Science of the Total Environment 626 (2018) 48–58

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Testing association between soil bacterial diversity and soil carbon storage on the Loess Plateau

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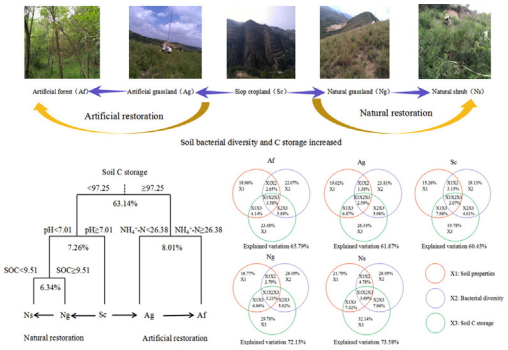
HIGHLIGHTS

- High-throughput 16S rRNA sequencing was used for soil bacterial community composition.
- Soil C storage and soil bacterial diversity increased due to vegetation restoration.
- A strong relationship between the dominant bacterial groups and soil C storage
- Soil bacterial diversity is closely related to soil C storage on the Loess Plateau.

GRAPHICAL ABSTRACT



Soil bacterial diversity was closely related to soil C storage on the Loess Plateau.

ARTICLE INFO

Article history:
Received 21 May 2017
Received in revised form 20 December 2017
Accepted 9 January 2018
Available online 12 January 2018

Editor: Ouyang Wei

Keywords:
Soil bacterial diversity
Soil C storage
Land use types
Loess Plateau
High-throughput sequencing

ABSTRACT

Bacteria are widely distributed and play an important role in soil carbon (C) cycling. The impact of soil bacterial diversity on soil C storage has been well established, yet little is known about the underlying mechanisms and the interactions among them. Here, we examined the association between soil bacterial diversity and soil C storage in relation to vegetation restoration on the Loess Plateau. The dominant phyla among land use types (artificial forest, Af; natural shrubland, Ns; artificial grassland, Ag; natural grassland, Ng; slope cropland, Sc) were *Acidobacteria*, *Actinobacteria*, *Alphaproteobacteria*, and *Betaproteobacteria*, which transited from *Acidobacteria*-dominant to *Actinobacteria*-dominant community due to vegetation restoration. Soil C storage and the Shannon diversity index of soil bacterial community ($H_{Bacteria}$) showed the order Ns > Ng > Af > Ag > Sc, whereas no significant difference was found in Good's coverage ($p > .05$). Further, a strong relationship was observed between the relative abundance of dominant bacterial groups and soil C storage ($p < .05$). Additionally, soil bacterial diversity was closely related to soil C storage based on the structural equation model (SEM) and generalized additive models (GAMs). Specifically, soil C storage had the largest deterministic effects, explaining >70% of the variation and suggesting a strong association between soil C storage and soil bacterial diversity. Overall, we propose that further studies are necessary with a focus on the soil bacterial groups with specific functions in relation to soil C storage on the Loess Plateau.

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https://doi.org/10.1016/j.scitotenv.2018.01.081
0048-9697/

1. Introduction

Soil bacteria, one of the most abundant and diverse groups of microorganisms, play a vital role in regulation of ecological processes, such as soil carbon (C) cycling from the Earth Microbiome Project (EMP, http://www.earthmicrobiome.org) (Lloydprice et al., 2017; Thompson et al., 2017). Soil C-fixing bacteria are widespread in soil, indirectly altering the rate of soil C sequestration and C storage. For example, *Bradyrhizobium japonicum*, *Mycobacterium* sp., and *Burkholderia* sp., also harbor the *cbbL* gene which can thus fix C via the Calvin cycle, resulting in the increase of soil C storage (Könneke et al., 2014; Lynn et al., 2016). In turn, soil C storage in terrestrial ecosystems considers as an overarching factor for soil bacteria and constitutes an important component of the global C balance (Kennedy and Smith, 1995; Torn et al., 1997; Treseder and Allen, 2000; Xia et al., 2016). Most soil bacteria rely on soil C storage to obtain energy, so there was a closely relationship between soil bacteria and soil C storage. Consequently, a large number of studies have shown the close links between soil bacteria and soil C storage (Horner-Devine et al., 2004; Wardle et al., 2004; Torsvik and Øvreås, 2002). For example, soil bacteria are directly responsible for the turnover and decomposition of soil organic matter and therefore contribute to the enhancement of soil C storage (Lange et al., 2015; Ling et al., 2017). Meanwhile, soil bacteria indirectly affect soil C storage by increasing soil aggregation due to the degradation of microbial byproducts (Exbrayat et al., 2014; van Groenigen et al., 2014). In recent years, traditional explanatory theories have focused on the stabilization, decomposition, and transformation of soil C storage owing to the growing interest in soil C cycling (Pan et al., 2009; Liu et al., 2010; Berlemont et al., 2014). It is generally accepted that the magnitude of soil C storage is dependent on microbial involvement (Burke, 2015; Doetterl et al., 2015; Lange et al., 2015), as soil C storage is ultimately affected by soil bacterial diversity and community composition (Treseder and Allen, 2000; Nave et al., 2010; Lange et al., 2015). Although soil bacterial diversity and community composition have been largely examined (Lupwayi et al., 1998; Zhao and Gillet, 2011; Bissett et al., 2013; Sun et al., 2015; Yao et al., 2017a; Yao et al., 2017b), there are still unclear gaps in understanding of the relationship between soil C storage and soil bacterial diversity.

China's Loess Plateau is one of the deepest loess deposits and also one of the most eroded areas in the world (Fu et al., 2017). Last century, increasing population pressure and environmental damage resulted in the serious degradation in this region. Government launched a series of ecosystem deterioration engineering projects in the 1980s (Deng et al., 2014; Feng et al., 2016; Fu et al., 2017). Since 1999, the Grain-for-Green program has made remarkable contribution to China's vegetation restoration, which aims to restore degraded ecosystem services, by improving carbon sequestration, soil conservation and reducing floods. Now, the Loess Plateau has become the most successful ecological restoration zone (Fu et al., 2017). Following the Grain-for-Green and Natural Forest Protection projects for vegetation restoration, large loessial areas of sloping farmland have been converted to artificial forest and grassland (artificial vegetation restoration) or natural grassland and shrubs (natural vegetation restoration) (Chen et al., 2015; Feng et al., 2016; Fu et al., 2017). In this case, soil C storage is a critical index for evaluating the efficiency of vegetation restoration, and soil bacteria are vulnerable to vegetation restoration (Jin et al., 2014; Feng et al., 2017). Hundreds of studies have reported that soil C storage has greatly increased due to vegetation restoration in this region (Chen et al., 2007; Wang et al., 2010; Feng et al., 2013; Deng et al., 2014). While there is considerable disagreement on soil bacterial diversity in relation to vegetation restoration (Houghton et al., 1999; Post and Kwon, 2000; Guo and Gifford, 2002). In fact, a wide range of biotic and abiotic factors influence soil bacterial diversity, including soil type, climate, nutrient management, and the decomposition of soil microorganisms (Nair and Ngouajio, 2012; Heijden and Wagg, 2013). For example, Zeng et al. (2016) reported that soil bacterial diversity was closely related to the edaphic properties on the Loess Plateau. Similarly, a strong relationship between soil nutrients and soil bacterial diversity was found in natural grassland, and soil nutrients contributed a great deal to soil bacterial diversity (Zhang et al., 2015). By contrast, some surveys have found that environmental conditions were determinants in regulating soil bacterial diversity, and this discrepancy could be attributed to soil C storage at large scales (Liang et al., 2017; Xu et al., 2017). At regional scale, different mechanisms have been proposed to explain how soil bacterial diversity affects soil C storage, although some studies also revealed that soil bacteria had some resilience to disturbance (Hartzog et al., 2017; Samaritani et al., 2017). Thus, a central issue in microbial ecology is to investigate the interactions between soil bacterial diversity and soil C storage on the Loess Plateau.

To examine the association between soil bacterial diversity and soil C storage, five land use types (artificial forest, Af; natural shrubland, Ns; artificial grassland, Ag; natural grassland, Ng; slope cropland, Sc) on the Loess Plateau were selected. Three objectives of this study were to (i) determine and compare the soil bacterial diversity and soil C storage in relation to vegetation restoration, (ii) explore the dominant factors shaping soil bacterial diversity and soil C storage, and (iii) test the association between soil bacterial diversity and soil C storage. Therefore, we tested three hypotheses: (i) soil bacterial diversity and soil C storage are related to vegetation restoration, (ii) the dominant driving factors (soil properties) can contribute to soil bacterial diversity and soil C storage, and (iii) soil C storage is positively associated with soil bacterial diversity.

2. Materials and methods

2.1. Sampling areas

We carried out this study in Zhifanggou (Yanhe river) catchment in Ansai county (36°46′28″–36°46′42″N, 109°13′03″–109°16′46″E), located in the middle of the Yellow river on the Loess Plateau. The study area occupies a total area of approximately 8.72 km^2 and has a semi-arid climate and a deeply incised hilly-gully Loess landscape with heavy seasonal rainfall and periodic flooding. Hills cover 90% of the region, and with the steep slopes (40%) near cliffs, only 7% of this area can be used in agriculture. The average annual rainfall from 1970 to 2000 was approximately 497 mm, and there are distinct rainy and dry seasons. The rainy season occurs from July to October, with the August rainfalls amounting to >20% of the annual total. The average annual temperature was 9.1 °C along the elevation gradient. Most of the area lies at an altitude between 900 m and 1500 m and the zonal soil in this region is Calcaric Cambisol according to FAO-UNESCO Soil Map of the World (FAO and ISRIC, 1988) or Orthic Entisol according to Chinese Soil Taxonomy (Cooperative Research Group on Chinese Soil Taxonomy, 2001), which developed directly from the parent wind-deposited yellow material.

We selected 45 sites within five land use types: artificial forest, Af; artificial grassland, Ag; natural shrubland, Ns; natural grassland, Ng; slope cropland, Sc. These land use types initially developed from similar parental material and under the same climate but were eventually changed by differences in long-term land-use regimes. In addition, there were no signs of fire or natural disasters in this area over the past several decades based on historical sources. The loess is perfectly arable due to its fine grains, loose texture and high content of mineral nutrients. The types of vegetation in this area include artificial restoration (Af, Ag) on account of the Grain-for-Green Project since 1974 and natural restoration (Ns, Ng) on account of the prevention of grazing by fencing since 1938.

2.2. Experimental design

A field survey was conducted at the peak of the growing season in 2016 between July and September. The sampling sites were located at

least 100 m apart. Each land use type had nine replicate sites, and we established homogeneous 100 × 100 m sites. Within each site, 15 × 15 m, 5 × 5 m, and 1 × 1 m in size plot were established in forest, shrub land, grassland, respectively, and five quadrats along a diagonal line were surveyed (Fig. 1). We divided soil samples into five depths: 0–20, 20–40, 40–60, 60–80 and 80–100 cm. Three replicates along an S-shaped curve were taken by using a soil corer (10 cm in diameter) and mixed to obtain one composite soil sample. Each soil sample was divided into three parts: one part was immediately stored at −80 °C using liquid nitrogen for DNA analysis, one part was used to measure soil water content (SW, %) by oven drying in aluminum containers, and the last part was sieved through a 2-mm mesh, air dried, then analyzed for soil C content ($g \cdot kg^{-1}$) and other properties. Soil bulk density (BD, $g \cdot cm^{-3}$) was calculated depending on the inner diameter of the core sampler, the sampling depth (0–20, 20–40, 40–60, 60–80 and 80–100 cm) and the oven-dried weight of the composite soil samples (105 °C for 48 h). In addition, a Global Position System (GPS) receiver (ST1000-1A, China) was used to obtain the basal gradient information, such as latitude, longitude, and altitude. A description of the plant characteristics, soil properties and land-use regime of the different land use types is provided in Supplementary Table S1.

2.3. Soil properties and C storage

Soil organic carbon (SOC, $g \cdot kg^{-1}$) was measured using the $K_2Cr_2O_7$-H_2SO_4 oxidation method (Nelson and Sommers, 1982). Soil pH was determined in a 1:2.5 (v/v) soil water aqueous extract. Soil total nitrogen (TN, $g \cdot kg^{-1}$) was quantified using the Kjeldahl method (UDK 140 Automatic Steam Distilling Unit, Automatic Titroline 96, Italy) (Bremner and Mulvaney, 1982). Soil total phosphorus (TP, $g \cdot kg^{-1}$) was measured using the molybdenum antimony colorimetric method. Soil available phosphorus (AP) was measured using the molybdenum blue method (Olsen et al., 1982). NH_4^+-N was measured using a Seal AutoAnalyzer (AA3 HR, Germany). Soil microbial biomass C and N (MBC, MBN, $mg \cdot kg^{-1}$) were measured using the fumigation-extraction method and calculated using the correction factors 0.35 (kC) and 0.4 (kN) (Brookes et al., 1985).

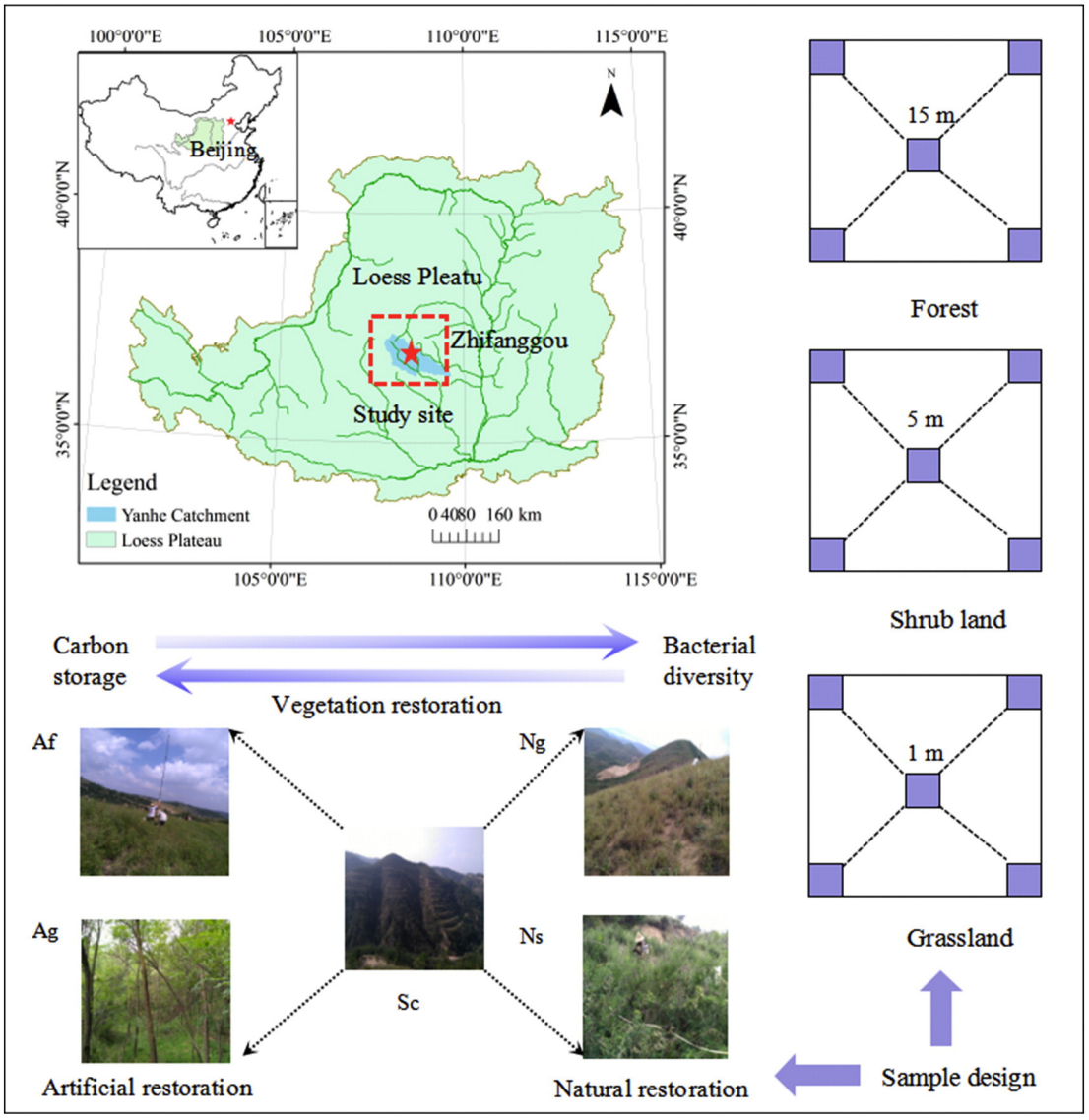

Fig. 1. Location of the study area and layout of the plots in terms of vegetation restoration. The pictures were generated using ArcMap version 10.2 (http://www.esri.com/). artificial forest, Af; natural shrubland, Ns; artificial grassland, Ag; natural grassland, Ng; slope cropland, Sc.

Soil C storage (in the 0–100 cm layer) was calculated by multiplying the soil depth (cm), soil bulk density ($g \cdot cm^{-3}$) and C content ($g \cdot kg^{-1}$).

Soil C storage was calculated as follows (Guo and Gifford, 2002):

$$\text{Soil C storage } (g \cdot m^{-2}) = SOC \times BD \times D \times 10$$

where SOC represents soil organic carbon concentration ($g \cdot kg^{-1}$), D represents soil thickness (cm), and BD means soil bulk density ($g \cdot cm^{-3}$).

2.4. Soil bacterial diversity and community composition

2.4.1. Soil bacterial DNA analysis

First, DNA was extracted from 0.5 g freeze-dried soil samples using a Mo Bio Power Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, USA) based on the manufacturer's protocol. Second, the quantity and quality of extracted DNAs were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. Finally, DNA was concentrated using a FLUOstar Optima (BMG Labtech, Jena, Germany) and stored at −80 °C for further molecular analysis.

2.4.2. Amplification and sequencing of soil DNA

Each soil DNA sample was amplified in triplicate by targeting the bacterial 16S rRNA gene using qPCR (BECKMAN AMPure XP beads), and the primer was then separated using electrophoresis in 1% agarose gel. PCR amplification of the bacterial 16S rRNA genes V4–V5 region was performed using the forward primer 515F (5′-GTGCCAGCMG CCGCGGTAA-3′) and the reverse primer 907R (5′-CCGTCAATTCM TTTRAGTTT-3′). Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. The PCR components contained 5 μl of Q5 reaction buffer (5×), 5 μl of Q5 High-Fidelity GC buffer (5×), 0.25 μl of Q5 High-Fidelity DNA Polymerase (5 U/μl), 2 μl (2.5 mM) of dNTPs, 1 μl (10 μM) of each Forward and Reverse primer, 2 μl of DNA Template, and 8.75 μl of ddH2O. Thermal cycling consisted of initial denaturation at 98 °C for 2 min, followed by 25 cycles consisting of denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension of 5 min at 72 °C. PCR amplicons were purified with Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). All raw sequences were deposited in the NCBI Sequence Read Archive under accession number SRP20170119. After the individual quantification step, amplicons were pooled in equal amounts, and pair-end 2 × 300 bp sequencing was performed using the Illlumina MiSeq platform with MiSeq Reagent Kit v3 at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

2.4.3. Processing of sequencing data

The raw sequences of all the soil samples were processed using various software tools on the Galaxy platform (Obi et al., 2017). Paired-end reads were assigned based on their unique barcodes. Sequences were analyzed using the QIIME software package (http://qiime.sourceforge.net) (Quantitative Insights Into Microbial Ecology, version 1.8.0), and then soil bacterial diversity indexes were calculated using in-house Perl scripts. The abundance-based coverage estimator (ACE) and Chao1 estimator were calculated, and then rarefaction curves were plotted using MOTHUR (http://www.mothur.org/). The identified species in each soil sample were analyzed using phylogenetic diversity (PD) and phylotype richness with the phylogenetic diversity whole tree metric. High-quality sequences were clustered into operational taxonomic units (OTUs) with 97% sequence similarity, and representative OTUs that were the most abundant in each soil sample were selected using PyNAST (DeSantis et al., 2006; Caporaso et al., 2010) and UCLUST.

2.5. Statistical analysis

All of the variables are presented using the mean ± standard deviation (SD). Analyses and testing were conducted using SAS software 9.3 (SAS Institute Inc., Cary, NC, USA). The software Origin 9.2 (IBM Corporation, Armonk, NY, USA) was used for plotting. We tested the normality and homogeneity of the variances and then conducted parametric tests using Fisher's least significant difference (*LSD*) test at $p < .05$ and $p < .01$. The explanatory power of the model was assessed based on significance (p-value) and the coefficient of determination (R^2). Phylogenetic diversity (PD) was estimated by using Chao1 (Chao, 1984) and Faith's index (Faith, 1992), and the abundance-based coverage estimator (ACE) and Chao1 estimator were calculated in the same way, providing several integrated indexes for the phylogenetic breadth among taxonomic levels (Chao, 1984; Faith, 1992; Turnbaugh et al., 2009). Matrices of the pairwise taxonomic distance (Bray-Curtis) and Euclidean distances among variables were constructed in R package 'vegan' (v.3.2.0, https://www.r-project.org/). Based on the calculated Bray-Curtis distances, principal coordinates analysis (PCoA) was used to analyze the distribution of soil bacterial community. Spearman's rank correlation analysis between soil properties and soil bacterial diversity was performed. The relationships between soil bacterial diversity and soil properties were tested using linear regression analysis by a significance level of 95% in SAS; then, the best-fitting models were performed in Origin. To identify the relative contributions of soil properties to soil bacterial diversity, a multivariate regression tree was applied. In addition, generalized additive models (GAMs) were constructed using the 'gam' package in R (v.3.2.0, https://www.r-project.org/). Prior to this analysis, forward selection was performed for three explanatory variable groups: soil properties, soil bacterial diversity and soil C storage, and these variable groups were treated as the independent variables in the final model. A structural equation model (SEM) was constructed in AMOS (version 20.0), and Mantel R values were used as the input variation. This technique is well suited to assessing the relationships among networks of variables, where variables can act as both predictor and response variables simultaneously, In this case, the direct and indirect effects of combinations of factors on soil C storage were calculated, including the significant regression weights from plausible interaction pathways. In SEM, we removed non-significant variables that demonstrated the lowest Akaike information criterion (AIC) values and assessed the fit of the regression models using chi-squared tests and the root mean square error of approximation (RMSEA, $p < .05$).

3. Results

3.1. Soil C storage and soil bacterial diversity in relation to vegetation restoration

We found that soil C storage was largely influenced by land use type (Fig. 2). Soil C storage showed the largest proportion in Ns (natural shrub) and Ng (natural grassland) compare with Sc (slope cropland) ($p < .05$), with the order of Ns > Ng > Af > Ag > Sc, but Ns and Ng did not significantly differ ($p > .05$). There was a similar vertical distribution of soil C storage among land use types, which gradually decreased with the increasing of soil depth. The highest soil C storage among land use types was detected in the 0–20 cm soil layer, varying within the range of 14.2–32.8 $g \cdot m^{-2}$. Soil C storage in the 80–100 cm soil layer was 26.8%–45.7% lower than that in the 0–20 cm soil layer. In addition, soil C storage showed the largest proportion in the 0–40 cm soil layer, suggesting that the main soil C storage occurred in the 0–40 cm soil layer across all land use types. In each soil layer, soil C storage also showed the order Ns > Ng > Af > Ag > Sc with slight fluctuation.

On the basis of high-throughput sequencing data, soil bacterial abundance and community composition are presented in Table 1. The results revealed with 13,407 sequences in total, and each soil sample had 1623-3501 sequences. Among soil bacteria, 586 genera were

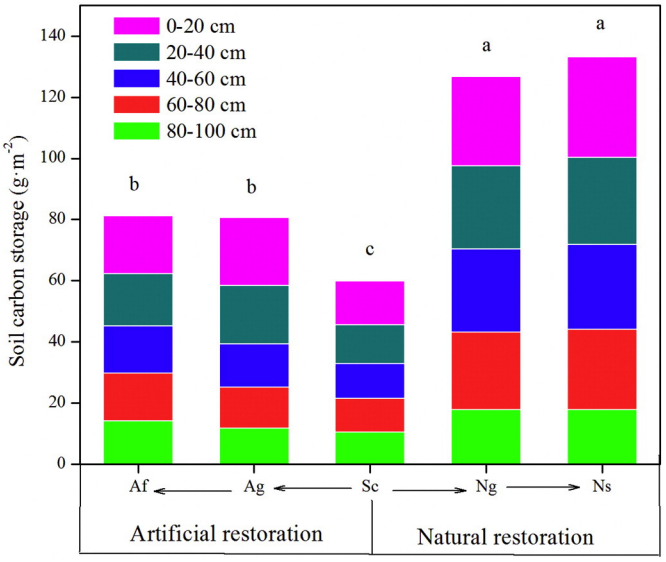

Fig. 2. Soil C storage among land use types. Different letters indicate a significant difference at the 0.05 level.

found with 99.1% explanation, belonging to 27 phyla, 119 classes, 213 orders, and 317 families. In addition, the lower bacterial diversity was found in relation to vegetation restoration. Natural restoration (Ns, Ng) had the higher soil diversity compared to the other land use types, while Heip's evenness index was lower compared to the other land use types ($p < .05$). Overall, ACE and $H_{Bacteria}$ showed the same trend with the order of Ns > Ng > Af > Ag > Sc. In contrast, Heip's evenness index showed the opposite order of Ns < Af < Ng < Ag < Sc, and there was no significant difference in Good's coverage among land use types ($p > .05$). Besides, we used rarefaction to compare the levels of soil bacterial diversity, as shown in Supplementary Fig. S1. For the Chao1 and PD estimators, natural restoration (Ns, Ng) and artificial restoration (Af, Ag) resulted in the higher values than Sc ($p < .05$), and there was a large significant difference among land use types for PD ($p < .05$). The total number of OTUs varied widely among land use types, and there were significant differences among land use types except for Ns and Ng ($p < .05$). Further, ternary plots showed the distribution of $H_{Bacteria}$ among land use types (Fig. 3). Intriguingly, $H_{Bacteria}$ was enriched following artificial restoration (Af, Ag) and natural restoration (Ns, Ng) compared to Sc, indicating that soil bacterial diversity largely increased due to vegetation restoration, and the effect of natural restoration on soil bacterial diversity was greater than that of artificial restoration.

3.2. Soil bacterial community composition in relation to vegetation restoration

We compared the relatedness among soil bacterial community using the unweighted pair group method (Supplementary Fig. S2). The dominant phyla across all land use types were *Acidobacteria*, *Actinobacteria*, *Alphaproteobacteria*, and *Betaproteobacteria*, accounting for >85% of all bacterial sequences. In addition, *Bacteroidetes* and *Gammaproteobacteria* were present in most soils but at relatively low abundances, and five other rarer phyla were identified (Supplementary Table S2). Further, the abundances of *Acidobacteria, Actinobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Bacteroidetes* and *Gammaproteobacteria* showed the order Ns > Ng > Af > Ag > Sc (Fig. 4A).

Similar results were revealed when the data were analyzed using principal coordinates analysis (PCoA) (Fig. 4B). The ordination results from PCoA identified the effects of soil properties on soil bacterial community composition. In the ordination plot, the first two axes explained 41.36% and 32.56% of the total variation in soil bacterial community composition. Through canonical variation partitioning, it was observed that soil C storage and pH were the major contributors to soil bacterial community variation. In addition, SOC, TN, NH_4^+, MBC, and MBN were significantly positively related to the relative abundance of dominant bacterial groups, such as *Acidobacteria*, *Actinobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, while negatively related to the relative abundance of *Bacteroidetes* and *Gammaproteobacteria*. AP was not significantly related to the relative abundance of the dominant bacterial groups (Fig. 4B). Of all the soil properties tested, soil pH showed the highest correlation coefficient (longest arrow) with soil community composition. Therefore, we can conclude that soil pH is the main dominant factor that drives soil bacterial community composition.

3.3. Relationship between bacterial diversity and soil C storage

Mantel test revealed significant correlations between soil bacterial diversity and soil properties, and Spearman correlation coefficients are listed in Table 2. Soil pH was negatively correlated with soil bacterial diversity (Heip's evenness index, $H_{Bacteria}$, ACE index, Good's coverage, PD, OTUs, and Chao1 index), and SOC, NH_4^+-N, soil C storage were significantly positively correlated with soil bacterial diversity ($p < .05$). However, AP and TP had no significantly correlation with soil bacterial diversity ($p > .05$). Additionally, there was a positive relationship between soil C storage and soil bacterial diversity ($p < .05$), suggesting that soil C storage have a large effect on soil bacterial diversity among land use types. Besides, a strong linear regression analysis between the relative abundance of specific phyla and soil C storage was performed, as shown in Fig. 5 ($p < .05$).

Table 1
Illumina MiSeq sequenced bacterial data based on the 16S rRNA gene and the diversity of soil bacterial communities among land use types. Different letters indicate significant differences ($p < .05$) for the individual factors based on a one-way ANOVA followed by an *LSD* test. $H_{Bacteria}$: Shannon diversity index value of bacterial community.

Item	Artificial forest (Af)	Artificial grassland (Ag)	Slope cropland (Sc)	Natural grassland (Ng)	Natural shrubland (Ns)
Quality sequences	2369	2617	1623	3297	3501
Number of phylotypes	516	484	493	552	586
Phyla	17	21	13	24	27
Classes	109	113	99	117	119
Orders	201	169	171	212	213
Families	309	302	291	313	317
Soil bacterial diversity					
Heip's evenness index	0.093 ± 0.008^{c}	0.112 ± 0.007^{b}	0.121 ± 0.013^{a}	0.115 ± 0.009^{ab}	0.089 ± 0.005^{c}
$H_{Bacteria}$	6.94 ± 1.23^{b}	6.78 ± 1.58^{b}	6.61 ± 0.36^{b}	7.51 ± 1.74^{a}	7.73 ± 1.05^{a}
ACE index	4652 ± 169^{b}	3924 ± 237^{c}	3887 ± 324^{c}	4357 ± 298^{b}	5269 ± 351^{a}
Coverage	0.999 ± 0.018^{a}	0.996 ± 0.013^{a}	0.994 ± 0.024^{a}	0.999 ± 0.078^{a}	1.002 ± 0.035^{a}
PD	72.76 ± 6.21^{b}	64.15 ± 5.85^{b}	53.08 ± 4.27^{c}	88.47 ± 6.93^{a}	96.59 ± 7.24^{a}
OTUs	935.1 ± 52.4^{c}	827.4 ± 68.2^{d}	662.5 ± 92.1^{e}	1262.0 ± 83.1^{b}	1451.2 ± 75.7^{a}
Chao1 index	3461.8 ± 256.5^{b}	2820.6 ± 368.6^{c}	2154.2 ± 341.2^{d}	4322.7 ± 358.6^{a}	4850.6 ± 412.3^{a}

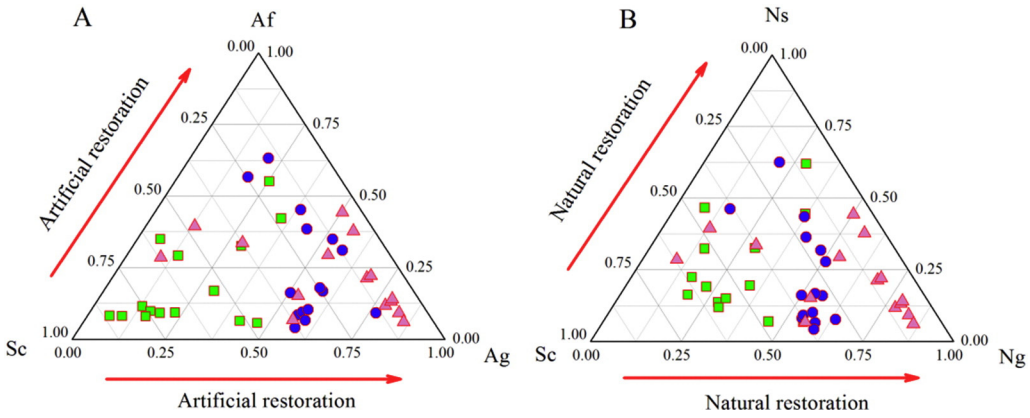

Fig. 3. Ternary plots depicting $H_{Bacteria}$ of artificial restoration (A, n = 15) and natural restoration (B, n = 15). Each point corresponds to $H_{Bacteria}$. Colored point represent $H_{Bacteria}$ enriched in one compartment compared with the others. The green points represent $H_{Bacteria}$ in Sc with no significant differences among land use types, the blue circles represent $H_{Bacteria}$ in Ag/Ng showing a significantly higher relative abundance, and the pink points represent $H_{Bacteria}$ in Af/Ns that had a significantly higher relative abundance, respectively.)

3.4. Structural equation model (SEM) of soil bacterial diversity and soil C storage

PCoA analysis and Spearman's correlation coefficients showed that soil C storage, soil pH, SOC and NH_4^+-N were the dominant driving factors to soil bacterial diversity. Thus, we selected soil C storage, soil pH, SOC and NH_4^+-N to make the multivariate regression analysis (Fig. 6A). The results explained 72.36% of the total variation, and soil C storage had the greatest deterministic effects, contributing 63.14% of the overall variation. In addition, soil bacterial diversity can be divided into two major groups based on soil C storage: Ns, Ng, Sc (group 1) and Ag, Af (group 2), the latter of which was then divided into two major groups based on the single parameter of soil pH. Additionally, SOC and soil pH played minor roles in soil bacterial diversity. In addition, variation partitioning analysis was conducted to examine the relative contributions to soil bacterial diversity (Fig. 6B). A subset of soil properties (soil C storage, SOC, soil pH, and NH_4^+-N) was selected; these properties together explained 73.81% of the total variation, whereas soil C storage alone explained 36.79%, leaving 36.19% unexplained. Of all the selected soil variables, SOC, soil pH, NH_4^+-N and other factors individually explained 13.48%, 15.12%, 5.89%, and 2.44% of the observed variation, respectively.

The combined stepwise regression and generalized additive models (GAMs) demonstrated that soil C storage is related to soil bacterial diversity (Fig. 7). Thus, the final structural equation model (SEM) was fitted to describe the pathways of interaction among soil properties, soil C storage and soil bacterial diversity (F = 157.89, df = 45, $p < .001$, GFI = 0.026, AIC = 97.16, RSMEA = 0.026). The variation in the explained factors of was >60% among all of land use types. Among the three variable groups, soil C storage had the highest variation, and soil bacterial diversity variation was higher than the variation in soil properties regardless of land use types. On the basis of the standardized values of statistically significant SEM paths, we obtained the direct, indirect and total effects on soil C storage (Table 3). Soil C storage was directly affected by soil properties (direct pathway effect of 0.811 and indirect pathway effect of 0.439) and indirectly affected by soil bacterial diversity (the direct pathway effect was 0.819, and the indirect pathway effect was 0.467). In these direct and indirect pathway effects, soil pH, SOC, $H_{Bacteria}$ and PD had the highest total pathway effect (0.158, 0.214, 0.204 and 0.168, respectively). In these direct and indirect pathway effects, SOC, Heip's evenness index, and PD had the highest total pathway effects (0.124, 0.127, and 0.104, respectively).

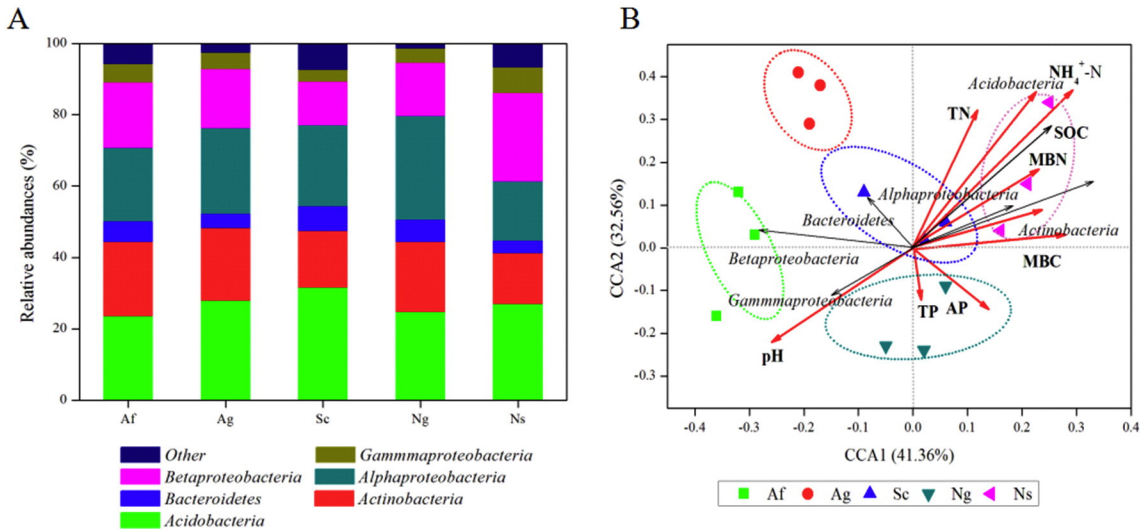

Fig. 4. Relative abundances of the dominant bacterial taxa at the phylum level in soils separated according to land use type. Relative abundances are based on the proportional frequencies of those DNA sequences that could be classified at the phylum level (A); Principal coordinates analysis (PCoA) of the bacterial community based on the dominant bacterial phyla (B).

Table 2
Spearman correlations (r) between soil properties and bacterial community composition (Bray-Curtis distance) determined by the Mantel test.

Item		pH	SOC ($g \cdot kg^{-1}$)	TN ($g \cdot kg^{-1}$)	TP ($g \cdot kg^{-1}$)	AP ($mg \cdot kg^{-1}$)	NH_4^+ ($mg \cdot kg^{-1}$)	MBC ($mg \cdot kg^{-1}$)	MBN ($mg \cdot kg^{-1}$)	Soil C storage ($g \cdot m^{-2}$)
Heip's evenness index	r	0.235	−0.023	−0.057	−0.158	0.147	−0.412*	−0.023	0.041	−0.569*
	p	0.078	0.123	0.156	0.197	0.265	0.002	0.569	0.512	0.000
$H_{Bacteria}$	r	−0.569*	**0.698****	0.069	0.147	0.054	**0.623****	0.126	0.125	**0.799****
	p	0.000	0.000	0.612	0.587	0.623	0.000	0.698	0.532	0.000
ACE index	r	**−0.689****	**0.741****	0.047	0.203	0.178	0.526*	0.231	0.025	**0.689****
	p	0.000	0.000	0.514	0.485	0.432	0.000	0.358	0.489	0.000
Coverage	r	−0.023	0.512*	0.230	0.147	0.156	0.230	0.159	0.302	0.236
	p	0.566	0.000	0.456	0.417	0.403	0.359	0.364	0.302	0.125
PD	r	−0.569*	**0.725****	0.023	0.149	0.265	0.601*	0.369	0.044	**0.781****
	p	0.000	0.000	0.621	0.589	0.205	0.000	0.255	0.603	0.000
OTUs	r	**−0.699****	0.501*	0.236	0.418	0.047	**0.687****	0.198	0.237	**0.688****
	p	0.000	0.000	0.147	0.087	0.678	0.000	0.104	0.156	0.000
Chao1 index	r	**−0.712****	**0.687****	0.025	0.241	0.325	**0.621****	0.158	0.243	**0.623****
	p	0.000	0.000	0.625	0.342	0.098	0.000	0.321	0.126	0.000

Note: Calculations based on the OTUs at 97% sequence similarity. r and p represent Spearman's correlation coefficient and the significance value, respectively (*, $p < .05$; **, $p < .01$). Values in bold indicate significant correlations.

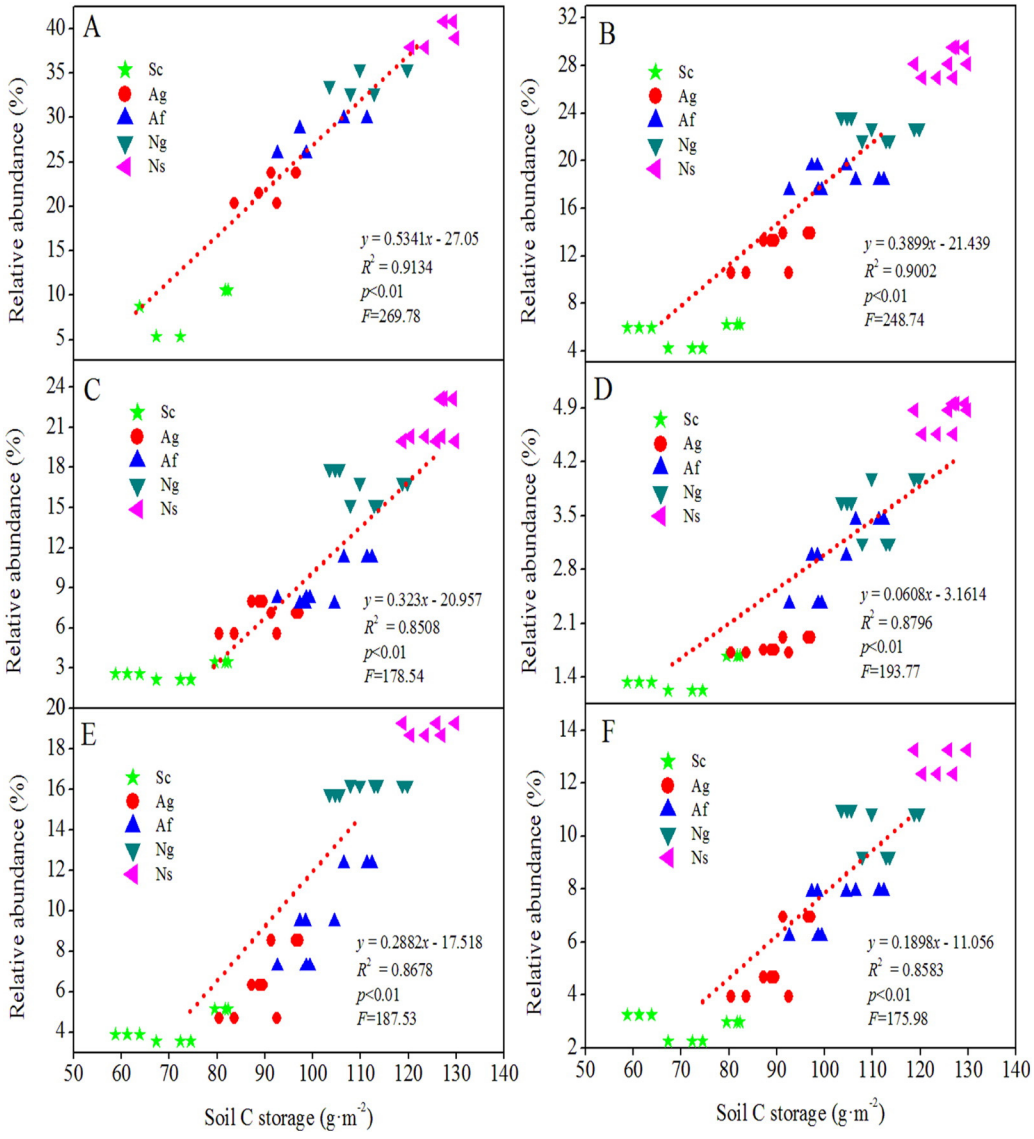

Fig. 5. Relationships between the relative abundances of dominant bacterial groups and soil C storage with symbols coded by land use type. Linear regressions were used to test the correlation between the relative abundances among taxa and soil C storage.

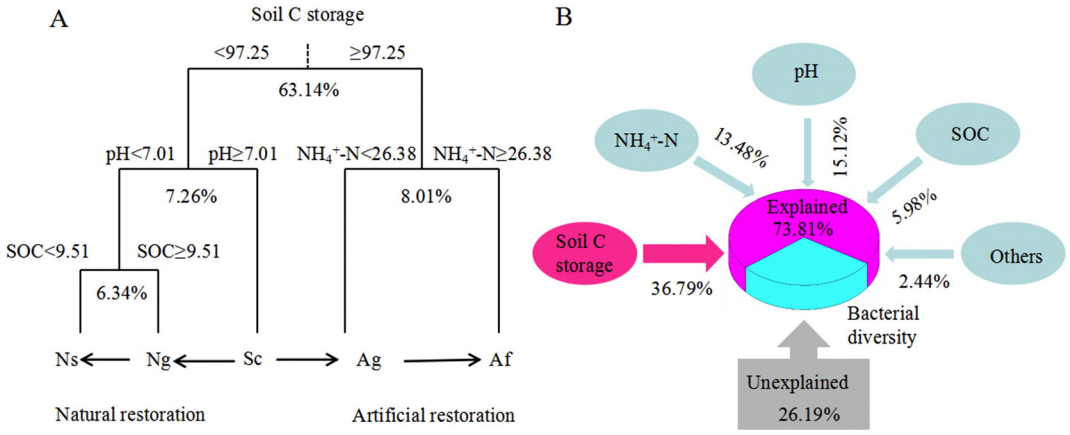

Fig. 6. Multivariate regression tree analysis of soil bacterial diversity among land use types. Numbers under the crosses of each split indicate percentages of variance explained by the split (A). Variation partitioning analysis of the effects of soil properties on the bacterial diversity (B).

4. Discussion

4.1. Soil C storage and soil bacterial community composition

This study investigated the effects of soil bacterial diversity and soil C storage by vegetation restoration on the Loess Plateau. The results supported the first hypothesis that vegetation restoration has significant effects on soil C storage and soil bacterial diversity (Fig. 2). These findings do agree with previous studies (Lehmann et al., 2008; Fu et al., 2011; Cheng et al., 2015), showing the higher soil C storage and soil bacterial diversity in natural restoration (Ns, Ng) than artificial restoration (Af, Ag). This may be associated with the relatively higher plant diversity in natural restoration, and the rich species provide more organic matter for soil bacteria (Schmidt et al., 2011; Liang et al., 2017; Xu et al., 2017).

In addition, large numbers of studies reported that there have been a lower soil C storage and bacterial diversity on the Loess Plateau (Fu et al., 2011; Deng et al., 2014; Zhang et al., 2015; Zeng et al., 2016). Thus, an interesting question has arisen regarding how changes in soil bacterial diversity and soil C storage due to vegetation restoration in this region. We found that *Acidobacteria*, *Actinobacteria*, *Alphaproteobacteria*, and *Betaproteobacteria* are the dominant phyla across the study area regardless of land use types (Fig. 3), which is consistent with previous findings (Sun et al., 2015; Burke, 2015; Yao et al., 2017a; Yao et al., 2017b). *Actinobacteria*, a phylum of gram-positive bacteria that help to

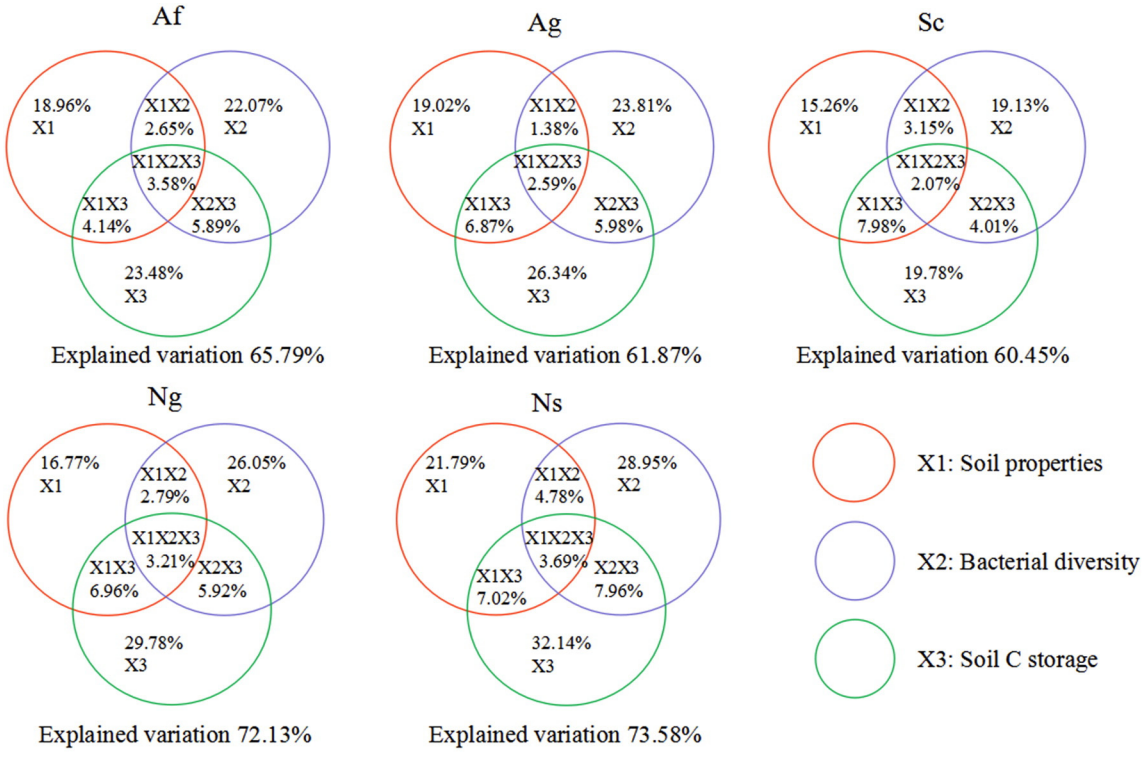

Fig. 7. The results of the structural equation model (SEM) and generalized additive models (GAMs) among different land use types. GAM analyses led to the following fractions: pure effect of soil properties (X1), pure effect of bacterial diversity (X2), pure effect of soil C storage (X3). X1X2 is the interactive effect of soil properties and bacterial diversity; X1X3 is the interactive effect of soil properties and soil C storage; X2X3 is the interactive effect of bacterial diversity and soil C storage; and X1X2X3 is the interactive effect of soil properties, bacterial diversity and soil C storage. Also included is the explained variation. Note: The path coefficients and explained variability were calculated after 999 bootstraps. Models with different structures were assessed using the goodness of fit statistic, which is a measure of the overall prediction performance.

Table 3
Direct, indirect and total effects on soil C storage on the basis of standardized values of statistically significant SEM paths. Values in bold indicate strong effects.

Item		Soil C storage		
		Direct pathway effect	Indirect pathway effect	Total effect
Soil properties	pH	0.217	0.076	0.293
	SOC	**0.254**	**0.124**	**0.378**
	TN	0.185	0.083	0.268
	TP	0.009	0.013	0.022
	AP	0.007	0.019	0.026
	NH_4^+-N	0.132	0.051	0.183
	MBC	**0.207**	**0.131**	**0.338**
	MBN	0.169	0.058	0.227
Bacterial diversity	Heip's evenness index	0.058	0.236	0.294
	$H_{Bacteria}$	**0.269**	**0.178**	**0.447**
	ACE index	0.234	0.135	0.369
	Coverage	0.056	0.032	0.088
	PD	**0.231**	**0.157**	**0.388**
	OTUs	0.205	0.084	0.289
	Chao1 index	0.216	0.127	0.343

decompose soil organic matter (House et al., 2014; Siegel et al., 2016), contributes a great deal to soil C storage (Melillo et al., 2002; Wei et al., 2014; Riggs and Hobbie, 2016; Arcand et al., 2016). In this study, the dominant phylum following artificial and natural restoration was *Actinobacteria*, while the dominant phylum of slope cropland (Sc) was *Acidobacteria* (Fig. 4A). From Sc to artificial restoration (Af, Ag) and natural restoration (Ns, Ng), soil bacterial community changed from *Acidobacteria*-dominant to *Actinobacteria*-dominant communities, which transited from slow-growing bacteria to fast-growing bacteria due to vegetation restoration on the Loess Plateau (Bending et al., 2002; Ceja-Navarro et al., 2010; Bissett et al., 2011).

4.2. Soil properties affecting soil bacterial community composition

There are currently debates on which are the driving factors to soil bacterial community composition (Fierer et al., 2007; Lauber et al., 2008; Lauber et al., 2009; Rousk et al., 2010; Liang et al., 2017; Xu et al., 2017). Traditional idea is that soil bacterial community composition is controlled by the minimum size and high local abundance, which is mainly driven by environmental conditions (Delgado-Baquerizo et al., 2013; Maestre et al., 2013; Wagg et al., 2014; Tian et al., 2017). In this study, soil properties play an important role in shaping soil bacterial community composition (Fig. 4B), supporting the second hypothesis. These findings were consistent with previous findings in northeast China (Wang et al., 2015; Zhang et al., 2016a, 2016b; Sun et al., 2016) but contrasted with the findings conducted on the Loess Plateau (Zeng et al., 2016). In recently studies, soil bacterial communities are determined at a large scale by latitudinal and latitudinal gradients along with geographic distance, climatic conditions, mean annual precipitation (MAP), and mean annual temperature (MAT) (Rousk et al., 2010; Liang et al., 2017; Xu et al., 2017). However, our study maintained unified climate and environmental conditions (and even the uniform soil type) but considered different land use types, which was appropriate for the analysis of soil properties affecting soil bacterial diversity and community.

According to PCoA, soil pH was the major factor determining soil bacterial community composition (Fig. 4B), which is supported by the correlation analyses (Table 2). Similarly, a large number of studies have shown that soil pH is also a good predictor for soil bacterial community composition in northeast China (Zeng et al., 2016; Zhang et al., 2016a, 2016b). This may be partly related to the fact that soil pH ranged from neutral to alkaline in our study area, also the result has been well explained by a long-term experiment in *Leymus chinensis* steppe (Yao et al., 2017a; Yao et al., 2017b). In addition, other soil properties (SOC, TN, NH_4^+-N, MBC, MBN) were positively correlated with the dominant soil bacterial groups (*Acidobacteria*, *Actinobacteria*, *Alphaproteobacteria*, and *Betaproteobacteria*) but negatively correlated with the abundance of *Bacteroidetes* and *Gammaproteobacteria*. However, there was no significant relationship between the abundance of these soil bacterial groups and TP or AP ($p > .05$), since P cycling on the Loess Plateau is weak (Vitousek et al., 2010). Besides, NH_4^+-N was closely correlated with the dominant soil bacterial groups, and TN was not correlated with the dominant soil bacterial groups, suggesting that not all the N well contributes to soil bacterial community composition on the Loess Plateau.

4.3. Association between soil bacterial diversity and soil C storage

In this study, soil C storage and soil bacterial diversity all increased due to restoration vegetation with leguminous plants (*Robinia pseudoacacia*, *Caragana korshinskii* and *Sophora viciifolia*), which formed dense roots and consistently released large amounts of C to improve the growth of soil bacteria (Delgado-Baquerizo et al., 2013; Maestre et al., 2013; Zechmeister-Boltenstern et al., 2015). Not surprisingly, a positive linear relationship between the relative abundance of dominant bacterial groups and soil C storage shown in Fig. 5, suggesting that soil C storage has a large influence on the dominant soil bacterial groups, supporting our third hypothesis, which is further confirmed by the results of structural equation model (SEM). Interestingly, previous studies have reported that soil C storage is governed by the metabolic activity of soil bacteria, which is mediated by C inputs, and then promotes soil bacterial activity (Wang et al., 2015; Zhang et al., 2016a, 2016b; Yang et al., 2017). Indeed, most soil microorganisms rely on C decomposition to obtain energy, and recently studies based on global-scale meta-analyses have concluded that soil C storage is an important driving factor for soil bacterial diversity (Delgado-Baquerizo et al., 2013; Maestre et al., 2013; Zechmeister-Boltenstern et al., 2015).

Besides, our results clearly demonstrate that soil C storage explained more of the variation in bacterial diversity than soil properties (Figs. 5 and 6), and generalized additive models (GAMs) and SEM can separate the individual and interactive effects among these driving factors. So we tested and highlighted a close interaction among soil bacterial diversity, soil properties and soil C storage via combined stepwise regression and SEM (Fig. 7). Of the selected driving factors, we observed that the independent effects of soil properties were lower than those of soil bacterial diversity and soil C storage, and the interaction between soil bacterial diversity and soil C storage was higher regardless of land use types. On the basis of the standardized values of statistically significant SEM paths, we obtained the direct, indirect and total effects on soil C storage (Table 3). Soil C storage was directly affected by soil properties (direct pathway effect of 0.811 and indirect pathway effect of 0.439) and indirectly affected by soil bacterial diversity (the direct pathway effect was 0.819, and the indirect pathway effect was 0.467). Of these direct and indirect pathway effects, soil pH, SOC, $H_{Bacteria}$ and PD had the highest total pathway effects (0.158, 0.214, 0.204 and 0.168, respectively). Among these direct and indirect pathway effects, SOC, Heip's evenness index, and PD had the highest total pathway effects (0.124, 0.127, and 0.104, respectively). For some reasons, soil bacterial diversity has direct effects on soil C storage owing to resource competition, and soil C storage promotes microbial activity, resulting in C accumulation (Zuppinger-Dingley et al., 2014; Kunstler et al., 2015). In contrast, soil properties appear to have direct effects on soil C storage, because soil properties (mainly soil nutrients) contribute to the decomposition of soil organic matter. Further, SOC and MBC also have a higher total pathway effect on soil C storage, providing a large nutrition source for soil C accumulation (Allison et al., 2010; Feng et al., 2016; Cheng et al., 2017). In total, these findings demonstrate and highlight the strong association between soil bacterial diversity and soil C storage on the Loess Plateau.

5. Conclusions

In summary, this study demonstrates the general and novel microbial ecology and pattern that soil bacterial diversity have a large effect on soil C storage regardless of land use type on the Loess Plateau. In fact, our data clearly show that soil C storage and soil bacterial diversity increased due to vegetation restoration, and there was a strong relationship between soil C storage and soil bacterial diversity. Specifically, soil pH was the dominant factor driving soil bacterial community composition in relation to vegetation restoration, and a strong relationship was observed between the relative abundance of dominant bacterial groups and soil C storage. Besides, soil bacterial diversity was closely related to soil C storage based on the structural equation model (SEM) and generalized additive models (GAMs). Soil C storage had the largest deterministic effects, explaining >70% of the variation and suggesting a strong relationship between soil C storage and soil bacterial diversity regardless of land use type. Overall, our findings provide a better understanding of the interaction between soil bacterial diversity and soil C storage. Future studies are necessary to investigate the processes and mechanisms that regulate soil bacteria and C storage over a large scale on the Loess Plateau.

Acknowledgements

This study was funded by the National Natural Science Foundation of China (41671280), the Special-Funds of Scientific Research Programs of State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau (A314021403-C6) and the Key Cultivation Project of the Chinese Academy of Sciences. Finally, we are grateful for the constructive reviewer comments that helped to improve the quality of our manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.01.081.

References

Allison, S.D., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to warming dependent on microbial physiology. Nat. Geosci. 3 (5), 336.
Arcand, M.M., Helgason, B.L., Lemke, R.L., 2016. Microbial crop residue decomposition dynamics in organic and conventionally managed soils. Appl. Soil Ecol. 107, 347–359.
Bending, G.D., Turner, M.K., Jones, J.E., 2002. Interactions between crop residue and soil organic matter quality and the functional diversity of soil bacterial community. Soil Biol. Biochem. 34 (8), 1073–1082.
Berlemont, R., Allison, S.D., Weihe, C., Lu, Y., Brodie, E.L., Martiny, J.B., 2014. Cellulolytic potential under environmental changes in bacterial community from grassland litter. Front. Microbiol. 5, 639.
Bissett, A., Richardson, A.E., Baker, G., Thrall, P.H., 2011. Long-term land use effects on soil bacterial community composition and function. Appl. Soil Ecol. 51, 66–78.
Bissett, A., Brown, M.V., Siciliano, S.D., Thrall, P.H., 2013. Bacterial community responses to anthropogenically induced environmental change: towards a systems approach. Ecol. Lett. 16 (s1), 128–139.
Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen—total. Methods of soil analysis. Part 2. Chemical and Microbiological Properties, pp. 595–624 (methodsofsoilan2).
Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure bacterial biomass nitrogen in soil. Soil Biol. Biochem. 17 (6), 837–842.
Burke, D.J., 2015. Effects of annual and interannual environmental variability on soil fungi associated with an old-growth, temperate hardwood forest. FEMS Microbiol. Ecol. 91 (6), 185–188.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Huttley, G.A., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7 (5), 335–336.
Ceja-Navarro, J.A., Rivera-Orduna, F.N., Patino-Zúniga, L., Vila-Sanjurjo, A., Crossa, J., Govaerts, B., Dendooven, L., 2010. Phylogenetic and multivariate analyses to determine the effects of different tillage and residue management practices on soil bacterial community. Appl. Environ. Microbiol. 76 (11), 3685–3691.
Chao, A., 1984. Nonparametric estimation of the number of classes in a population. Scand. J. Stat. 265–270.
Chen, L., Gong, J., Fu, B., Huang, Z., Huang, Y., Gui, L., 2007. Effect of land use conversion on soil organic carbon sequestration in the loess hilly area, loess plateau of China. Ecol. Res. 22 (4), 641–648.
Chen, Y., Wang, K., Lin, Y., Shi, W., Song, Y., He, X., 2015. Balancing green and grain trade. Nat. Geosci. 10 (8), 739–741.
Cheng, M., Xue, Z., Xiang, Y., Darboux, F., An, S.S., 2015. Soil organic carbon sequestration in relation to revegetation on the Loess Plateau, China. Plant Soil 397 (1–2), 31–42.
Cheng, L., Zhang, N., Yuan, M., Xiao, J., Qin, Y., Deng, Y., He, Z., 2017. Warming enhances old organic carbon decomposition through altering functional microbial communities. ISME J. 11 (8), 1825.
Cooperative Research Group on Chinese Soil Taxonomy, 2001. Chinese Soil Taxonomy. Science Press.
Delgado-Baquerizo, M., Maestre, F.T., Gallardo, A., Bowker, M.A., Wallenstein, M.D., Quero, J.L., García-Palacios, P., 2013. Decoupling of soil nutrient cycles as a function of aridity in global drylands. Nature 502 (7473), 672–676.
Deng, L., Liu, G.B., Shangguan, Z.P., 2014. Land-use conversion and changing soil carbon stocks in China's 'Grain-for-Green'Program: a synthesis. Glob. Chang. Biol. 20 (11), 3544–3556.
DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72 (7), 5069–5072.
Doetterl, S., Stevens, A., Six, J., Merckx, R., Oost, K.V., Pinto, M.C., 2015. Soil carbon storage controlled by interactions between geochemistry and climate. Nat. Geosci. 8 (10), 8–12.
Exbrayat, J.F., Pitman, A.J., Abramowitz, G., 2014. Response of bacterial decomposition to spin-up explains cmip5 soil carbon range until 2100. Geosci. Model Dev. 7 (3), 3481–3504.
Faith, D.P., 1992. Conservation evaluation and phylogenetic diversity. Biol. Conserv. 61 (1), 1–10.
FAO, ISRIC, 1988. Revised legend of the FAO-UNESCO soil map of the world. ISRIC Report 1988/01. ISRIC-World Soil Information.
Feng, X., Fu, B., Lu, N., Zeng, Y., Wu, B., 2013. How ecological restoration alters ecosystem services: an analysis of carbon sequestration in China's Loess Plateau. Sci. Rep.-UK 3, 2846.
Feng, X., Fu, B., Piao, S., Wang, S., Ciais, P., Zeng, Z., Wu, B., 2016. Revegetation in China Loess Plateau is approaching sustainable water resource limits. Nat. Clim. Chang. 6 (11), 1019–1022.
Feng, X., Fu, B., Li, J., Shao, M., Cheng, W., 2017. Evaluation of AMSR-E retrieval by detecting soil moisture decrease following massive dryland re-vegetation in the Loess Plateau, China. Remote Sens. Environ. 196, 253–264.
Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88 (6), 1354–1364.
Fu, B.J., Liu, Y., Lü, Y., He, C., Zeng, Y., Wu, B., 2011. Assessing the soil erosion control service of ecosystems change in the Loess Plateau of China. Ecol. Complex. 8 (4), 284–293.
Fu, B.J., Wang, S., Liu, Y., Liu, J., Liang, W., Miao, C., 2017. Hydrogeomorphic ecosystem responses to natural and anthropogenic changes in the loess plateau of China. Annu. Rev. Earth Planet. Sci. 45 (1), 223–243.
van Groenigen, K.J., Qi, X., Osenberg, C.W., Luo, Y., Hungate, B.A., 2014. Faster decomposition under increased atmospheric CO_2 limits soil carbon storage. Science 344 (6183), 508–509.
Guo, L.B., Gifford, R.M., 2002. Soil carbon stocks and land use change: a meta analysis. Glob. Chang. Biol. 8 (4), 345–360.
Hartzog, Paul E., Sladek, Margaret, Kelly, John J., Larkin, Daniel J., 2017. Bottle effects alter taxonomic composition of wetland soil bacterial community during the denitrification enzyme activity assay. Soil Biol. Biochem. 87–94.
Heijden, M.G., Wagg, C., 2013. Soil bacterial diversity and agro-ecosystem functioning. Plant Soil 363 (1), 1–5.
Horner-Devine, M.C., Lage, M., Hughes, J.B., Bohannan, B.J., 2004. A taxa-area relationship for bacteria. Nature 432 (7018), 750.
Houghton, R.A., Hackler, J.L., Lawrence, K.T., 1999. The US carbon budget: contributions from land-use change. Science 285 (5427), 574–578.
House, C.H., Pellegrini, M., Fitz-Gibbon, S.T., 2014. Genome-wide gene order distances support clustering the gram-positive bacteria. Front. Microbiol. 5.
Jin, Z., Dong, Y., Wang, Y., Wei, X., Wang, Y., Cui, B., 2014. Natural vegetation restoration is more beneficial to soil surface organic and inorganic carbon sequestration than tree plantation on the loess plateau of china. Sci. Total Environ. 485-486 (485), 615–623.
Kennedy, A.C., Smith, K.L., 1995. Soil microbial diversity and the sustainability of agricultural soils. Plant Soil 170 (1), 75–86.
Könneke, M., Schubert, D.M., Brown, P.C., Hügler, M., Standfest, S., Schwander, T., 2014. Ammonia-oxidizing archaea use the most energy-efficient aerobic pathway for CO_2 fixation. PNAS 111 (22), 8239.
Kunstler, G., Falster, D., Coomes, D.A., Hui, F., Kooyman, R.M., Laughlin, D.C., Aiba, M., 2015. Plant functional traits have globally consistent effects on competition. Nature 529, 204.
Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., 2015. Plant diversity increases soil bacterial activity and soil carbon storage. Nat. Commun. 6, 6707.
Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal community across land-use types. Soil Biol. Biochem. 40 (9), 2407–2415.
Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community composition at the continental scale. Appl. Environ. Microbiol. 75 (15), 5111–5120.
Lehmann, J., Skjemstad, J., Sohi, S., Carter, J., Barson, M., Falloon, P., Krull, E., 2008. Australian climate–carbon cycle feedback reduced by soil black carbon. Nat. Geosci. 1 (12), 832–835.
Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nat. Microbiol. 2, 17105.

Ling, N., Chen, D., Guo, H., Wei, J., Bai, Y., Shen, Q., 2017. Differential responses of soil bacterial community to long-term n and p inputs in a semi-arid steppe. Geoderma 292, 25–33.
Liu, G.T., Cheng, L., Wang, B.L., Zhao, Q.G., Qu, D., 2010. Changes of soil ammonia-oxidizing bacterial diversity in response to long-term fertilization in dry highland of Loess Plateau. China Agri. Sci. 43 (13), 2706–2714.
Lloydprice, J., Mahurkar, A., Rahnavard, G., Crabtree, J., Orvis, J., Hall, A.B., 2017. Strains, functions and dynamics in the expanded human microbiome project. Nature 550 (7674), 61.
Lupwayi, N.Z., Rice, W.A., Clayton, G.W., 1998. Soil bacterial diversity and community composition under wheat as influenced by tillage and crop rotation. Soil Biol. Biochem. 30 (30), 1733–1741.
Lynn, T.M., Ge, T., Yuan, H., Wei, X., Wu, X., Xiao, K., 2016. Soil carbon-fixation rates and associated bacterial diversity and abundance in three natural ecosystems. Microb. Ecol. 73, 1–13.
Maestre, F.T., Escolar, C., Guevara, M.L., Quero, J.L., Lázaro, R., Delgado-Baquerizo, M., Gallardo, A., 2013. Changes in biocrust cover drive carbon cycle responses to climate change in drylands. Glob. Chang. Biol. 19 (12), 3835–3847.
Melillo, J.M., Steudler, P.A., Aber, J.D., Newkirk, K., Lux, H., Bowles, F.P., Morrisseau, S., 2002. Soil warming and carbon-cycle feedbacks to the climate system. Science 298 (5601), 2173–2176.
Nair, A., Ngouajio, M., 2012. Soil bacterial biomass, functional bacterial diversity, and nematode community composition as affected by cover crops and compost in an organic vegetable production system. Appl. Soil Ecol. 58 (58), 45–55.
Nave, L.E., Vance, E.D., Swanston, C.W., Curtis, P.S., 2010. Harvest impacts on soil carbon storage in temperate forests. For. Ecol. Manag. 259 (5), 857–866.
Nelson, D.W., Sommers, L., 1982. Total carbon, organic carbon, and organic matter. Methods of soil analysis. Part 2. Chemical and Microbiological Properties, pp. 539–579 (methodsofsoilan2).
Obi, C.C., Adebusoye, S.A., Amund, O.O., Ugoji, E.O., Ilori, M.O., Hedman, C.J., Hickey, W.J., 2017. Structural dynamics of bacterial community in polycyclic aromatic hydrocarbon-contaminated tropical estuarine sediments undergoing simulated aerobic biotreatment. Appl. Microbiol. Biotechnol. 1-16.
Olsen, S.R., Sommers, L.E., Page, A.L., 1982. Methods of soil analysis. Part 2. Chemical and Microbiological Properties of Phosphorus. 9. ASA Monograph, pp. 403–430.
Pan, X.L., Huang, S., Fang, H., Xu, J., Guo, X.F., Chen, Y., 2009. Diversity of bacterial community composition in soils of loess plateau. J. Eco. 25 (3), 39–685.
Post, W.M., Kwon, K.C., 2000. Soil carbon sequestration and land-use change: processes and potential. Glob. Chang. Biol. 6 (3), 317–327.
Riggs, C.E., Hobbie, S.E., 2016. Mechanisms driving the soil organic matter decomposition response to nitrogen enrichment in grassland soils. Soil Biol. Biochem. 99, 54–65.
Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J. 4 (10), 1340.
Samaritani, E., Mitchell, E.A.D., Rich, J., Shrestha, J., Fournier, B., Frey, B., 2017. Soil bacterial community and ecosystem functioning change more strongly with season than habitat in a restored floodplain. Appl. Soil Ecol. 112, 71–78.
Schmidt, M.W., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Nannipieri, P., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478 (7367), 49.
Siegel, S.D., Liu, J., Ton-That, H., 2016. Biogenesis of the gram-positive bacterial cell envelope. Curr. Opin. Microbiol. 34, 31–37.
Sun, R., Zhang, X.X., Guo, X., Wang, D., Chu, H., 2015. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. Soil Biol. Biochem. 88 (4), 9–18.
Sun, R., Dsouza, M., Gilbert, J.A., Guo, X., Wang, D., Guo, Z., Chu, H., 2016. Fungal diversity composition in soils subjected to long-term chemical fertilization is most influenced by the type of organic matter. Environ. Microbiol. 18, 5137–5150.
Thompson, L.R., Sanders, J.G., Mcdonald, D., Amir, A., Ladau, J., Locey, K.J., 2017. A communal catalogue reveals earth's multiscale microbial diversity. Nature 551:457–463 (https://www.nature.com/articles/nature24621).
Tian, J., He, N., Hale, L., Niu, S., Yu, G., Liu, Y., Zhou, J., 2017. Soil organic matter availability and climate drive latitudinal patterns in bacterial diversity from tropical to cold temperate forests. Funct. Ecol. 6 (2), 1–15.
Torn, M.S., Trumbore, S.E., Chadwick, O.A., Vitousek, P.M., Hendricks, D.M., 1997. Mineral control of soil organic carbon storage and turnover. Nature 389 (6647), 170–173.
Torsvik, V., Øvreås, L., 2002. Bacterial diversity and function in soil: from genes to ecosystems. Curr. Opin. Microbiol. 5 (3), 240.
Treseder, K.K., Allen, M.F., 2000. Mycorrhizal fungi have a potential role in soil carbon storage under elevated co2 and nitrogen deposition. New Phytol. 147 (1), 189–200.
Turnbaugh, P.J., Ridaura, V.K., Faith, J.J., Rey, F.E., Knight, R., Gordon, J.I., 2009. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci. Transl. Med. 1 (6), 614–619.
Vitousek, P.M., Porder, S., Houlton, B.Z., Chadwick, O.A., 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. Ecol. Appl. 20 (1), 5–15.
Wagg, C., Bender, S.F., Widmer, F., van der Heijden, M.G., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proc. Natl. Acad. Sci. U. S. A. 111 (14), 5266–5270.
Wang, Y., Fu, B., Lü, Y., Song, C., Luan, Y., 2010. Local-scale spatial variability of soil organic carbon and its stock in the hilly area of the Loess Plateau, China. Quat. Res. 73 (1), 70–76.
Wang, X., Van Nostrand, J.D., Deng, Y., Lü, X., Wang, C., Zhou, J., 2015. Scale-dependent effects of climate and geographic distance on bacterial diversity patterns across northern China's grasslands. FEMS Microbiol. Ecol. 91 (12), 213–222.
Wardle, D.A., Yeates, G.W., Williamson, W.M., Bonner, K.I., Barker, G.M., 2004. Linking aboveground and belowground community: the indirect influence of aphid species identity and diversity on a three trophic level soil food web. Oikos 107 (2), 283–294.
Wei, H., Guenet, B., Vicca, S., Nunan, N., Asard, H., AbdElgawad, H., Janssens, I.A., 2014. High clay content accelerates the decomposition of fresh organic matter in artificial soils. Soil Biol. Biochem. 77, 100–108.
Xia, Z., Bai, E., Wang, Q., Gao, D., Zhou, J., Jiang, P., 2016. Biogeographic distribution patterns of bacteria in typical Chinese forest soils. Front. Microbiol. 7 (e69705).
Xu, M., Li, X., Cai, X., Li, X., Christie, P., Zhang, J., 2017. Land use alters arbuscular mycorrhizal fungal communities and their potential role in carbon sequestration on the Tibetan Plateau. Sci. Rep. UK 7, 1–11.
Yang, T., Adams, J.M., Shi, Y., He, J.S., Jing, X., Chen, L., Chu, H., 2017. Soil fungal diversity in natural grasslands of the Tibetan Plateau: associations with plant diversity and productivity. New Phytol. 215, 756–765.
Yao, Q., Liu, J., Yu, Z., Li, Y., Jin, J., Liu, X., 2017a. Changes of bacterial diversities after three years of biochar application in a black soil of northeast china. Appl. Soil Ecol. 113, 11–21.
Yao, Q., Liu, J., Yu, Z., Li, Y., Jin, J., Liu, X., 2017b. Three years of biochar amendment alters soil physiochemical properties and fungal diversity in a black soil of northeast china. Soil Biol. Biochem. 56–67.
Zechmeister-Boltenstern, S., Keiblinger, K.M., Mooshammer, M., Peñuelas, J., Richter, A., Sardans, J., Wanek, W., 2015. The application of ecological stoichiometry to plant–bacterial–soil organic matter transformations. Ecol. Monogr. 85 (2), 133–155.
Zeng, Q.C., Dong, Y.H., An, S.S., 2016. Bacterial community responses to soils along a latitudinal and vegetation gradient on the Loess Plateau, China. PLoS One 11 (4), e0152894.
Zhang, X., Liu, W., Zhang, G., Jiang, L., Han, X., 2015. Mechanisms of soil acidification reducing bacterial diversity. Soil Biol. Biochem. 81 (81), 275–281.
Zhang, X., Johnston, E.R., Liu, W., Li, L., Han, X., 2016a. Environmental changes affect the assembly of soil bacterial community primarily by mediating stochastic processes. Glob. Chang. Biol. 22 (1), 198–207.
Zhang, C., Liu, G., Xue, S., Wang, G., 2016b. Soil bacterial community dynamics reflect changes in plant community and soil properties during the secondary succession of abandoned farmland in the loess plateau. Soil Biol. Biochem. 97, 40–49.
Zhao, L.P., Gillet, F., 2011. Long-term effects of grazing exclusion on aboveground and belowground plant species diversity in a steppe of the Loess Plateau, China. Plant Ecol. Evol. 144 (3), 313–320.
Zuppinger-Dingley, D., Schmid, B., Petermann, J.S., Yadav, V., De Deyn, G.B., Flynn, D.F., 2014. Selection for niche differentiation in plant communities increases biodiversity effects. Nature 515, 108–111.