Bacterial communities drive the resistance of soil multifunctionality to land use change

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Abstract

Bacterial communities play a key role in maintaining ecosystem multifunctionality. With increasing land use intensity, soil biogeochemical and microbial characteristics change significantly and may influence the resulting multifunctionality of the ecosystem. The relationship between soil microbial communities and resistance of multiple ecosystem functions under land use change have not previously been assessed in the Karst region of Southwest China. Soils from four karst ecosystems (Primary forest; Secondary forest; Abandoned land; Cultivated land) were analyzed for microbial communities as predictor of multifunctional resistance to land use change by using high-throughput sequencing, structural equation modelling and random forest modelling. The resistance of Proteobacteria was highest in the forest, and the resistance of Verrucomicrobia highest in the abandoned and cultivated land. With increasing land use intensity, C-cycling functional resistance decreased by 77%, nitrogen and phosphorus functional resistance increased by 17% and 19% in abandoned land, compared to secondary forest. Structural equation modellings suggested the bacteria communities have the largest direct positive effect on multifunctional, and N-related functional resistance. Among bacterial communities, Verrucomicrobia and Chloroflexi were the two most important groups that affected soil multifunctional resistance. Regrading specific ecosystem function, Chthonomonadetes, Chloroflexia and OPB35_soil_group were the best predictor of TOC,TN and TP, respectively. Our results suggest strong links between microbial community composition and multifunctional resistance in various karst ecosystems, and provide insights into the importance of microbial community composition for recovering the ecosystems following human intervention.

1 INTRODUCTION

Changes in land use, and its main vegetation cover, modify soil physico-chemical properties, including soil organic carbon content (Guillaume et al., 2015), phosphorous content and fractions (Maranguit et al., 2017). Land use change is a major driver of soil bacterial and fungal communities (Szoboszlay et al., 2017; Tian et al., 2017; Meng et al., 2019), leading to changes in microbial community composition. These changes are likely to influence ecosystem functioning (Sala et al., 2000; Lauber et al., 2008; Zhu et al., 2011; Xue et al., 2017). Increased land-use intensity, such as conversion of natural vegetation to arable agriculture, hampers disturbance-sensitive species, thereby selecting for species tolerant of such disturbance (Balmford, 1996; Williams et al., 2017). It leads to decreased populations and often to reduced soil microbial diversity (De Vries et al., 2012; Tsiafouli et al., 2015; Williams et al., 2017; Delgado-Baquerizo et al., 2017a; Newbold et al., 2018) as well as homogenization of microbial assemblage biodiversity across space (McKinney et

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al., 1999; Newbold et al., 2018; Tian et al., 2018). This effect have been consistently observed across the continental scale (Szoboszlay et al., 2017).

The effects of land use change on soil microbial communities has the potential to affect soil ecosystem multifunctionality and stability (Naeem & Li, 1997; Lefcheck et al., 2015; Maestre et al., 2015). Ecological stability consists of two components: resistance (the amount of change caused by a disturbance), and resilience (the speed with which a system returns to its pre-disturbance level) (Pimm, 1984).Recent studies have shown that changes in microbial properties, such as bacterial [?]-diversity (Jing et al., 2015) affected multifunctional resistance of ecosystem(De Vries & Shade, 2013; Luo et al., 2019). At the same time, microbial communities can regulate the multifunctional resistance of ecosystems to global change (Delgado-Baquerizov et al., 2017b). Microbial diversity is a strong predictor of ecosystem multifunctionality (Luo et al., 2017). Soil microbial communities drive biogeochemical processes, including carbon dynamics and nutrient transformation (Camenzind et al., 2018), litter decomposition (Delgado-Baquerizov et al., 2016), and plant productivity (Creamer et al., 2010). Increased bacterial abundance raises the protozoa abundance (Valencia et al., 2018) and conversely, reduces bacterial abundance is related to multifunctionality (Wagg et al., 2014). As a result, microbial function has a positive linear relationship with the multifunctional resistance of ecosystems (Luo et al., 2019).

Karst ecosystems account for 15-20% of global ice-free surface area (Hu et al., 2016). The karst area in southwestern China is one of the largest in the world (Liang et al., 2015). Widespread soil degradation observed in the southwest Chinese karst has been driven by rapid population growth, intensive agriculture and urbanization over the last 50 years (Huang & Cai,2006; Chen et al., 2011; Gao et al., 2013). Increasing land use intensity in the region has caused increased exposure of carbonate rock outcrops leading to loss and limitation of nitrogen and phosphorus nutrients (Zhang et al., 2015), decreasing soil organic carbon and microbial biomass carbon concentration in aggregates (Xiao et al., 2017). Recent studies have reported effects of land use change on soil microbial communities in the Chinese karst (Yun et al., 2016; Xiao et al., 2017; Wang et al., 2017; Li et al., 2018). However, direct evidence on how microbial communities regulate the multifunctional resistance of ecosystems following land-use change in karst areas is still lacking, particularly on the quantifiable impact of microbial communities on multifunctional resistance of ecosystems and the key bacterial taxa involved.

We hypothesize that (1) changes in land use reduces multifunctional resistance of ecosystems by affecting microbial communities; (2) the composition of microbial communities are a significant driver of ecosystem multifunctional resistance. A specific aspect is that oligotrophic bacteria have high resistance with increasing land use disturbance because they are well adapted to soils with low substrate availability (Fierer et al., 2007; Tian et al., 2017). To test our hypotheses, the present study focused on the link between bacterial community structure and ecosystem multifunctionality along a land-use change gradient in the karst region. We (1) evaluated multifunctional resistance of the soil ecosystems and the resistance of major bacterial taxa and (2) determined the main predictors of multifunctional resistance of ecosystems and quantified the ability of specific bacterial groups to predict both multifunctional and single function resistance of ecosystems including carbon, nitrogen, and phosphorus cycling.

2 MATERIALS AND METHODS

2.1 Study area and soil sampling

The sampling site was located in Puding county, Anshun city, southwest Guizhou Province, China (26°22'–26deg26'N and 105deg34'–105deg41'E). The region is characterized by a subtropical monsoon climate, with annual average sunshine of 1165 h, annual average temperature of 15.1degC, frost-free period of 301 d, and annual average rainfall of 1378 mm. The karst landforms in the territory are widely developed and complete. The soil type is limestone soil according to Chinese soil general classification, which is similar to Mollic Inceptisols according to USDA Soil Taxonomy (Soil Survey Staff, 2010) .

Four ecosystems covering different land-use intensity levels were selected according to the degree of anthropogenic perturbation: (1) primary forest (PF, undisturbed natural vegetation, dominant plant species:

Lithocarpus confinis, Itea yunnanensis and Carpinus pubescens); (2) secondary forest (SF, without culti-vation and fertilization >15 y, dominant plant species: Rhus chinensis, Populus adenopoda Maxxim and Rosa cymosa); (3) abandoned land (AL, without cultivation and fertilization <6 y, dominant plant species: Conyza canadensis, Imperata cylindrica and Artemisia dubia); (4) cultivated land (CL, cultivation and fer-tilization with urea/compound fertilizer with some excreta, dominant plant species: Brassica napus and Zea mays). In April 2017, soil samples (n = 6) from 0-10 cm depth were collected using a trowel in each land use type. In brief, Typical plots (10 mx20 m) of each land-use type were sampled from six established sites at six slope positions at least 30 m apart along one altitudinal transect. The 4 replicate soil samples taken at each site were composited to provide one representative sample for each site. The fresh soil samples were sieved to 2 mm and visible roots and stones were removed. For each samples, one portion was air-dried and used for soil chemistry and mineral content analyses, while the other portion was immediately frozen at -80 degC for subsequent microbial analyses.

2.2 Soil physiochemical analyses

Soil pH was determined in 1:2.5 (weight:volume) suspensions by ULTRAMETERII6PFCE (Myron L, CA, USA). The samples used for elemental analysis of iron (Fe), calcium (Ca), magnesium (Mg), aluminium (Al) and sulphur (S) were prepared by dissolution in HCl-HNO3-HF-HClO4 (Zhao et al., 2016) and analyzed using an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 5300 DV, Perkin Elmer, Waltham, MA, USA).

2.3 Characterizing soil microbial communities

For each sample, DNA was extracted from 0.25 g of frozen soil (Power soil DNA Isolation Kit, MoBio Laboratories, Carlsbad, CA, USA), and quantified by spectrophotometry (Nanodrop Technologies, USA). The V3-V4 hypervariable regions of bacterial 16S ribosomal RNA (rRNA) encoding gene were amplified using the primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'- GGACTACHVGGGTWTCTAAT-3'). PCR was performed in triplicate using the following thermal program: 95 degC for 3 min, followed by 27 cycles at 95 degC for 30 s, 55 degC for 30 s, 72 degC for 45 s and a final extension at 72 degC for 10 min. The PCR products were subjected to electrophoresis on 2% agarose gels for detection, purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and then quantified using QuantiFluor-ST (Promega, USA).

Illumina next-generation amplicon sequencing was used to sequence the samples (Illumina MiSeq platform, Illumina, San Diego, CA, USA) . Raw sequences >200 bp with an average quality score >20 and without ambiguous base calls were quality processed, using the Quantitative Insights into Microbial Ecology (qiime) pipeline (version 1.17). Operational taxonomic units (OTUs) were picked at 97% sequence similarity. The resultant OTU abundance tables from these analyses were rarefied to an even number of sequences per samples to ensure equal sampling depth (14371 for 16S rDNA)

2.4 Assessing multiple ecosystem functions and the resistance of multifunctionality to human disturbance

On all soil samples, thirteen functions related to C, N and P cycling were measured: activity of extracellular soil enzymes α-1,4-glucosidase (αG), β-1,4-glucosidase (βG), β-xylose (βX), cellulose diglucosidase (CBH), N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP) and phosphatase (AP), dissolved and total organic carbon (C), total nitrogen (N), extractable ammonium (NH_4^+) and nitrate (NO_3^-) concentrations and total P concentration (TP). The total C and N contents, enzyme activities, inorganic nitrogen concen-trations and AP and TP contents were obtained using methods as described by Wang et al. (2019) and presented in Table S1. Collectively, these variables constitute a good proxy for the processes contributing to nutrient cycling (Maestre et al., 2012).

We then used the Orwin & Wardle (2004) index (RS) to evaluate the multifunctionality resistance as:

RS$=1 - \frac{2|D0|}{(C0+|D0|)}$ (1)

where, D0 is the difference between the natural ecosystem (C0, value of each functional variable of primary

forest) and the disturbed ecosystem (P0, secondary forest, abandoned land or cultivated land) soils. This index has the advantage of being: (1) standardized by the control and (2) bound between -1 (lowest resistance) and +1 (maximal resistance) even when extreme values are encountered (Orwin & Wardle 2004). The resistance of each function was calculated independently for each land-use intensity level. In addition, the resistance of the thirteen functions measured was then averaged for each land-use intensity level to obtain a standard index of multifunctionality resistance, which is more integrative than the response of individual functions and better reflects overall ecosystem functioning. The same method has been used to determine indexes of multistability (Durán, et al., 2017) as well as multifunctionality (Delgado-Baquerizo et al., 2016). Finally, the resistance of bacterial taxa also was calculated using equation (1). In this case D0 is the difference between the natural ecosystem (C0, bacterial relative abundance of primary forest) and the disturbed ecosystem (P0, secondary forest, abandoned land or cultivated land) bacterial relative abundance.

2.5 Statistical analyses

Structural equation modelling (SEM; Grace 2006) was used to evaluate the direct and indirect relationships between land-use intensity, soil pH, soil elemental characteristics(Fe, Mg, Ca S and Al) and soil microbial composition (phylum- and class-level predictors) and [?]-diversity on multifunctional resistance. The overall goodness-of-fit of the SEM results were evaluated by Chi square test (?2 ; the model has a good fit when 0 [?] ?2 /df [?] 2 and 0.05 < P [?] 1.00), root mean square error of approximation (RMSEA; the model has a good fit when 0 [?] RMSEA [?] 0.05 and 0.10 < P [?] 1.00 –Schermelleh-Engelet al., 2003). Additionally, the standard total effects (STE) was calculated for each variables included in the SEM.

To gain a mechanistic understanding of the drivers of resistance of multifunctionality, within the bacterial community, we conducted a Random Forest classification analysis (Breiman 2001; Delgado-Baquerizo et al., 2016) to identify the best class-level microbial predictors based on the relative abundance of bacterial taxa. We analyzed the importance of soil microbial communities as the predictor of both multifunctional resistance and to individual functions. These analyses were conducted using the random forest package (R Core Team, 2019) of the R statistical software, version 3.0.2. The significance of the importance of each predictor was assessed using the rfPermute package (Archer, 2016).

3 RESULTS

3.1 Resistance of ecosystem multifunctionality and major microbial taxa

After conversion from primary forest to the other three land use, we found similar multifunctional resistance among SF, AL and CL (Fig. 1). C-cycling functional resistance decreased by 77%, nitrogen and phosphorus functional resistance increased by 17% and 19% in abandoned land, compared to secondary forest. Cultivated land had the lowest N functional resistance, while SF had lowest P functional resistance (Fig. 1).

At the level of the major bacterial phylum, in addition to Verrucomicrobia, the groups with relatively high abundance had higher resistance than the groups with low relative abundance, and the proteobacteria (p<0.001) had the highest resistance (Fig. 2). Land-use intensity levels affected the resistance of the major bacterial phylum (Fig. 3a). Compared with SF, the resistance of Actinobacteria (p < 0 .001), Acidobacteria (p = 0.001) and Chloroflexi (p = 0.049) were 40%, 11% and 78% lower, respectively, in AL, and 41%, 8.0% and 53% lower in CL. In contrast, Verrucomicrobia (p<0.001) was 49% more resistant in AL than in SF.

Significant effects of land-use intensity levels were also found for bacterial multifunctionality resistance at the class level (Fig. 3b). Deltaproteobacteria, Actinobacteria and Alphaproteobacteria had the highest resistance (Fig. 2). Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Thermoleophilia showed a pattern of lesser resistance in AL and CL than in SF (p < 0.05, Fig. 3b). In contrast, Actinobacteria was more resistant in AL (42%, p < 0.05) as well as in CL (10%, p < 0.05) compared to SF.

3.2 Linkage of bacteria communities with resistance of ecosystem multifunctionality

NMDS ordination of the bacterial Bray-Curtis distance matrices clearly separates the PF and SF from the AL and CL (Fig. 4a). Soil physicochemical properties and soil enzyme activities were also clearly separated

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by land-use in the PCA plot (Fig. 4b) but, in contrast to the NMDS plot, the two forest sites (PF and SF) were not grouped close together. Structural equation modelling was constructed to explore the direct and indirect effects of land-use and bacterial communities on multifunctional resistance (Fig. 4c). The bacterial communities (NMDS1, NMDS2, $p<0.05$) showed the largest direct positive effect on multifunctional and N-related functional resistance (Fig. 4e, Fig. S1). The positive direct effect of soil Mg content (path coefficient = 1.029) on C-functional resistance was the greatest, followed by bacterial community structure and Fe content (Fig. 4d, Fig. S1). Besides bacterial community, land use presented as the second main predictor influencing the N functional resistance via direct (path coefficient = 0.676) and indirect effects (path coefficient = -0.237) on soil pH, minerals and bacterial community (Fig. 4d, Fig.S1). Among soil variables, the positive direct effect of bacterial community on P functional resistance was the greatest, while Mg was related to the largest negative indirecteffect (Fig. 4f, Fig. S1).

3.3 Bacterial groups predicting multifunctional resistance of ecosystem and soil single function

To further elucidate the role of bacterial community structure in driving multifunctional resistance, we used Random Forest modelling to quantify the influence of bacterial abundance at both phylum and class level on multifunctional resistance of the different ecosystems in the land-use gradient (Fig. 5). The model explained 46.4% variance on multifunctional resistance (Fig. 5). The minimum cross-validation error was obtained when using 20 important classes, And the number of classes against the cross-validation error curve stabilized (Fig. 5a). Thus, we defined these 20 classes as biomarker taxa in the model. The list of the top 20 bacterial taxa at the class level across multifunctional resistance, in order of multifunctional resistance importance, is shown in Fig.5a. Verrucomicrobia showed the highest contribution to multifunctional resistance (Fig. 5a), and was mainly present in secondary forest and abandoned land (Fig. 5b). This was followed in importance by Chloroflexia, Anaerolineae and Chloroplast (Fig. 5a), which started to accumulate in the AL and CL stages (Fig. 5b). We also found Thermoleophilia and Alphaproteobacteria showed a contribution to multifunctional resistance (Fig. 5a), and started to accumulate in PF and SF (Fig. 5b). These indicates that some biomarker taxa showed high relative abundance in the corresponding land-use gradient (Fig. 5b).

Based on 20 bacterial groups as important predictors identified by random forests(Fig. 5a), we further predicted the soil single function (e.g. TOC, TN, and TP contents) and listed the corresponding significant microbial species (Fig. 6, $p<0.05$).Our Random Forest models indicate that the relative abundance of Chthonomonadetes and OPB35_soil_group were the best predictors of TOC and DOC, respectively. The relative abundance of Chloroflexia, Alphaproteobacteria and OPB35_soil_group were the best predictors of TN, NH4+ and NO3-, respectively. OPB35_soil_group was also the best predictor for TP.

4 DISCUSSION

4.1 The resistance of multifunctional and bacterial groups under different land use intensity

Our study showed that land-use change affected the resistance of ecosystem functions related to C, N and P cycling, resulting in changes in ecosystem multifunctional resistance as proposed in our first hypothesis. It is interesting that the main bacterial species (>3%) are the same different land uses. This indicates that the bacterial community has self-recovering ability during the succession process (Da C Jesus et al., 2009). However, the resistance of bacterial groups differed among different land use (Fig. 3). This is consistent with previous studies that different groups showed particular responses to land use change (Shange et al., 2012; Griffiths, 2012; Gomez-Acata et al., 2016). The responses are generally classified as copiotroph and oligotroph (Fierer et al., 2007). Proteobacteria and Actinobacteria can be categorized as copiotrophic, being well adapted to nutrient-rich environment (Zhang et al., 2016; Tian et al., 2017;Zheng et al., 2019). Studies have shown that the higher the organic matter content and pH in forest soil, the more the system tends to have larger relative populations of Actinomycetes and Proteobacteria (De Vries et al., 2012; Ramirez et al., 2014). In this study, the resistance of Actinomycetes and Proteobacteria in secondary forests were the highest phylums (Fig. 3a), which is consistent with the higher TOC content in secondary forests than that in soils that have been cleared and used for agriculture (AL and CL). Alphaproteobacteria, Betaproteobacteria and Deltaproteobacteria at class level were in accord with the Proteobacteria at phylum level (Fig. 3b). In

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contrast to copiotrophic groups, Acidobacteria grow rapidly in low nutrient environments (Navarrete et al., 2015a; Zheng et al., 2019), and is generally classified as oligotroph. Therefore, the resistance of Acidobacteria in abandoned land is also higher than in SF in this study (Fig. 3a). And at the class level, the resistance of Acidobacteria is consistent with their position at the phylum level (Fig. 3b). Verrucomicrobia has higher relative abundance in cultivated and abandoned land (Fig. 3a), being categorized as oligotroph and having strong resilience in low nutrient conditions (Battistuzzi & Hedges, 2009; Pan et al., 2014). Chloroflexi has strong adaptability in low-carbon soils (ability to survive desiccation and low nutrient availability conditions; Battistuzzi, 2009). Bacterial resistance corresponds to its correlation with its abundance (Fig. 2), that is, the stronger the resistance of the bacteria, the higher the abundance in the soil, the resistance and abundance of the bacteria have a close relationship with land use. This indicates that microbial communities can regulate the stability of ecosystems based on the resistance of the bacteria.

4.2 The linkage of the composition of microbial communities and ecosystem multifunctional resistance

The SEM analysis showed important effects of bacterial community structure on the multifunctional resistance of ecosystems, confirming our second hypothesis. This is consistent with previous studies emphasizing that multifunctionality resistance was regulated by changes in microbial composition (Delgado-Baquerizo et al.,2017b). In the response to global climate change, microbial functional capacity displayed a positive linear relationship with multifunctionality resistance, and buffered negative impacts on ecosystem functioning caused by climate change via regulating rich microbial communities (Luo et al., 2019). Our research indicates that in addition to protecting ecosystems against impacts of climate change, microbial communities also have a regulatory effect on the negative effects of land use change on ecosystem functions.

Of 98 bacterial classes selected through random forest models, 20 bacterial species were identified as the main predictors of multifunctional resistance (Fig.5a). This indicates that multifunctionality resistance is driven by multiple key microbial taxa, consistent with De Vries & Shade (2013). Consistent with our second hypothesis, oligotrophic bacteria (Verrucomicrobia and Chloroflexi) contributed more to predicting the multifunctional resistance of ecosystems than copiotrophic bacteria (Fig. 5a). Among bacterial species, OPB35_soil_group (phylum Verrucomicrobia) contributed the most (Fig. 5a), and were also selected as main drivers of soil single function (including DOC, TN, NO3- and TP; Fig. 6). Research shows Verrucomicrobial abundance is extremely sensitive to changes in chemical factors linked to soil fertility, such as soil total carbon, nitrogen, and phosphorus (Navarrete et al., 2015b), which supports our research. At the same time, Verrucomicrobia may exert a great impact with regard to nitrogen availability in certain ecosystem, including oligotrophic environments (Wertz et al., 2011). Chloroflex is another main predictor of multifunctional resistance, together with 8 other taxa at class level (Fig.5a). Chloroflexia and Anaerolineae were significantly positively correlated with β-1,4-glucosidase and phosphatase, promoting starch degradation and mineralization of organic phosphorus (Table S2). Ardenticatenia was significantly positively related to N-acetylglucosaminidase, which promotes the degradation of chitin. These findings support previous studies that oligotrophic bacteria promote the resistance of functions related to C cycle (De Vries & Shade 2013; Trivedi et al., 2013), but our research further shows that oligotrophic bacteria also contribute to the nitrogen and phosphorus cycles of the ecosystem. Actinobacteria and Proteobacteria, as copiotrophic bacteria (Navarrete et al., 2015c; Zheng et al., 2019), also play an important role in predicting multifunctional resistance (Fig. 5a). Thermoleophilia affected ecosystem functions by affecting starch degradation, leucine decomposition and organic phosphorus mineralization (Table S2). Our research showed Thermoleophilia not only significantly predicted multifunctional resistance (Fig. 5a), but also TOC, TN, TP (Fig. 6). Furthermore, Actinobacteria are significantly related to the total nutrient content in the soil and play an important role in soil carbon and nitrogen storage. Alphaproteobacteria contribute more for NH_4^+ concentrations (Fig. 6), catalyzing the rate of N cycle and mediating multifunctionality resistance by regulating NH_4^+. At the same time, cyanobacteria are not only predictors of multifunctional resistance (Fig. 5a), but also predictors of TOC and DOC (Fig. 6), which indicates that they also participate in the soil carbon cycle. Chamizo et al. (2018) considered that they play multiple roles in soil including synthesis of extracellular polysaccharides and contributing to accumulation of total organic carbon and total nitrogen. In addition, previous studies have shown that functional genes associated with nitrification and denitrification are the major predictors of multifunctional resistance (Luo

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et al., 2019); consistent with this, our research also shows that the nitrifier Nitrospira (phylum Nitrospirae) can predict multifunctionality (Fig. 5a).

4.3 Soil pH and elements driving multifunctional resistance

Besides soil bacterial communities, the SEM further indicated a significant effect of soil pH and certain elemental concentrations on multifunctional resistance (Fig. 4). pH is an important driving factor affecting many soil properties and microbial populations and acttivities (Fierer et al., 2007) and could affect multifunctional resistance via direct and indirect effects (Li et al., 2019). In this study, pH had a significant negative effect on N-related function resistance (Fig. 4e). This may be because pH significantly influenced substrate availability (Kemmitt et al., 2006).Pearson correlation analysis shows that pH has a negative effect on NO3- and NAG (Fig. S2), indicating that in neutral or slightly alkaline soils (average pH of the soil in the study area is 7.09), pH can reduce NO_3^- content and nitrogen functional enzyme activity to reduce N-functional resistance.

Soil vegetation cover and land management directly influence the cycling of chemical elements, and are key factors for soil biogeochemistry and also Al behavior in soil (Tejnecký et al., 2020). We found significant differences in soil Al, Mg and Ca concentration between land uses and these had significant effects on functional resistance (Fig. 4). Don et al. (2011) considered that the accumulation of some mineral elements has a negative impact on multifunctional resistance. Our study also showed that Al had a negative effect on multifunctional resistance (Fig. 4), which may be because Al reduced the activity of soil functional enzymes responsible for carbon, nitrogen and phosphorus transformations (Fig. S2). At the same time, the differences in soil organic matter content affect the differences in cation exchange capacity, especially Al_3^+ (Jiang et al., 2018). In our study, aluminum was positively correlated with TOC, DOC (Fig. S2), this explained the promoting effect of aluminum on C-functional resistance (Fig. 4). Similar to Al, Mg also promotes resistance to carbon function. This is because Mg can increase the content of DOC in the soil (Zhu et al., 2019), significantly correlated with DOC content (Fig. S2).We also found that soil Ca was significantly related to N-functional resistance (Fig. 4) and positively related to soil nitrogen content (Fig. S2).Previous studies have shown a positive correlation between soil Ca and nitrogen metabolism (Tang et al., 2019), soil Ca_2^+ was beneficial to nitrogen saturation and nitrate leaching in long-term soil nitrogen enrichment (Perakis et al., 2013), and Ca is related to the process of soil microbial nitrogen-fixation (Xie et al., 2016).

5 CONCLUSIONS

In summary, there is a strong relationship between the bacterial community structure and the multifunctional resistance of soil. The most significant predictor of multifunctional resistance within the ecosystems studied is the relative abundance of oligotrophic bacteria, Verrucomicrobia and Chloroflexi being the most important. Regrading specific ecosystem functions, Chthonomonadetes, Chloroflexia and OPB35_soil_group were the best predictor of total organic carbon, total nitrogen and total phosphorus, respectively. The interplay among bacterial community and soil attributes modulates the rates of soil nutrient cycles, which promotes the ultimate maintenance of multifunctional resistance. In summary, this study suggests that maintain microbial diversity can mitigate the negative impacts of land use on multifunctional resistance of ecosystem.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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