensive review demonstrated that the soil microbial composition varies to alter acquisition, metabolism, and degradation processes in response to changes in soil P due to grassland afforestation (Chen et al. 2008). The C:N:P ratio of soils with different vegetation types also reflect different transformation processes and rates between plant and soil organic matter, and these biogeochemical processes are mediated by soil microbial community structure and functions (Zechmeister-Boltenstern et al. 2015). More importantly, environmental conditions and existing microbial diversity determine the ecological function and nutrient transformation efficiency of soil microbiota (Zechmeister-Boltenstern et al. 2015). Therefore, quantifying the effects of afforestation on changes in soil properties, microbial composition, and ecofunctions would accelerate the understanding of plant-soil-microbe interactions.

Afforestation has been suggested to have a greater impact on soil biological properties than soil chemical properties (Gunina et al. 2017; Jangid et al. 2011). These underground biological changes are mainly ascribed to changes in litter amount and chemistry, which stimulate the development of the fungal community rather than the bacterial community (Klein et al. 1995). The response of the prokaryotic microbiome to changes in vegetation type is small, likely because microbial assemblies

are based mainly on functional genes rather than species (Burke et al. 2011), and the changes in soil properties due to afforestation are relatively small and occur slowly (Gunina et al. 2017; Jangid et al. 2011). In addition, abundant microbial "core species" with highly conserved core functions (Falkowski et al. 2008) and rarely occurring species (Ai et al. 2013) may act as confounders in statistical analyses of the effects of afforestation or soil properties on microbiome change. Therefore, habitat generalists (core species) and specialists (divergent species) in microbial communities must be classified before analysis, particularly when using high-throughput sequencing (HTS) technology (e.g., 16S rRNA metagenomic sequencing), which can generate huge amounts of data, to outline the composition and structure of microbial communities (Székely & Langenheder 2014).

In this study, to quantify the effect of afforestation on soil properties and soil microbial composition and functions, the soil physicochemical and microbial compositions were measured in an afforested region and adjacent grassland. Based on previous studies that have demonstrated functional assembly of bacterial communities (Burke et al. 2011) and a small response of bacterial communities to afforestation, with low sensitivity to soil properties (Gunina et al. 2017; Jangid et al. 2011; Klein et al. 1995), we hypothesized that (1) the soil prokaryotic microbiome is indirectly affected by afforestation mediated by soil physicochemical properties and (2) soil microbial ecofunctions are more sensitive to changes in vegetation type than changes in soil microbial composition. To test these hypotheses, the 16S rRNA metagenomes of the afforested and adjacent grassland soil microbiomes were sequenced, and the relative abundances of microorganisms and predicted ecophysiological functions were quantified by multivariate and regression analyses. Based on these analyses, we provide a possible explanation of the link between soil physicochemical properties and microbiome changes in grassland afforestation.

Materials and Methods

Study sites and sampling

Mongolia pine is native to Honghua'erji in Inner Mongolia in China and is widely planted as a forestation tree in temperate Asia. The study site was located 6 km west of the town of Honghua'erji, in an artificial forest produced by seedling afforestation in a large area of thin grassland (savanna). Field experiments were approved by the Honghua'erji Nature Reserve (permit number 200/66150221). The tree ages of the Mongolian pines in this afforestation forest ranged from 27 to 33 years, with a DBH of 21.21±4.19 cm (12.09~32.10 cm) and tree height of 12.86±0.86 (11.2~14.6 m). In this seedling afforestation area, almost no seedlings were found (personal observation), implying that this region lacks naturally symbiotic fungi to assist the germination and growth of Mongolian pines except in the planted soils. Although the lack of fungal symbionts of Mongolian pines requires further investigation and confirmation, the absence of seedlings suggests that this afforestation area was likely not a Mongolian pine forest in the past.

At the study site, we collected soil samples from 20 quadrants, including 10 inside the forest (i.e., forest soils) and 10 in the adjacent grassland (i.e., grassland soils). At each location, soil samples were collected at a depth of 50 cm from 10 quadrants located 10 to 100 m from each other. The soil samples were separated into two parts: one part was dried for quantification of the soil physicochemical properties, and the other was used to quantify the microbiome. The latter samples were stored in RNAlater stabilization solution on ice immediately after collection until transfer to the laboratory, where the samples were stored at -20 °C before metagenomic DNA extraction.

Quantifying soil physicochemical properties

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Organic carbon (C) was measured by the external-heat potassium dichromate oxidation method, and total nitrogen content (N) was measured by the Kjeldahl distillation method. The inductively coupled plasma (ICP) method was used with soils digested in a mixture of HF–HClO<sub>4</sub>–HNO<sub>3</sub> to quantify the contents of the soil elements K<sup>+</sup>, P, Ca<sup>2+</sup>, Fe, Mg<sup>2+</sup>, and Na. Soil pH was determined using a Sartorius pH meter PB-10 (Germany). All soil properties were determined and quantified by the State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy Sciences.

### 16S rRNA metagenome sequencing

Microbial metagenomic DNA was extracted with an EZNA<sup>®</sup> Soil DNA Kit (Qiagen, Valencia, CA, USA), and the concentration was adjusted to 50 ng/μL. The metagenomic DNA was quantified using Qubit<sup>®</sup> 2.0 (Invitrogen, Life Technologies, CA, USA). Primers 341F and 805R were used to amplify the V3-V4 hypervariable 16S rRNA region, and the PCR products were used to construct a DNA library with the Roche GS FLX Titanium emPCR kit (Roche Applied Science). The DNA libraries were then sequenced by Sangon Biotech Co. (Shanghai, China) on an Illumina MiSeq 2X300. The sequencing procedures followed the manufacturer's instructions.

Before analysis, the raw HTS data were cleaned by removing sequence fragments shorter than 200 bp or with missing barcodes or polyN or polyA/T in Ribosomal Database Project (RDP) (Cole et al. 2007). We also discarded reads with PHRED quality scores <Q25 (Ewing & Green 1998; Ewing et al. 1998). The Mothur package was used to remove non-prokaryotic sequences and de-noise and trim the sequences (Mothur ver. 1.30.1, [http://www.mothur.org/wiki/MiSeq\\_SOP](http://www.mothur.org/wiki/MiSeq_SOP) (Schloss et al. 2009)). Chimeric sequences were removed using Uchime (Edgar et al. 2011). After quality filtering, we clustered sequences using a criterion of >97% sequence similarity as operational taxonomic units (OTUs) defined as representing the same species by the RDP classifier (Cole et al. 2007). The

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rarefied OTU table was generated using Qiime (Caporaso et al. 2010) and deposit in Mendeley (doi:10.17632/gjskh8wswz.1). Each OTU was annotated and classified according to the RDP classifier and SILVA database. The raw sequence data were deposited in NCBI GenBank under Bioproject PRJNA317430 (Accession number: SAMN04607375).

### **Predictive functional profiling of microbial communities**

PICRUSt v. 1.0.0, a functional prediction tool for estimating shared gene content according to the corresponding microbiome phylogeny, was used to predict the molecular functions of each sample (Langille et al. 2013). PICRUSt generates the composition of gene families for each metagenome using an extended ancestral-state reconstruction algorithm. The online version of PICRUSt implemented in Galaxy (<https://huttenhower.sph.harvard.edu/galaxy/>) was used to assist the algorithms. The quality-filtered sequences were assigned to a closed reference OTU table against the Greengenes v. 13.5 OTU database (DeSantis et al. 2006) for PICRUSt prediction implemented in QIIME v. 1.8.0 (Caporaso et al. 2010). Each OTU was normalized by its copy number. The functional contribution of each OTU member was reconstructed and predicted by mapping the 16S sequences to their nearest reference genome. ‘Virtual’ metagenomes with gene content abundance were then generated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) Ortholog and Clusters of Orthologous Groups (COGs) databases.

### **Statistical analyses**

This study included two types of measurements, the soil physicochemical properties and soil microbiomes (i.e., relative abundances of microbial OTUs), and one treatment, the grassland afforestation (i.e., vegetation type). We first assessed the differences in soil properties between vegetation types by the Kruskal-Wallis (KW) test. A logistic regression model was further used to



determine if the soil properties predicted the grassland-afforestation treatment. The likelihood ratio test (LRT) was performed to identify the best-fitting model of the logistic regression. To test the hypotheses of a stochastic process of microbial assembly (random distribution model) or resource-governed assembly (niche-based mechanism), we used Rank-Abundance Dominance (RAD) analysis to display logarithmic species abundances against species rank order (McGill et al. 2007). Bray-Curtis distance-based redundancy analysis (dbRDA) was used to examine the explanatory proportions of the treatment for these two measurements. Because soil physicochemical properties can also affect the soil microbiome (Stutter & Richards 2012), we further performed a partial dbRDA to assess the effects of grassland afforestation on the soil microbiome conditioned by soil physicochemical properties and the effects of the soil physicochemical properties on the soil microbiome conditioned by vegetation type. Type II ANOVA was used to evaluate the fit of the model of each constraint factor. We further identified divergent microbes (i.e., forest and grassland specialists) using the supermajority rule ( $>2/3$  difference in abundance) with the assistance of the multinomial species classification method (CLAM) test (Chazdon et al. 2011). The soil elements that significantly explained the soil microbiome by partial dbRDA were then used as independent factors to predict the abundance of microbial specialists by the generalized linear model (GLM). Because the ecophysiological functions (COGs and KEGG modules) of the soil microbiomes were also predicted, all tests were repeated by replacing the soil microbial OTUs with the COGs and KEGG modules.

## Results

### Effects of afforestation on soil physicochemical properties

To characterize the soil physicochemical properties, the contents of eight elements (organic C, total N, P, K, Ca, Mg, Fe, and Na) and the pH value of each soil sample were determined (Table 1).

Among these soil variables, the contents of C, P, Ca, Mg, and Fe were significantly higher in the forest soils than in the grassland soils ( $P = 0.0003$ ,  $0.008$ ,  $0.003$ ,  $0.0004$ , and  $0.00006$ , respectively), and the pH of the grassland soils was significantly higher than the pH of the forest soils ( $P = 0.004$ ) (Fig. 1). To estimate the explanatory proportions of the afforestation effect on the variance of soil properties, we performed dbRDA using vegetation type (i.e., forest and grassland) as the independent factor. Vegetation type significantly explained 50.18% of the variance of soil properties ( $P = 0.002$ , Table 2), indicating that afforestation has changed the soil properties.

To determine if the contents of soil elements can predict the vegetation type (i.e., afforestation effect), we compared the simple logistic regression (SLR) model using a single soil element as the independent factor and the multivariate logistic regression (MLR) model using all soil elements as independent factors (i.e. the full model M1) to the empty (null) model M0. Most of the SLR models and M1 rejected the null model M0 ( $P < 0.005$ ), except the SLR models with K (LRT:  $P = 0.084$ ) or Na (LRT:  $P = 0.108$ ) as the independent factor. We further compared the SLR models with each single soil element (C, N, P, Ca, Mg, Fe, and pH) as the independent factor to the MLR model using all C, N, P, Ca, Mg, Fe, and pH as independent factors (full model M2) by LRT. The SLR models with Mg or Fe as the independent factor could not be rejected by M2 ( $P = 0.278$  and  $0.130$ , respectively, Fig. 2). Although the other SLR models with C, N, P, Ca, or pH as the independent factor were rejected by M2, none of the independent factors in M2 could significantly predict the presence of forestation ( $Z < 10^{-6}$ ,  $P > 0.9999$  in each term). These results indicated that the contents of Mg ( $0.456 \pm 0.099$  and  $0.313 \pm 0.035$  g/kg in forest and grassland soils, respectively) and Fe ( $8.355 \pm 1.047$  and  $5.607 \pm 1.308$  g/kg in forest and grassland soils, respectively) could singly reflect the changes in soil properties due to afforestation (Table 1 and Fig. 2).

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#### Afforestation effects on the relative abundances of soil microorganisms

We further examined how afforestation affected the biological properties of soils. We estimated the relative abundances (RAs) of the soil microbiome according to 16S rRNA metagenome sequencing.

The RAs of microbial OTUs were estimated from the reads of the 16S rRNA gene with >97% similarity. Divergence of the soil microbiome was inferred by significant or marginal differences in the diversity indices (Shannon–Wiener index  $H$ ,  $KW\chi^2 = 3.291$ ,  $P = 0.070$ ; Reciprocal Simpson's index  $1/D$ :  $KW\chi^2 = 4.166$ ,  $P = 0.041$ ; Pielou's evenness  $J$ :  $KW\chi^2 = 9.864$ ,  $P = 0.002$ , Table 3).

However, no difference was estimated in the species richness of the soil microbiome between forest and grassland ( $KW\chi^2 = 0$ ,  $P = 1$ , Table 3). The significant differences in  $H$ ,  $1/D$ , and  $J$  but not

richness between the grassland and forest soil microbiomes suggest that the change in the soil

microbiome is species composition (RA) rather than species number. Hence, we used RA as an

indicator to compare the microbiomes of the soil samples. Hierarchical cluster analysis (H-cluster) and DAPC (five first PCs of PCA used, which conserved 70% of the variance of the microbial RA) presented similar patterns of clear divergence of soil microbiomes between the forest and grassland (Fig. 3). These results confirm that the soil microbial composition has changed due to grassland afforestation despite no change in microbial richness.

Because most of the 16S rRNA sequences with <97% sequence identity and hence assigned as different OTUs were rarely found among different samples, we performed the CLAM test (Chazdon et al. 2011) to classify these OTUs as four types of microbes using the supermajority (2/3) rule: too-rare microbes, generalist microbes, and grassland- and forest-specialist microbes. In this classification, a large proportion of OTUs (95.46%) belonged to the too-rare microbes, which accounted for 38.42% and 36.73% of the abundance of grassland and forest soil microbes, respectively; only 2.98% of the OTUs were generalists, but they accounted for 35.55% and 35.88% of the abundance of grassland and forest soil microbes, respectively. Only 1.56% of the OTUs belonged to specialists, of which 1061 (0.7%) and 1224 (0.8%) microbial OTUs were identified as forest and grassland specialists, respectively. The RA of grassland-specialist microbes was 22.84%

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in grassland soils and 2.84% in forest soils, while the RA of forest-specialist microbes was 3.19% in grassland soils and 24.55% in forest soils.

We further used the RAs of the microbial OTUs as the dependent response to access the impact of afforestation on the soil microbiome. When vegetation type was used as the categorical independent factor, 16.3% of the variance of microbial RA was explained significantly ( $P = 0.001$ ). However, the significant explanatory effect of vegetation type was lost after removing (conditioning on) soil properties ( $P = 0.466$ , Table 2). When soil properties were used as a constraint factor, 64.4% of the variance of microbial RA was explained by soil properties, in which the organic C (11.4% explanation), P (10.0%), Ca (9.0%), Mg (7.1%), and Fe (10.5%) significantly fit the model by type II ANOVA (Table 2). Similar significant explanations by C (6.7%), P (9.5%), Ca (8.8%), Mg (6.7%), and Fe (5.4%) were obtained when the effect of vegetation type was removed (i.e., partial dbRDA, Table 2). These analyses suggested that afforestation has changed the soil physicochemical properties, which has indirectly affected the soil microbiome.

### **Effects of soil properties on the divergence of microbial phyla between forest and grassland soils**

To identify the divergent soil microorganisms, the habitat-specialist microbial phyla were classified under the supermajority rule (i.e., 2/3 majority). At the phylum level, five phyla were habitat specialists, including one forest specialist (Thaumarchaeota, 2.3%) and four grassland specialists (Chloroflexi, Fibrobacteres, Nitrospirae, and Parcubacteria, 9.3%). The forest-specialist phylum Thaumarchaeota belongs to Archaea, while the four grassland specialists are Eubacteria.

We further tested the effects of soil properties on the abundance of these habitat specialists by GLM. Because organic C, P, Ca, Mg, and Fe significantly explained the RA of soil microbes in dbRDA, these five soil variables were used as independent predictors in GLM. The variances of the

microbial abundance of the sampled soils were significantly or marginally significantly greater than the means, suggesting overdispersion of these responses ( $P = 0.056, 0.001, 0.051, 0.002, \text{ and } 0.002$ ;  $\alpha = 52.675, 25.871, 1.065, 23.101, \text{ and } 76.612$  in Thaumarchaeota, Chloroflexi, Fibrobacteres, Nitrospirae, and Parcubacteria, respectively). Hence, we used the quasi-Poisson model for GLM, which led to the same coefficient estimates as the standard Poisson model but with adjustment of the dispersion parameter for overdispersion. Under the quasi-Poisson model, Fe content was marginally correlated with the abundance of the forest specialist Thaumarchaeota; for the grassland specialists, the P content was correlated with the abundances of all four phyla (Table 4). In addition, C, Ca, and Mg were significantly correlated with Chloroflexi, Fe was significantly correlated with Nitrospirae, and Ca and Mg were marginally correlated with Parcubacteria (Table 4).

#### **Afforestation effects on the soil ecophysiological functions predicted by soil microbiome**

To understand the ecophysiological functions of the soil microbiomes, we predicted their functional composition using 16S rRNA gene and databases of reference genomes. A total of 4659 clusters of orthologous groups (COGs) and 306 KEGG modules (Level-3 KEGG orthology) were identified. Similar to the analyses for testing the effects of afforestation and soil properties on the soil microbiome, we used vegetation type and soil elements as predictors to test the explanatory proportion and significance of each predictor on the RAs of the COGs and KEGG modules. dbRDA indicated that 22.0% and 21.2% of the variation of COGs and KEGG modules was significantly explained by vegetation type, respectively, whereas the explanatory proportion decreased to 3.4% and 4.5% when conditioning the soil-property effect (Table 5). Soil properties explained 83.0% and 82.4% of the variation of the COGs and KEGG modules in dbRDA, respectively, in which C, P, Ca, Mg, Fe, and N significantly or marginally fit the model according to type II ANOVA (Table 5). When conditioned on vegetation type, the explanatory proportion decreased slightly to 64.4% and 65.8% for the COGs and KEGG modules, respectively, and the remaining significant fitting factors

were P, Ca, and Fe (Table 5).

Among all COGs, 44 and 21 were identified as forest and grassland specialists, respectively, whereas among all KEGG modules, only two forest specialists and no grassland specialists were identified. Further testing of the correlation of soil elements with these specialist COGs and KEGG modules revealed that 50 of the 65 COGs and both KEGG modules were significantly correlated with at least one soil element under Poisson or quasi-Poisson regression. These significant correlations indicate that soil properties, particularly P, Ca, and Fe, account for the changes in ecophysiological functional due to grassland afforestation.

## Discussion

The closure of the tree canopy and increased litter accumulation that accompany the ecosystem change from grassland to forest may directly alter the soil environment (Bond & Midgley 2012; Cunningham et al. 2015). The significantly higher contents of soil physicochemical factors (C, P, Ca, Mg, Fe, and pH) in the Mongolian pine plantation areas than in the unplanted region suggest a great influence of grassland afforestation on secondary salinization. Soil mineral elements are usually increased in tree-plantation regions where groundwater is insufficient to meet water requirements (Nosetto et al. 2008). High contents of soil elements in a forest suggest not only a larger amount of litter biomass but also a rapid decomposition rate of pine litters compared to other broadleaf flora (Berger et al. 2015). However, despite significant differences in the contents of soil elements between forest and grassland, these soil elements, except Mg and Fe (Fig. 2), could not singly predict the ecosystem change due to afforestation by logistic regression analysis.

Significantly high predictable contents of Fe and Mg in forest soils reflect the characteristics of litter and humus accumulation in forests (Song et al. 2008). Litter decomposition accelerates the conversion and accumulation of soil non-organic elements (Fenchel et al. 2012b). In 2<sup>nd</sup>-to-3<sup>rd</sup>-year

needle litters of *P. sylvestris* L., a decrease in the rate of biomass loss but an increase in the release of Fe and Mg were recorded (De Marco et al. 2007). Consequently, we suggest that the high contents of Fe and Mg in the forest soils of our study sites are due to the long, steady accumulation and decomposition of needle litter. The soil element cycling affects and is affected by the composition of the soil microbiota (Fenchel et al. 2012b). For example, the iron bacteria *Siderocapsa* (Siderocapsaceae) and *Leptothrix* (Comamonadaceae), which are able to deposit iron metal oxides under natural conditions, can grow rapidly in iron-rich and acidic substrates (Fenchel et al. 2012a; Hanert 2006). The distributions of the soil microbial abundances in our samples best fit to Zipf and Zipf–Mandelbrot rank abundance models (Table S1 and Fig. 4). The Zipf and Zipf–Mandelbrot rank abundance models belong to the family of random-branching processes; these models suggest that individuals are always derived from ancestor individuals (McGill et al. 2007) and that microbial community assembly is explained by the niche-based mechanism (McGill et al. 2007; Mendes et al. 2014). These models indicate that decades of grassland afforestation have generated soil properties that provide a divergent but stable resource supply for the soil microbial community.

Classification by the supermajority rule revealed that generalist microbes represented more than 1/3 of the RA but only <3% of microbial OTUs. The low richness but relatively high abundance of generalists suggests that a great proportion of residents utilize broad resources or are highly tolerant of the environment (Verberk 2011). It has been suggested that microbes that are present in all or the majority of microbial communities with high abundance represent the core set of genes responsible for key elements of most metabolic pathways (Falkowski et al. 2008). Similarly, specialist microbes exhibited <1% richness but accounted for approximately 1/5 to 1/4 of the RA in the grassland and forest soils. These specialist OTUs with low richness and high abundance are probably more susceptible than generalists to environmental change. Since the original vegetation was scattered grasses, the grassland specialists rarely found in forest soils were those selected against by the

afforestation effect; by contrast, forest specialists should be enriched after forestation. The environmental differences (e.g., the contents of soil C, P, Ca, Mg, and Fe, Table 2) could result in resource (niche) divergence to differentiate the microbial composition descended from the original microbiome, reflecting the microbial abundance distribution in the Zipf and Zipf–Mandelbrot models (Table S1).

In particular, the Fe content was significantly correlated with the abundance of the forest-specialist Archaea phylum Thaumarchaeota, and P was correlated with the four grassland-specialist Eubacteria phyla Chloroflexi, Fibrobacteres, Nitrospirae, and Parcubacteria (Table 4), suggesting that these two soil elements are key factors differentiating soil microbial composition. encodes the genes ammonia monooxygenase A (*amoA*, encoding subunit A of AMO) and *amoB*, which are distantly related to one another (Stieglmeier et al. 2014). The high abundance of *amoA* and its transcripts suggests that ammonia-oxidizing Archaea (AOA) outnumber ammonia-oxidizing bacteria (Shen et al. 2008) and that nitrogen cycling is enhanced in the forest (Konneke et al. 2005; Stieglmeier et al. 2014). A high abundance of AOA with a high content of Fe (e.g., ferrate, an ammonia oxidation reagent) could accelerate ammonia oxidation (Sharma et al. 1998).

Soil P is closely related to plant growth (Shen et al. 2011). However, much of soil P is stable and insoluble and hence recalcitrant to uptake by plants (Holford 1997). Microbes transform P to improve uptake by plants, which is also influenced by a combination of factors, including plant species and soil type (Chen et al. 2008). However, the specialists that were correlated with soil P content were primarily not responsible for P transformation but were light and aerobic thermophiles (e.g., Chloroflexi), symbionts with ruminant animals (e.g., Fibrobacteres), or involved in the nitrogen cycle (e.g., Nitrospirae). Although not directly linked to P content, these microbial ecofunctions reflect the differences between grassland and forest ecosystems. The major changes in the canopy, amounts of litterfall, plant composition, and root-microbe interactions due to grassland afforestation may explain the differences in soil P content as well as the abundances of these

**Comentado [ADM20]:** This sentence is completely outside of the context.

**Comentado [ADM21]:** Please, re-write this sentence to be clear.

**Comentado [ADM22]:** Why the authors not determined *amoA* genes by qPCR and didn't analyzes the  $\text{NH}_4^+\text{-N}$  contents in the soil? This is a critical gap.

**Comentado [ADM23]:** P metabolism in the soil is more related to fungi community than bacteria, that's why you find no correlation. You should highlight this in your paper.



microbial phyla (Chen et al. 2008; Li et al. 2004).

By contrast, a high proportion of “too-rare” microbial OTUs (95% richness) occupied roughly  $>1/3$  of the RA of soil microbes, reflecting transient changes in microorganisms in the environments.

This rarity could result from stochasticity (Ai et al. 2013), fitness trade-off (Gobet et al. 2012; Gudelj et al. 2010), or biological interactions (García-Fernández et al. 2004; Narisawa et al. 2008; Schluter et al. 2015). These rare microbes are still relevant in ecological functions, including microbiome assembly and function and biogeochemical cycling (Jousset et al. 2017). The high richness of rare microbes contributes to the alpha-diversity of the soil microbiome. Although the ecological functions of these rare species in grassland or forest are not known, the high rarity provides adequate options for selection under environmental change, and these species may play an important role in maintaining fundamental ecosystem functions.

Based on the dbRDA of the relationships among vegetation type, soil properties, and microbial composition, we concluded that the grassland afforestation affected the soil physicochemical properties (Table 2) and soil microbiome composition and ecofunctions. In addition, these effects of afforestation on the microbiome were modulated by changes in soil physicochemical properties (Table 2 and Table 5). This conclusion was reached because the explanation of soil microbiome by vegetation type decreased or was even lost when conditioned on soil properties (Table 2). Albeit indirectly, afforestation indeed altered the ecofunction of the soil microbiome (Table 5).

As discussed above, soil physicochemical properties interact with the soil microbiome. Because the original vegetation before forestation was grassland, the changes in the soil properties are probably attributable to the vegetation changes produced by afforestation. Several studies have suggested that afforestation can influence biotic and abiotic changes in micro- and macro-ecosystems (Jousset et al. 2017; Noretto et al. 2008; Wang et al. 2016; Zheng et al. 2017). Here, we suggest that the underground biotic change was indirectly affected by forestation mediated by soil property changes

(Table 2), especially the contents of soil P, Ca, and Fe, which are further related to ecofunctional changes in soil microbiomes for different vegetation types (Table 5 and Table S2). These results are similar to the bacterial abundance changes and compositional shifts reported for a long-term poplar plantation, which were suggested to be highly correlated with the changes in soil properties caused by afforestation (Zheng et al. 2017). Soil bacterial composition has been suggested to be more closely related to plant diversity-controlled abiotic soil properties because of the highly resilient characteristics of bacterial communities due to their fast life cycle (de Vries et al. 2012; Lange et al. 2014).

## Conclusions

The change in vegetation type was linked, at least in part, to ecofunctional changes in the soil microbiome, despite an indirect impact on microbial composition. Aboveground changes in the canopy, the composition and abundance of litterfall, and biotic activities (e.g., succession rate, fauna activity, and root-microbe interactions) may be responsible for these changes in underground ecofunctions. However, the relatively small proportion of microbial specialists and high proportion of microbial generalists with respect to ecofunction compared to microbial OTUs indicates that the vegetation change still preserved a high proportion of the core functions of the soils (Li et al. 2004). This preservation occurred because the core functional genes were distributed widely across a variety of microbial taxa. However, the high proportion of functions that were correlated with changes in soil properties indicates that microbial ecofunctions are highly sensitive to environmental change.

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**Comentado [ADM24]:** I suggest that you try to re-write specific parts in conclusion, because this part should be appropriately stated, should be connected to the original question investigated, and should be limited to those supported by the results.

**Comentado [ADM25]:** You didn't analyze litterfall.

**Comentado [ADM26]:** You didn't analyze fauna activity.

**Comentado [ADM27]:** I highly recommend you include a sentence of "future prospects" here.

105-2628-B-003-001-MY3 and MOST 105-2628-B-003-002-MY3) (to PCL). This article was also subsidized by the National Taiwan Normal University (NTNU). We also thank Dr. Dawn Schmidt for English editing of the manuscript.

## References

- Ahrends A, Hollingsworth PM, Beckschäfer P, Chen H, Zomer RJ, Zhang L, Wang M, and Xu J. 2017. China's fight to halt tree cover loss. *Proceedings of the Royal Society B: Biological Sciences* 284: 20162559.
- Ai D, Chu C, Ellwood MDF, Hou R, and Wang G. 2013. Migration and niche partitioning simultaneously increase species richness and rarity. *Ecological Modelling* 258:33-39. <http://dx.doi.org/10.1016/j.ecolmodel.2013.03.001>
- Arnáez J, Lana-Renault N, Lasanta T, Ruiz-Flaño P, and Castroviejo J. 2015. Effects of farming terraces on hydrological and geomorphological processes. A review. *CATENA* 128:122-134. <http://dx.doi.org/10.1016/j.catena.2015.01.021>
- Berger TW, Duboc O, Djukic I, Tatzber M, Gerzabek MH, and Zehetner F. 2015. Decomposition of beech (*Fagus sylvatica*) and pine (*Pinus nigra*) litter along an Alpine elevation gradient: Decay and nutrient release. *Geoderma* 251-252:92-104. 10.1016/j.geoderma.2015.03.024
- Bond WJ, and Midgley GF. 2012. Carbon dioxide and the uneasy interactions of trees and savannah grasses. *Philosophical Transactions of the Royal Society B: Biological Sciences* 367:601-612.
- Burke C, Steinberg P, Rusch D, Kjelleberg S, and Thomas T. 2011. Bacterial community assembly based on functional genes rather than species. *Proceedings of the National Academy of Sciences* 108:14288-14293.
- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JL, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, and Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7:335-336. 10.1038/nmeth.f.303
- Chazdon RL, Chao A, Colwell RK, Lin SY, Norden N, Letcher SG, Clark DB, Finegan B, and Arroyo JP. 2011. A novel statistical method for classifying habitat generalists and specialists. *Ecology* 92:1332-1343. Doi 10.1890/10-1345.1
- Chen CR, Condron LM, and Xu ZH. 2008. Impacts of grassland afforestation with coniferous trees on soil phosphorus dynamics and associated microbial processes: A review. *Forest Ecology and Management* 255:396-409. <http://dx.doi.org/10.1016/j.foreco.2007.10.040>
- Chudzińska E, Diatta JB, and Wojnicka-Półtorak A. 2014. Adaptation strategies and referencing trial of Scots and black pine populations subjected to heavy metal pollution. *Environmental Science and Pollution Research* 21:2165-2177. 10.1007/s11356-013-2081-3
- Cibils L, Principe R, Márquez J, Gari N, and Albariño R. 2015. Functional diversity of algal communities from headwater grassland streams: How does it change following afforestation? *Aquatic Ecology* 49:453-466. 10.1007/s10452-015-9538-z
- Cole JR, Chai B, Farris RJ, Wang Q, Kulam-Syed-Mohideen AS, McGarrell DM, Bandela AM, Cardenas E, Garrity GM, and Tiedje JM. 2007. The ribosomal database project (RDP-II): introducing myRDP space and quality controlled public data. *Nucleic Acids Research* 35:D169-172. 10.1093/nar/gkl889
- Cunningham SC, Mac Nally R, Baker PJ, Cavagnaro TR, Beringer J, Thomson JR, and Thompson RM. 2015. Balancing the environmental benefits of reforestation in agricultural regions. *Perspectives in Plant Ecology, Evolution and Systematics* 17:301-317. <http://dx.doi.org/10.1016/j.ppees.2015.06.001>
- De Marco A, Vittozzi P, Rutigliano FA, and Virzo De Santo A. 2007. Nutrient dynamics during decomposition of four different pine litters. *OPTIONS MÉDITERRANÉENNES SÉRIE A: SÉMINAIRES MÉDITERRANÉENS* 75:73-77.
- de Vries FT, Manning P, Tallwin JRB, Mortimer SR, Pilgrim ES, Harrison KA, Hobbs PJ, Quirk H, Shipley B, Cornelissen

- JHC, Kattge J, and Bardgett RD. 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. *Ecology Letters* 15:1230-1239. 10.1111/j.1461-0248.2012.01844.x
- DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, and Andersen GL. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* 72:5069-5072. 10.1128/AEM.03006-05
- Edgar RC, Haas BJ, Clemente JC, Quince C, and Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27:2194-2200. 10.1093/bioinformatics/btr381
- Ewing B, and Green P. 1998. Base-calling of automated sequencer traces using Phred. II. error probabilities. *Genome Research* 8:186-194. 10.1101/gr.8.3.186
- Ewing B, Hillier L, Wendl MC, and Green P. 1998. Base-calling of automated sequencer traces using Phred.I. Accuracy assessment. *Genome Research* 8:175-185. 10.1101/gr.8.3.175
- Falkowski PG, Fenchel T, and Delong EF. 2008. The microbial engines that drive earth's biogeochemical cycles. *Science* 320:1034-1039.
- Fenchel T, King GM, and Blackburn TH. 2012a. Chapter 1 - Bacterial Metabolism. *Bacterial Biogeochemistry (Third Edition)*. Boston: Academic Press, 1-34.
- Fenchel T, King GM, and Blackburn TH. 2012b. Chapter 6 - Biogeochemical Cycling in Soils. *Bacterial Biogeochemistry (Third Edition)*. Boston: Academic Press, 89-120.
- García-Fernández JM, de Marsac NT, and Diez J. 2004. Streamlined regulation and gene loss as adaptive mechanisms in *Prochlorococcus* for optimized nitrogen utilization in oligotrophic environments. *Microbiology and Molecular Biology Reviews* 68:630-638.
- Gobet A, Boer SI, Huse SM, van Beusekom JEE, Quince C, Sogin ML, Boetius A, and Ramette A. 2012. Diversity and dynamics of rare and of resident bacterial populations in coastal sands. *ISME Journal* 6:542-553. <http://www.nature.com/ismej/journal/v6/n3/supinfo/ismej2011132s1.html>
- Gudelj I, Weitz JS, Ferenci T, Claire Horner-Devine M, Marx CJ, Meyer JR, and Forde SE. 2010. An integrative approach to understanding microbial diversity: from intracellular mechanisms to community structure. *Ecology Letters* 13:1073-1084. 10.1111/j.1461-0248.2010.01507.x
- Gunina A, Smith AR, Godbold DL, Jones DL, and Kuzyakov Y. 2017. Response of soil microbial community to afforestation with pure and mixed species. *Plant and Soil* 412:357-368. 10.1007/s11104-016-3073-0
- Hanert HH. 2006. The Genus *Siderocapsa* (and Other Iron- and Manganese-Oxidizing Eubacteria). In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, and Stackebrandt E, eds. *The Prokaryotes: Volume 7: Proteobacteria: Delta, Epsilon Subclass*. New York, NY: Springer New York, 1005-1015.
- Holford ICR. 1997. Soil phosphorus: its measurement, and its uptake by plants. *Australian Journal of Soil Research* 35:227-240. 10.1071/S96047 <http://www.publish.csiro.au/?paper=S96047>
- Hurme P, Repo T, Savolainen O, and Pääkkönen T. 1997. Climatic adaptation of bud set and frost hardiness in Scots pine (*Pinus sylvestris*). *Canadian Journal of Forest Research* 27:716-723. 10.1139/x97-052
- Hurme P, Sillanpää MJ, Arjas E, Repo T, and Savolainen O. 2000. Genetic basis of climatic adaptation in Scots pine by bayesian quantitative trait locus analysis. *Genetics* 156:1309-1322.
- Jangid K, Williams MA, Franzluebbers AJ, Schmidt TM, Coleman DC, and Whitman WB. 2011. Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. *Soil Biology and Biochemistry* 43:2184-2193. <http://dx.doi.org/10.1016/j.soilbio.2011.06.022>
- Jousset A, Bienhold C, Chatzinotas A, Gallien L, Gobet A, Kurm V, Kusel K, Rillig MC, Rivett DW, Salles JF, van der Heijden MGA, Youssef NH, Zhang X, Wei Z, and Hol WHG. 2017. Where less may be more: how the rare biosphere pulls ecosystems strings. *ISME Journal* 11:853-862. 10.1038/ismej.2016.174
- Khamzina A, Lamers JPA, and Martius C. 2016. Above- and belowground litter stocks and decay at a multi-species afforestation site on arid, saline soil. *Nutrient Cycling in Agroecosystems* 104:187-199. 10.1007/s10705-016-9766-1
- Klein DA, McLendon T, Paschke MW, and Redente EF. 1995. Saprophytic fungal-bacterial biomass variations in

- successional communities of a semi-arid steppe ecosystem. *Biology and Fertility of Soils* 19:253-256. 10.1007/BF00336168
- Konneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, and Stahl DA. 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* 437:543-546. [http://www.nature.com/nature/journal/v437/n7058/supinfo/nature03911\\_S1.html](http://www.nature.com/nature/journal/v437/n7058/supinfo/nature03911_S1.html)
- Krakau U-K, Liesebach M, Aronen T, Lelu-Walter M-A, and Schneck V. 2013. Scots Pine (*Pinus sylvestris* L.). In: Pâques LE, ed. *Forest Tree Breeding in Europe: Current State-of-the-Art and Perspectives*. Dordrecht: Springer Netherlands, 267-323.
- Lafleur B, Labrecque M, Arnold AA, and Bélanger N. 2015. Organic carbon accumulation in topsoil following afforestation with willow: emphasis on leaf litter decomposition and soil organic matter quality. *Forests* 6:769-793. 10.3390/f6030769
- Lange M, Habekost M, Eisenhauer N, Roscher C, Bessler H, Engels C, Oelmann Y, Scheu S, Wilcke W, Schulze E-D, and Gleixner G. 2014. Biotic and abiotic properties mediating plant diversity effects on soil microbial communities in an experimental grassland. *PLOS ONE* 9:e96182. 10.1371/journal.pone.0096182
- Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, Beiko RG, and Huttenhower C. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology* 31:814-821. 10.1038/nbt.2676 <http://www.nature.com/nbt/journal/v31/n9/abs/nbt.2676.html#supplementary-information>
- Li Q, Lee Allen H, and Wollum AG. 2004. Microbial biomass and bacterial functional diversity in forest soils: effects of organic matter removal, compaction, and vegetation control. *Soil Biology and Biochemistry* 36:571-579. <http://dx.doi.org/10.1016/j.soilbio.2003.12.001>
- Li Y, Zhao M, Mildrexler DJ, Motesharrei S, Mu Q, Kalnay E, Zhao F, Li S, and Wang K. 2016. Potential and Actual impacts of deforestation and afforestation on land surface temperature. *Journal of Geophysical Research: Atmospheres* 121:14,372-314,386. 10.1002/2016JD024969
- Lu S, Zhang Y, Chen C, Xu Z, and Guo X. 2017. Plant-soil interaction affects the mineralization of soil organic carbon: evidence from 73-year-old plantations with three coniferous tree species in subtropical Australia. *Journal of Soils and Sediments* 17:985-995. 10.1007/s11368-016-1602-3
- Ma E, Liu A, Li X, Wu F, and Zhan J. 2013. Impacts of vegetation change on the regional surface climate: A scenario-based analysis of afforestation in Jiangxi Province, China. *Advances in Meteorology* 2013:796163. 10.1155/2013/796163
- McGill BJ, Etienne RS, Gray JS, Alonso D, Anderson MJ, Benecha HK, Dornelas M, Enquist BJ, Green JL, He F, Hurlbert AH, Magurran AE, Marquet PA, Maurer BA, Ostling A, Soykan CU, Ugland KI, and White EP. 2007. Species abundance distributions: moving beyond single prediction theories to integration within an ecological framework. *Ecology Letters* 10:995-1015. 10.1111/j.1461-0248.2007.01094.x
- Mendes LW, Kuramae EE, Navarrete AA, van Veen JA, and Tsai SM. 2014. Taxonomical and functional microbial community selection in soybean rhizosphere. *ISME Journal* 8:1577-1587. 10.1038/ismej.2014.17
- Márquez JA, Cibils L, Principe RE, and Albariño RJ. 2015. Stream macroinvertebrate communities change with grassland afforestation in central Argentina. *Limnologica - Ecology and Management of Inland Waters* 53:17-25. <http://dx.doi.org/10.1016/j.limno.2015.05.002>
- Narisawa N, Haruta S, Arai H, Ishii M, and Igarashi Y. 2008. Coexistence of antibiotic-producing and antibiotic-sensitive bacteria in biofilms is mediated by resistant bacteria. *Applied and Environmental Microbiology* 74:3887-3894.
- Nosetto MD, Jobbágy EG, Tóth T, and Jackson RB. 2008. Regional patterns and controls of ecosystem salinization with grassland afforestation along a rainfall gradient. *Global Biogeochemical Cycles* 22:GB2015. 10.1029/2007GB003000
- Oleksyn J, Reich PB, Zytewski R, Karolewski P, and Tjoelker MG. 2003. Nutrient conservation increases with latitude of origin in European *Pinus sylvestris* populations. *Oecologia* 136:220-235. 10.1007/s00442-003-1265-9
- Pedley SM, Martin RD, Oxbrough A, Irwin S, Kelly TC, and O'Halloran J. 2014. Commercial spruce plantations support a limited canopy fauna: Evidence from a multi taxa comparison of native and plantation forests. *Forest Ecology and Management* 314:172-182. <http://dx.doi.org/10.1016/j.foreco.2013.12.010>

- Peng S-S, Piao S, Zeng Z, Ciais P, Zhou L, Li LZX, Myneni RB, Yin Y, and Zeng H. 2014. Afforestation in China cools local land surface temperature. *Proceedings of the National Academy of Sciences of the United States of America* 111:2915-2919.
- Ren C, Sun P, Kang D, Zhao F, Feng Y, Ren G, Han X, and Yang G. 2016. Responsiveness of soil nitrogen fractions and bacterial communities to afforestation in the Loess Hilly Region (LHR) of China. *Scientific Reports* 6:28469. 10.1038/srep28469  
<https://www.nature.com/articles/srep28469#supplementary-information>
- Savolainen O, Bokma F, García-Gil R, Komulainen P, and Repo T. 2004. Genetic variation in cessation of growth and frost hardiness and consequences for adaptation of *Pinus sylvestris* to climatic changes. *Forest Ecology and Management* 197:79-89. <http://dx.doi.org/10.1016/j.foreco.2004.05.006>
- Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, and Weber CF. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 75:7537-7541. 10.1128/AEM.01541-09
- Schluter J, Nadell CD, Bassler BL, and Foster KR. 2015. Adhesion as a weapon in microbial competition. *ISME Journal* 9:139-149. 10.1038/ismej.2014.174
- Sharma VK, Bloom JT, and Joshi VN. 1998. Oxidation of ammonia by ferrate(vi). *Journal of Environmental Science and Health, Part A* 33:635-650. 10.1080/10934529809376752
- Shen J-p, Zhang L-m, Zhu Y-g, Zhang J-b, and He J-z. 2008. Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam. *Environmental Microbiology* 10:1601-1611. 10.1111/j.1462-2920.2008.01578.x
- Shen J, Yuan L, Zhang J, Li H, Bai Z, Chen X, Zhang W, and Zhang F. 2011. Phosphorus dynamics: From soil to plant. *Plant Physiology* 156:997-1005.
- Šnajdr J, Dobíášová P, Urbanová M, Petránková M, Cajthaml T, Frouz J, and Baldrian P. 2013. Dominant trees affect microbial community composition and activity in post-mining afforested soils. *Soil Biology and Biochemistry* 56:105-115. <http://dx.doi.org/10.1016/j.soilbio.2012.05.004>
- Song B, Yin X, Zhang Y, and Dong W. 2008. Dynamics and relationships of Ca, Mg, Fe in litter, soil fauna and soil in *Pinus koraiensis*-broadleaf mixed forest. *Chinese Geographical Science* 18:284-290. 10.1007/s11769-008-0284-1
- State Forestry Administration of China. 2011. China National Progress Report to the UNFF Secretariat on the implementation of NLBI and other relevant resolutions. In: Administration SF, editor. Beijing: S. F. A. China.
- Stieglmeier M, Alves RJE, and Schleper C. 2014. The Phylum Thaumarchaeota. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, and Thompson F, eds. *The Prokaryotes: Other Major Lineages of Bacteria and The Archaea*. Berlin, Heidelberg: Springer Berlin Heidelberg, 347-362.
- Stutter MI, and Richards S. 2012. Relationships between soil physicochemical, microbiological properties, and nutrient release in buffer soils compared to field soils. *Journal of Environmental Quality* 41:400-409. 10.2134/jeq2010.0456
- Székely AJ, and Langenheder S. 2014. The importance of species sorting differs between habitat generalists and specialists in bacterial communities. *FEMS Microbiology Ecology* 87:102-112. 10.1111/1574-6941.12195
- Verberk WCEP. 2011. Explaining general patterns in species abundance and distributions. *Nature Education Knowledge* 3:38.
- Wang F, Zhu W, and Chen H. 2016. Changes of soil C stocks and stability after 70-year afforestation in the Northeast USA. *Plant and Soil* 401:319-329. 10.1007/s11104-015-2755-3
- Xiao H, Li Z, Dong Y, Chang X, Deng L, Huang J, Nie X, Liu C, Liu L, Wang D, Liu Q, and Zhang Y. 2017. Changes in microbial communities and respiration following the revegetation of eroded soil. *Agriculture, Ecosystems & Environment* 246:30-37. <https://doi.org/10.1016/j.agee.2017.05.026>
- Zechmeister-Boltenstern S, Keiblinger KM, Mooshammer M, Peñuelas J, Richter A, Sardans J, and Wanek W. 2015. The application of ecological stoichiometry to plant-microbial-soil organic matter transformations. *Ecological Monographs* 85:133-155. 10.1890/14-0777.1

Zheng J, Chen J, Pan G, Wang G, Liu X, Zhang X, Li L, Bian R, Cheng K, and Zheng J. 2017. A long-term hybrid poplar plantation on cropland reduces soil organic carbon mineralization and shifts microbial community abundance and composition. *Applied Soil Ecology* 111:94-104. <https://doi.org/10.1016/j.apsoil.2016.11.017>

#### Figure legend

**Fig. 1.** Tukey's Honest Significant Differences (HSD) test revealing significant differences in soil properties, i.e., contents of organic C, P, Ca, Mg, and Fe and pH, between the forest (Fr) and grassland (Gr) soils.

**Fig. 2.** A simple logistic regression model revealing significant prediction of the absence (0) and presence (1) of forestation based on the contents of the soil elements (a) Fe and (b) Mg.

**Fig. 3.** Divergence of the microbiome between the forest and grassland soils as revealed by (a) hierarchical cluster analysis and (b) discriminant analysis of principal components (DAPC).

**Fig. 4.** Rank-abundance dominance (RAD) model tests showing that the sampled microbial communities were best fit to the Zipf or Zipf–Mandelbrot model. The blue dots and the line in each panel are the observed values and the expectations simulated from the observed data, respectively. Gr and Fr indicate samples from the grassland and forest soils, respectively. The Zipf and Zipf–Mandelbrot models are both niche-based models. Rejection of the null model indicates rejection of the hypothesis of a stochastic process of microbial assembly.