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The microbially-mediated soil organic carbon loss under degenerative succession in an alpine meadow

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Abstract

Land-cover change has long been recognized as having marked effect on the amount of soil organic carbon (SOC). However, the microbially-mediated processes and mechanisms on SOC are still unclear. In this study, the soil samples in a degenerative succession from alpine meadow to alpine steppe meadow in the Qinghai-Tibetan Plateau were analyzed using high-throughput technologies, including Illumina sequencing and GeoChip functional gene arrays. The soil microbial community structure and diversity were significantly ($P < 0.05$) different between alpine meadow and alpine steppe meadow, the microbial α-diversity in alpine steppe meadow was significantly ($P < 0.01$) higher than in alpine meadow. Molecular ecological network analysis indicated that the microbial community structure in alpine steppe meadow was more complex and tighter than in the alpine meadow. The relative abundance of soil microbial labile carbon degradation genes (e.g., pectin and hemicellulose) was significantly higher in alpine steppe meadow than in alpine meadow, but the relative abundance of soil recalcitrant carbon degradation genes (e.g. chitin and lignin) showed the opposite tendency. The Biolog Ecoplate experiment showed that microbially-mediated soil carbon utilization was more active in alpine steppe meadow than in alpine meadow. Consequently, more soil labile carbon might be decomposed in alpine steppe meadow than in alpine meadow. Therefore, the degenerative succession of alpine meadow because of climate change or anthropogenic activities would most likely decreased SOC and nutrients medicated by changing soil microbial community structure and their functional potentials for carbon decomposition.

Introduction

Land-cover change is a common phenomenon in land ecosystems and it has been recognized that this aboveground change would markedly affect the belowground soil organic carbon (SOC) pool. The aboveground change includes processes such as the plant succession

and degradation, reflecting the ecological processes caused by the combined effects of natural climate change, over-grazing, deforestation and other human activities (Yu et al., 2013; Yan et al., 2005). About 1.2 Pg carbon (C) in every year, or about 12% to 15% of total anthropogenic fluxes, was released as CO_2 to the atmosphere by land-cover change (Powers et al., 2011). Both soil fertility loss and CO_2 release (Powers et al., 2011) were the consequences of the changes in plant residues and the immobilization of organic C mediated by microorganisms (Tate, 1987; Van der Werf GR et al., 2009). Therefore, the study of the effect of land-cover change on SOC and its effect on processes and mechanisms is critically important to understand the global C balance and contribute to sustainable land-cover management.

Microorganisms are one of the most abundant and diverse organisms and are essential to soil ecological function, particularly in SOC and nutrient cycling (Vand der Heijden et al., 2008; Feeney et al., 2006). Many studies have revealed the changes in SOC, CO_2 release, microbial biomass and microbial species diversity by land-cover changes (Lundquist et al., 1999; Wang et al., 2003; Michelsen et al., 2004; Fierer *et al*., 2010; Lopez-Lozano *et al*., 2013). Because of the high microbial diversity in soil ecosystem and technical limitations, soil microbial activities and processes involved in soil C cycling have been assessed by indirect indicators in most previous studies, such as soil respiration (Li et al., 2010; Bastida et al., 2006), metabolic quotient (Bini et al., 2013), exo-enzyme activities (Nayak et al., 2007) and microbial phospholipid fatty acid (Smith et al., 2014). In recent years, some researchers have focused on the changes soil microbial community based on 16S rDNA, ITS or functional genes sequencing (Zifcakova, et al., 2015; Xue et al., 2016). However, the soil microbial activities and processes mediating the conversion of SOC to CO_2 and biomass are still a "black box" (Waldrop et al., 2004; Ding *et al*., 2013; Lange M, et al., 2015; Sulman et al., 2014). Therefore, although land-cover changes significantly affect SOC, little is known about the influence of land-cover changes on the metabolic activities and processes of the belowground microbial community (Reeve et al., 2010). Further study on the change in soil microbial potential and metabolic traits following land-cover change is needed (Lopez-Lozano *et al*., 2013).

The Qinghai-Tibet Plateau is the highest and the largest low-latitude plateau in the world (Wang *et al*., 2012), and it is an extremely sensitive region to the impact of global warming and environmental changes (Zhang et al., 2013). The alpine meadow (AM), widely distributed on the Tibetan Plateau, occupies over 40% of the Qinghai-Tibetan Plateau area and plays a critical role in regional sustainable development, biodiversity and water resource conservation (Kang et al., 2007; Zhou *et al*., 2005). The AM is also a large SOC pool. Wang et al. (2002) found that soil (0 - 75cm) organic C content reached 23.2 Pg in the meadow and steppe grasslands in the Tibetan Plateau, accounting for 23.44% of China's total organic soil-stored C or 2.5% of the global soil C pool. As one of the most important and vulnerable soil C pools, about 3.02 Pg of C have been emitted from the grasslands of the Qinghai-Tibetan plateau because of the changes in land-cover and grassland degeneration in the last 30 years (Wang et al., 2002). In recent decades, succession and degradation have been gradually occurring between different AM types, such as AM has appeared in the alpine steppe meadow (ASM) region. This might be the consequences of the climate warming and anthropogenic activities (Guo *et al*., 2011; Zhou *et al*., 2005; Wang et al., 2012).

In this study, we adopted Illumina sequencing and functional gene microarray (GeoChip) to analyze the processes and mechanisms of changes in microbially-mediated SOC in the degenerative succession from AM to ASM in Qinghai-Tibetan Plateau. The aims of this study were to determine: (1) the effect of degenerative succession from AM to ASM on SOC and soil microbial community structure; (2) the divergence of soil C utilization by microbes and microbial functional gene diversity related to C cycling; and (3) the major environmental factors affecting soil microbial community structure and microbially-mediated SOC loss.

Material and methods

Site and sampling

The study sites were situated in Sanjiangyuan Natural Reserve (97°402″ - 100°05′27″ E, 34°08′16″ - 35°56′06″ N), Qinghai Province, China, which was located in the center of Qinghai-Tibetan Plateau (Zhang *et al*., 2013). The annual mean air temperature is -5.6~3.8℃, and the average precipitation is 262.2~772.8mm (Lu *et al*., 2015).

Soil sampling sites were set up in AM (35°41′26″N, 99°33′01″E, elevation: 3880 m) and ASM (35°40′10″N, 99°55′13″E, elevation: 3490 m). At each site, 10 plots (1 m×1 m) were established and the diagonal method was used to collect soil samples at the depth of 0 -10 cm in each plot. Ten to fifteen soil cores were taken from each plot and combined to obtain about 400 g of soil. Roots and stones were removed from samples, and then the samples from each plot were thoroughly mixed. Ten replicate soil samples were collected from the same site. To avoid contamination during sampling, the sterile gloves, sterilized paper and water was used for sampling from each plot. At the same time, plant properties were investigated and recorded in each plot, including the plant species, plant number, canopy of each grass and plant height (Fang et al., 2004). To survey the plant biomass, all the grass was harvested in each plot, dried in the oven at 65 ^{o}C for about 24h and weighed.

Soil property measurements

All soil samples were air-dried and then sieved to 2 mm. Soil moisture was measured by the drying method (Bao, 1999). Soil pH was measured by pH meter according to the ratio of 1: 2.5 soil: H_2O. Total organic C, total nitrogen (TN), total phosphorus (TP), total sulfur (TS), rapidly available phosphorus (RAP), available N (AN), nitrate N (NO_3^--N) and ammonium N (NH_4^+-N) were measured (Bao, 1999). The vegetation properties and soil physicochemical properties were presented in Table S1.

Soil microbial carbon utilization

A Biolog Eco-plate experiment was performed to examine the microbial functional diversity of carbon metabolism (Cookson *et al*., 2008). Each well of the plate was scanned at the wavelength of 595 nm with the Biolog plate reader (Microlog ReL 3.5) at 12 h intervals through to 168 h (Liu *et al*., 2013). C utilization was monitored by average well color development (AWCD) = $\sum(C_i - R)/n$, where *Ci* was the absorption value of the *i* th well, *R* was the control absorption well and *n* was the number of plates (*n*=31). AWCD values of 168h were used to calculate the microbial functional diversity of C metabolism (Garland & Mills, 1991). Several indexes were used to analyze diversity and richness of the communities: the Shannon-Wiener diversity (*H*): H=- $-\sum P_i \times (\ln P_i)$, where *P i* was the ratio of the relative

absorption of the *i* th divided by the sum of all relative color development of the plate at 168 h; the McIntosh index (*U*): $U=\sqrt{\sum ni^2}$, where *ni* was the relative color development of the *i*th and the richness index (*S*) was the number of wells with *Ci* -*R* > 0.25.

Soil microbial DNA extraction, purification, and quantification

Soil microbial DNA extraction was conducted by using the Fast DNA Spin kit for soil following the manufacturer's instructions (MP Biomedical, Carlsbad, CA, USA). Soil microbial DNA was further purified twice by using 0.5% low melting point agarose gels and was determined by analyzing the ratios of absorbance at 260nm/280nm and 260nm/230nm. Finally, microbial DNA was quantified using a FlUOstar Optima (BMG Labtechm Jena, Germany).

Illumina sequencing and data processing

Purified DNA extracts from soil samples were used as a template and the primers were designed for amplification according to the V4 hypervariable region of the bacterial 16S rDNA gene. The sequence of forward primer was 5'- GTGCCAGCMGCCGCGGTAA-3' (515F), and the reverse primer was 5'- GGACTACHVGGGTWTCTAAT-3' (806R) (Caporaso *et al*., 2011, 2012). The reverse primer was combined with a barcode sequence. PCR amplification was used in a 25 μl reaction, containing 1 μl of each primer, 2.5 μl AccuPrime PCR buffer II (Invitrogen, Grand Island, NY, USA), 5 μl DNA and 0.1 μl AccPrime Taq Polymerase. The reaction mixture was denatured at 94 ^{o}C for 1 min, followed by 30 cycles of 94 ^{o}C for 20 s, 53 ^{o}C for 25 s and 68 ^{o}C for 45s, and extension at 68 ^{o}C for 10 min (Ding et al., 2015). The PCR products were purified and run using a Miseq (Illumina, San Diego, CA, USA) (Cong et al., 2015; Ding et al., 2015).

Raw data were separated into samples according to the barcode sequence. Adapters, low quality and ambiguous reads ("N") were trimmed; for example, reads that did not perfectly match the PCR primer, had non-assigned tags, or had reads < 250 bp were removed (Kong, 2011). The forward and reverse reads were integrated into a whole sequence by FLASH (Magoč & Salzberg, 2011). Operational taxonomic units (OTUs) were defined at 97%

similarity level by using UCLUST (Edgar, 2010). The singletons were removed. The ribosomal database project (RDP) classifier was used to determine the taxonomic identity of each phylotype (Wang *et al*., 2007). The number of detected OTUs and sequences at different levels of classification were counted. Random resampling was processed with 15,000 sequences per soil sample. All these data were tested with the Galaxy Illumina sequencing pipeline.

GeoChip hybridization and data processing

Geochip 4.0 was used for detecting soil microbial DNA functional gene diversity. Geochip 4.0 contained 82,000 oligonucleotide probes covering 141,995 functional genes involved in 410 gene categories involved in C, N cycling and other biogeochemical processes. The detailed GeoChip information is presented on the website (http://ieg.ou.edu). Purified DNA was labeled with Cy3 fluorescent dye using a random priming method (Tu et al., 2014). All hybridizations were carried out at 42 ^{o}C for 16 h using a hybridization station (MAUI, BioMicro Systems, Salt Lake City, UT, USA) and arrays were scanned at full laser power and 100% photomultiplier tubes with a NimbleGen MS200 Microarray scan (Roche, Madison, WI, USA). Scanned images were gridded by NimbleScan software (Tu et al., 2014).

Raw GeoChip data were uploaded to the GeoChip data analysis manager (http://ieg.ou.edu/microarray/). Data was pre-processed data using the following steps: (i) the poor-quality spots with a signal-to-noise ratio of less than 2.0 or the signal intensity value less than 1000 were discarded; (ii) genes that were detected in no more than 6 out of 10 replicate samples from the same sampling site were removed; (iii) normalizing the signal intensity of each spot by dividing the mean value of each sample of total signal intensity; and (iv) transformation of the data to the natural logarithmic form (He et al., 2010; Cong et al., 2015; Ding et al., 2015).

Statistical analysis

Plant diversity was calculated by Simpson index, and the number of plant species was calculated in all samples based on the survey data in the fields. Shannon index, Simpson's index, Pielou's evenness, Simpson evenness and OTUs richness index were used to test soil

microbial diversity based on Illumina sequencing data of 16S rDNA and *gyr*B gene in GeoChip 4.0. Data analysis was performed by t-test analysis, and *P* values of t-tests were adjusted by a false discovery rate (FDR) of < 5% (Kong et al., 2013). Principal coordinate analysis (PCoA) was used to assess the distribution of microbial communities based on the Bray-Curtis dissimilarity matrix. The Mantel test was used to analyze the correlation between microbial community structure and environmental factors; variance partitioning analysis (VPA) was performed to analyze the contributions of environmental variables to the microbial community structure. Canonical correspondence analysis (CCA) was used to determine the major environmental attributes contributing to the microbial community structure. Before performing CCA, the environmental variables were firstly filtrated according to the variance inflation factors (VIF) (Yang *et al*., 2014). All data were tested in R v. 3.1.2 using the Vegan package (v.3.1.2).

Soil microbial network construction

Based on random matrix theory (Deng *et al*., 2012), ecological networks was constructed using sequencing data of 16S rDNA. In the network construction, only 3 out of 10 replicates of OTUs data were used. Various network properties, such as average clustering coefficient, average degree, modularity index, and average path distance, were counted. Among the topological properties in the ecological network, modularity could be used to measure the extent of species interactions and it could characterize the ecosystem quality and stability (Olesen *et al*., 2007; Alon, 2003). Average degree was used to describe the properties of nodes (Guimera *et al*., 2007), and average clustering coefficient was used to measure the extent of module structure present in a network (Deng *et al*., 2012), while harmonic geodesic distance (HD) could represent the path length of different nodes in disjointed graph (Deng *et al*., 2012).

The network modules were generated using rapid greedy modularity optimization. Hub and connector genes were determined by among-module connectivity (*Pi*) and within-module connectivity (*Zi*) (Olesen et al., 2007). The *Zi* described the degree of connectivity between a node and other nodes in its own module, and *Pi* reflected the extent that a node was connected

to the other modules. The network parameters and properties were obtained from the website (http://ieg2.ou.edu/mena/). According to the parameters and properties, the visualized network graphs were constructed by Cytoscape 2.8.0 software (Cline *et al*., 2007).

Results

SOC and soil geochemical properties

The soil and plant characteristics were remarkably different between AM and ASM (Table S1). *Kobresia pygmaea*, *Potentilla bifurca* and *Leontopodium pusillum* were the dominant species in AM, while *Poa annua*, *Oxytropis deflexa* and *Carex tristachya* were dominant in ASM. The plant biomass and plant α-diversity were significantly ($P < 0.01$) in AM sites than in ASM sites.

Among the measured soil parameters (Table S1), SOC content was significantly ($P < 0.01$) higher in AM samples than in ASM samples, and most of the other soil nutrient contents followed the same trend, such as soil TP, TN and RAP (Table S1). All these results indicated that the soil degenerative process from AM to ASM caused the decreased of SOC and nutrient content, even though the aboveground grass biomass might be temporarily increased. .

Soil microbial community composition and structure between AM and ASM

To compare soil microbial community composition and structure in the two meadow sites, 16S rDNA high-throughput sequencing was performed. A total of 13, 307 and 15, 754 operational taxonomic units (OTUs) were separately obtained at 97% similarity level, ranging from 2663 to 4407 OTUs per sample in AM and from 3615 to 4759 OTUs per sample in ASM. For taxonomic identification, all detected OTUs could be classified into 34 bacterial phyla and 2 archaeal phyla. The dominant phyla were *Acidobacteria*, *Proteobacteria*, *Actinobacteria* and *Planctomycetes* in both AM and ASM; the soil microbial richness (number of OTU) was significantly ($P < 0.05$) higher in ASM than in AM (Table S2). Total 21 subgroups of phylum *Acidobacteria* were detected and 7 of them were dominant (Table S3). At the family classification level, a total of 176 families (average number of OTU over 1 in 10 replicate samples) were detected in the two sites, with 154 families in AM and 171 families in ASM. The most dominant families (average number of OTU over 100 in 10 replicate samples)

in these sites were *Planctomycetaceae*, *Actinomycetales*, *Solirubrobacterales*, *Chitinophagaceae*, *Sphingomonadaceae* and *Acidimicrobiales* (Dataset S1). At the genus classification level, a total of 369 genera (average number of OTU over 1 in 10 replicate samples) were found in the two sites, and 311 genera in AM and 342 genera in ASM. The most dominant genera (average number of OTU over 80 in 10 replicate samples) in these sites were *Acidimicrobineae*, *Conexibacteracae*, *Zavarzinella* and *Gemmatimonas* (Dataset S2). However, the microbial diversity based on *gyr*B gene was lower than the 16S rDNA and had no significantly difference between the two alpine meadow soils (Table 1).

The relative abundances of *δ -Proteobacteria*, *Planctomycetes Chloroflexi and Firmicutes* were significantly ($P < 0.05$) higher in ASM than in AM. The relative abundance of *α--Proteobacteria* and *β-Proteobacteria*, were significantly ($P < 0.05$) higher in AM than in ASM, while the others had no significant difference between the two meadow sites (Table S2). Therefore, the composition and relative abundance of soil microbial community were significantly difference between AM and ASM.

The α-diversity indexes of microbial community structures were calculated (Table 1). The Shannon index and Simpson index were significantly ($P < 0.01$) higher in ASM (7.59 and 844.31, respectively) than in AM (7.33 and 636.15, respectively). PCoA of the overall microbial community structure showed that the microbial communities of the two meadow sites were well separated (Fig. 1). Furthermore, three non-parametric multivariate statistical tests (MRPP, ANOSIM and Adonis) indicated that there were significant ($P < 0.01$) differences between these two sites (Table S4). Therefore, the diversity and structure of the soil microbial communities were significantly different between AM and ASM soil.

Ecological networks analysis of soil microbial communities between AM and ASM

The ecological networks that were constructed had 613 and 828 nodes for AM and ASM, respectively, under the identical thresholds (0.89) (Table S5). In this study, modularity, average degree and average clustering coefficient were higher in the network of ASM than in AM (Table S5), which indicated that soil microbial community structure in ASM site might be more complex and tighter than in AM site.

In the *Z P*-plot, peripherals representing a node in this category have lower connectivity and lower value of *Pi* and *Zi*. According to the network topological structure graph, the majority of nodes belonged to the peripherals and did not contact with the external module (*Pi* = 0). The connector category describes the nodes with lower *Zi*, but higher *Pi*. In AM sites, no nodes were be detected that belonged to connector category, while seven connectors were observed in ASM site (Fig. 2). Among these connectors, four of seven connectors were derived from *Proteobacteria*, and the other three connectors were derived from *Acidobacteria*, *Gemmatimonadetes* and *Actinobacteria* respectively. Module hubs represented the nodes with higher *Zi* but lower *Pi*. For AM, five nodes were detected that belonged to the module hub category, which were composed of three *Proteobacteria*, one *Acidobacteria* and one *Actinobacteria*. In ASM, seven module hubs were observed, which were composed of three *Actinobacteria*, three *Acidobacteria* and one *Chloroflexi*. According to the results, the network interaction of soil microbial taxa had been substantially changed in the process of degenerative succession from AM to ASM.

Carbon utilization of soil microbial communities

The average well color development (AWCD) showed that the C sources were rapidly used from 24 to 168 h incubation and reached the maximum values at 168 h (Fig. 3). Compared with AM, samples in ASM had higher AWCD values across all the incubation time points (Fig. 3). The Shannon index, McIntosh index and richness index were significant ($P < 0.01$) higher in ASM than in AM (Table 1). These results indicated that soil microbial diversity and activity to SOC utilization was higher in ASM than in AM.

Carbon sources analysis showed that the utilizations of most C sources was significantly ($P < 0.01$) higher in ASM than in AM, such as polymers, carbonhydrates, phenolic acids, carboxylic acids and amino acids (Fig. S1). These results implied that the soil microbial communities in ASM might consume a broader range of C substrates to satisfy their ecological function. Furthermore, the top three C sources utilized by microbes in ASM were polymers, amino acids and phenolic acids, while the top three C substrates in AM were polymers, amino acids and carbohydrates (Fig. S1). The range of C sources metabolized

indicates different ecological functions of the soil microbial communities in these two meadow sites.

Difference s in soil microbial functional gene related to C and N cycling

A total of 6425 microbial genes involved in different C degradation pathways, such as starch, pectin, hemicellulose, cellulose, chitin and lignin degradations, were detected by Geochip 4.0 in the meadow samples. The detected relative abundances of many genes involved in labile C degradation were significantly ($P < 0.05$) higher in ASM than in AM (Fig. 4), such as the *pectinase, rgh and rgl* genes involved in pectin degradation and the *ara* gene involved in hemicellulose degradation. However, the detected relative abundances of genes involved in recalcitrant C degradation were significantly ($P < 0.05$) higher in AM than in ASM, such as endochitinase gene involved in chitin degradation, *mnp* and phenol oxidase genes involved in lignin degradation (Fig. 4). These results apparently indicated that soil microorganisms in AM might have a higher potential ability to use some recalcitrant C (e.g., chitin and lignin), while after conversion of AM to ASM, the microorganisms may tend to decompose more labile C (e.g., pectin and hemicellulose).

Microbial genes related to N cycling were analyzed. The detected relative abundance of *amoA* gene related to nitrification was significantly higher ($P < 0.05$) in ASM than AM. In contrast, the detected relative abundance of *napA* and *nrfA* genes related to N reduction, and *hzo* gene involved in anammox were significantly ($P < 0.05$) higher in AM than in ASM (Fig. 5). These variations in N cycling genes might lead to the difference in transformation from NO_3^- synthesis to NH_4^+ synthesis. . These results indicated that many N cycling genes might be changed and influence the N bioprocess under the degenerative succession from AM to ASM.

Relationship between soil microbial community and environmental factors

To identify the relationship between environmental factors and soil microbial community, Mantel test and CCA were performed (Table 2). The results indicated that soil properties, such as pH, TN, TP, TS, SOC and RAP were significantly ($P < 0.05$) affected by both soil microbial taxonomic and functional gene structures. The CCA results indicated that SOC,

plant diversity and TP might be the most important factors in forming microbial taxonomic structure (P =0.05) (Fig. S2A) and microbial functional genes (P =0.01) (Fig. S2B).

VPA was performed to analyze the contributions of environmental variables to microbial community structure. A substantial proportion (52.92%) of the variations in soil microbial community structure could be explained by the selected environmental factors, specifically, 9.88%, 33.39% and 4.76% of the variations could be explained by vegetation factors (including plant biomass and plant diversity), soil nutrients (including TN, TP, NH_4^+-N, NO_3^--N, SOC and RAP) and soil pH, respectively (Fig. S3A). A even higher proportion (86.2%) variations could be explained for the microbial functional gene structure (Fig. S3B). These results showed that soil nutrients were highly associated with soil microbial taxonomic structure and potential metabolic function in both sampling sites.

Discussion

In recent decades, our knowledge on soil microbial communities has expanded rapidly with the development of new sequencing methods by passing the need for isolations of microorganisms (Torsvik et al., 2002; Drenovsky et al., 2004). In this study, the microbial taxonomic composition obtained by Illumina sequencing showed that species diversity significantly increased under the degeneration from AM to ASM, but the responses of different phyla could be varied. The phylum *Acidobacteria* is one of the most abundant soil bacteria and the relative abundance of dominant subgroups 3, 4, 6, 7 and 10 was increased in ASM, with low soil organic matter content, when compared with AM. Recent studies showed that the phylum *Acidobacteria* are in general oligotrophic ecosystems and revealed their adaptation to low substrate envrionments; for example, the proportion of *Acidobacteria* was reported to be significantly lower in nutrient-rich rhizosphere than in bulk soil (Kielak et al., 2009), and they have low abundance in nutrient-rich agricultural soil (Lopez-Lozano et al., 2013; Kielak et al., 2009). However, some dominant subbroups of *Acidobacteria* were also known to have a decreasing response to the soil environments with decreased available nutrients (Navarrete, 2013, 2015; Zhang et al., 2014a). In our study, the dominant subgroup 17 was significantly decreased in ASM. These results suggested that a differential response of

the *Acidobacteria* subgroups to ecosystem or environment changes could be used to as early warning indicators of soil managements and plant type successions (Navarrete, 2013, 2015; Zhang et al., 2014a).

For the complicated and diversified interactions among different species (Olesen *et al*., 2007), ecological network analysis is a sensitive, reliable and robust tool to reveal the interactions of microorganisms in complex biogeochemical processes (Zhou *et al*., 2010; Deng *et al*., 2012). The modularity, average degree, and harmonic geodesic distance (HD) are crucial indicators in reflecting the stability, robustness and resistance of complicated ecosystem networks (Deng *et al*., 2012). According to the analysis results, the ecological network relationship was significantly difference between AM and ASM. The network topological properties were more complicated in ASM than in AM, implying that the microorganisms in ASM might have more complicated interactions, in which microbial species could more stably coexist (Ding *et al*, 2015). With the degenerative succession of AM to ASM, soil microbial community structures were changing toward a more complicated ecosystem, and this succession might be conducive to strengthening the resistance to external disturbance.

Understanding the mechanism of land-cover changes on the soil microbially-mediated C cycling is essential for estimation of the soil C pool. Analyzing the differences in microbial C utilization ability was helpful to understand functional changes in the soil microbial community (Liu *et al*, 2013). In the Biolog C utilization study, AWCD represented the utilization of C sources by microbes, and reflected the activity and physiological function of microbial communities (Liu *et al*., 2013), while the Shannon index, McIntosh index and richness index could reflect the functional diversity of microbial metabolisms (Wang *et al*, 2011). Our studies indicated that soil microbial communities in ASM had higher activity than in AM for C utilization of different C components, such as polymers, carbohydrates, phenolic acids and carboxylic acids. The analysis of utilization of sole C substrates by microbial community structure, indicated that soil microbes might have greater ability to decompose SOC in ASM than in AM, which could be the reason why SOC decreased in ASM.

Directly Revealing the microbial metabolic activities and processes that mediated the SOC cycles caused by land-cover change is still difficult. In our study, the microbially-mediated soil C cycling processes were further analyzed using GeoChip technology. In previous studies, GeoChip was used to show that the detected functional gene signal intensities had significant correlations with environmental nutrient contents and that GeoChip could be used to link microbial communities with ecosystem processes and functions to a certain extent; for example, Yergeau et al. (2007) showed a significant correlation between cellulase enzyme activity and the number of cellulase gene variants; Reeve et al. (2007) found a significant correlation between cellulose gene signal intensity and cellulose activity in the soil; Zhang et al. (2014b) showed oxidizable organic carbon was significantly linked ($P < 0.05$) to the total abundance of genes involved in active organic carbon degradation (cellulose, hemicellulose and starch); Ding et al. (2015) explored the total abundances of nitrification genes (*amo*A and *hao*) were negatively correlated ($r = -0.46$, $P = 0.023$) with soil NH_4^+-N, and total abundances of denitrification gens (*nirS* and *nirK*) were also negatively correlated ($r = -0.54$, $P = 0.008$) with soil NO_3^--N. In this study, the relative abundance of microbial C degradation genes related to labile C degradation were significantly higher in ASM site than in AM site, but the relative abundance of C degradation genes related to recalcitrant C were significantly lower in ASM site than in AM site. Therefore, a significant difference in soil C metabolic processes might occur with degenerative succession and soil microbes could be the facilitators of this process in the AM. Compared with 16S rDNA sequencing, however, GeoChip provided limited information for complex ecosystems due to the limitation of microbial functional gene probe number and type, sensitivity and quantitative capability, it might be preferable to use a combination of technologies to better reveal the interaction in complex soil ecosystems in the future.

Plant diversity and soil nutrients are important environmental factors that influence the soil microbial community and their ecological functions (Liu *et al.*, 2008; Wardle *et al.*, 2004). Species diversity and productivity of vegetation might greatly affect organic compounds and the litter diversity, which are the major soil resources and substrate satisfying microbial

requirements (Bardgett & Shine, 1999). Changes in the plant community often lead to a corresponding change in both quantity and quality of soil organic matter (Yang *et al*., 2014; Carney & Matson, 2005; Chabrerie *et al*., 2003). SOC, as an important intermediating substance between plant and microorganisms, could be considered as the primary driving force in shaping microbial diversity and activity (Eilers *et al*., 2010; Benizri & Amiaud, 2005). Some studies have confirmed that soil C availability, which could effectively regulate the changes in microbial community structure and microbial growth, had substantial impact on microbial community composition and activity (Zhang *et al*., 2014; Liu *et al*., 2014). Consistently, SOC was not only significantly correlated with microbial community composition, but influenced the functional genes involving in C and N cycling. Soil phosphorus as a limiting factor might indirectly affect the availability of other nutrients, such as N element (Janssens *et al*., 1998). Recent studies have reported that the availability of phosphorus not only limited microbial growth, but was also an important factor in driving microbial community structure and biogeochemical function (Demetz & Insam, 1999; DeForest *et al*., 2012; Kuramae *et al*., 2010; Liu *et al*., 2014). In this study, we found that soil nutrients, including TN, TP, NH_4^+-N, NO_3^--N, SOC and RAP, were highly associated with soil microbial taxonomic structure and potential metabolic function in both AM and ASM sites, suggesting that the soil degenerative succession might dramatically affect both soil nutrients and microbial communities synchronously.

In summary, to understand the effect of land-cover change on the soil microbial community and microbially-mediated SOC loss, the soil microbial community structure and metabolic function related to C cycling in AM and ASM on Qinghai-Tibetan Plateau, were analyzed by Illumina sequencing, Biolog Ecoplate and GeoChip technologies. The results showed that the soil microbial community structure and diversity were significantly increased under degenerative succession from AM to ASM. Both microbial functional genes involved in C cycling and Biolog experiments indicated that ASM might decompose more SOC and released it in the form of CO_2, which could further intensify the greenhouse effect. Therefore, the changes in land-cover not only affected soil microbial community structure, but also

affected their functional potential for C decomposition. This might alter organic C dynamics, leading to increasing soil C losses and greenhouse gas emissions with degenerative succession of vegetation based on climate change or anthropogenic activities.

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Authors' contributions

Y. Z., J. Z and D. L. designed the experiments. Y. Z., X. L. and Y. D wrote the main manuscript text. Y. Z., J. C., H. L., H. Y., X. Wand Y. S performed the experiments. Y. Z., X. L. and D. Y analyzed the data. All authors reviewed the manuscript.

Data accessibility

Sequencing data are accessible in NCBI SRA database with Accession No. SRP096658. GeoChip data are accessible in NCBI database with Accession No. GSE93158. The OTU table as well as the input and output files of the network analysis is accessible in Dryad database with doi:10.5061/dryad.h781v.

Supporting information

Table S1. Summary of environmental parameters analyzed by two-tailed t-test.

Table S2. The number of soil microbial OTUs and relative abundances in the two meadow sites.

Table S3. The number of OTUs and relative abundance of phylum Acidobacteria in two meadow sites

Table S4. Dissimilarity report of the overall microbial community structure with three different statistical approaches between alpine meadow and alpine steppe meadow.

Table S5. Topological properties of OTU in the two meadow sites.

Fig. S1. Relationship between taxonomic diversity index and functional diversity index. The difference between alpine steppe meadow and alpine meadow were tested by two-tailed unpaired t-test.

Fig. S2. Canonical correspondence analysis (CCA) of (a) high-throughput sequencing data and (b) C, N cycling genes with environmental factors.

Fig. S3. Variation partitioning analysis (VPA) of (a) high-throughput sequencing data and (b) C, N functional gene data with environmental factors.

Table 1. Overall of microbial community diversity detected by Illumina sequencing and Biolog Ecoplate data in the two meadow sites

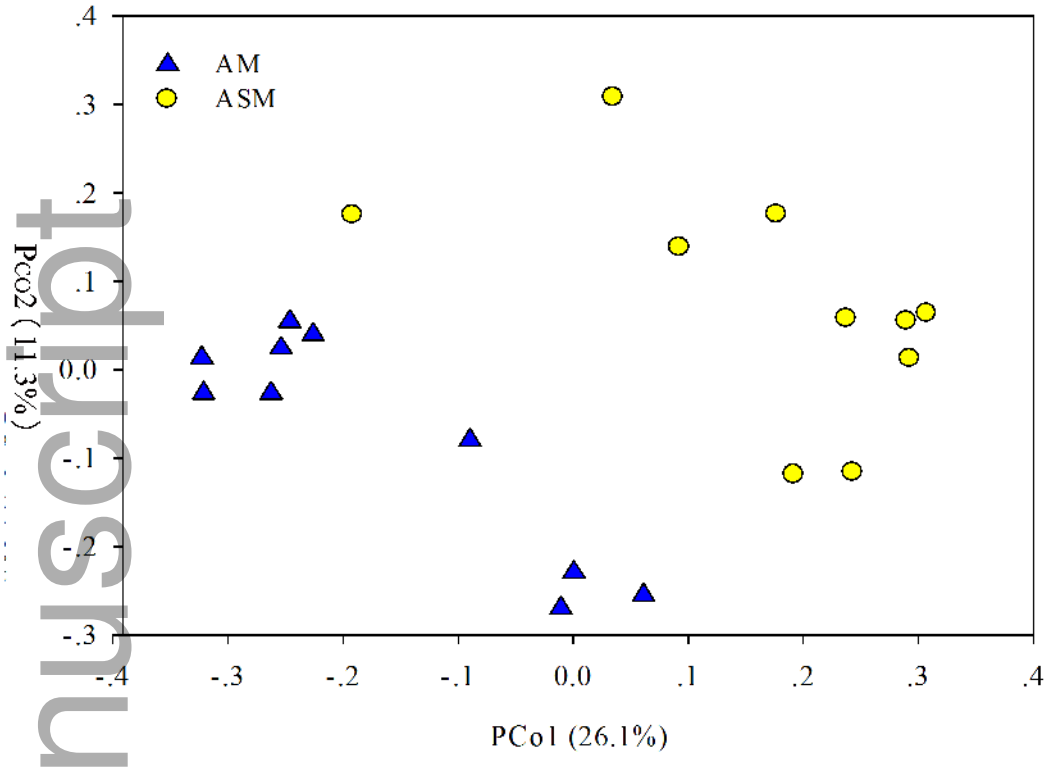
	Indices	AM	ASM	FDR
Taxonomic diversity (16S rDNA)	Shannon Index(H)	7.33±0.06	7.59±0.03	0.003
	Simpson Index(D)	636.15±36.47	844.31±22.45	0.000
	Pielou evenness(J)	0.89±0.003	0.91±0.001	0.003
	Simpson evenness(Si)	0.17±0.01	0.19±0.01	0.012
	Richness Index	3680.80±559.69	4374.40±301.34	0.006
Phylogentic diversity (gyrB)	Shannon Index (H)	6.25±0.16	6.05±0.16	0.333
	Simpson Index (D)	585.32±78.45	463.02±61.50	0.283
Biolog data	Shannon Index	3.22±0.01	3.29±0.01	0.006
	McIntosh Index	6.85±0.28	8.69±0.31	0.000
	Richness Index	25.13±0.44	27.40±0.40	0.003

Data present the mean value and standard error.

Note: AM, Alpine meadow; ASM, Alpine steppe meadow.

Table 2. Mantel test between 16S rDNA OTUs and functional genes of carbon and nitrogen cycling genes with environmental factors

Environmental parameters		16S OTUs		Functional genes	
		R	P	R	P
Vegetation properties	Plant biomass	0.102	0.124	0.444	0.001
	Plant diversity	0.181	0.027	0.391	0.001
	Plant species	-0.055	0.719	0.007	0.410
Soil properties	Moisture	-0.046	0.654	0.001	0.428
	pH	0.213	0.015	0.338	0.002
	Total Nitrogen	0.317	0.001	0.654	0.001
	Total Phosphorus	0.376	0.001	0.707	0.001
	Total Sulfur	0.294	0.004	0.435	0.001
	NH_4^+-N	0.012	0.434	-0.017	0.549
	NO_3^--N	0.104	0.156	0.028	0.311
	Soil organic carbon	0.352	0.003	0.674	0.001
	Available Nitrogen	0.080	0.198	0.289	0.004



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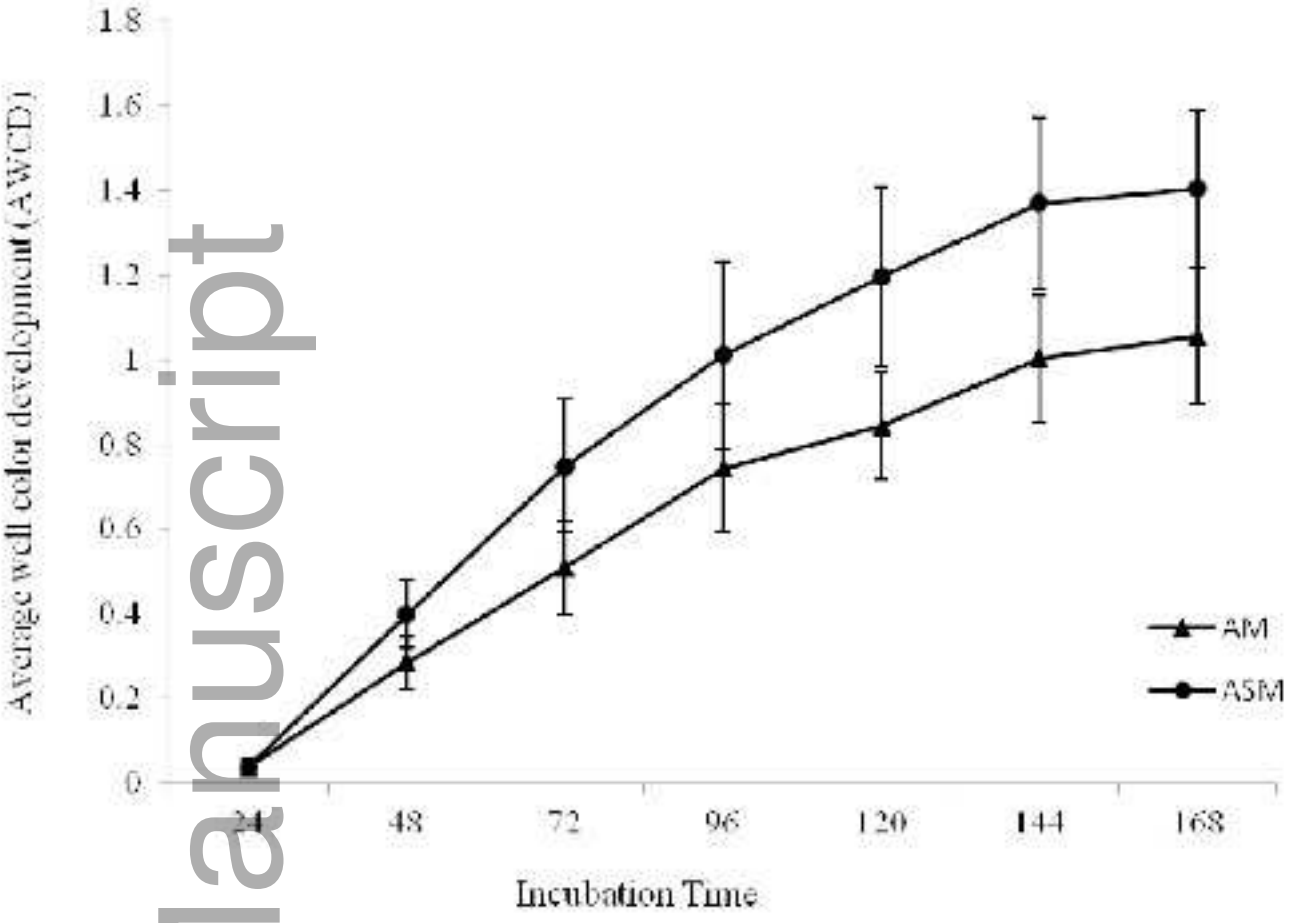

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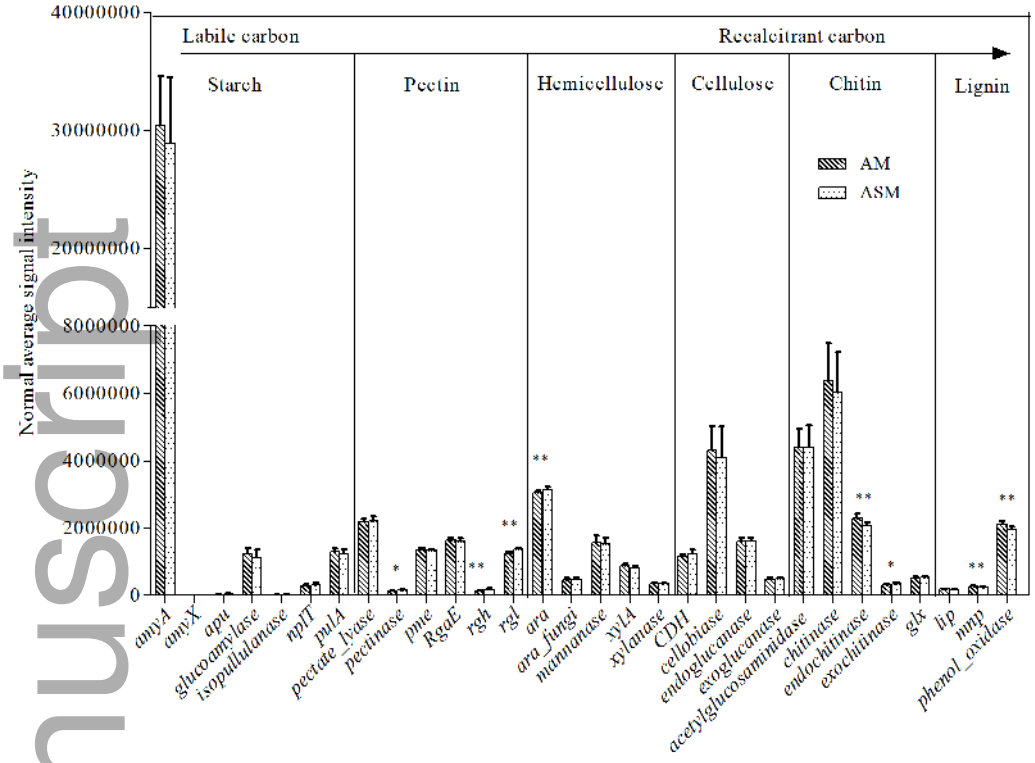

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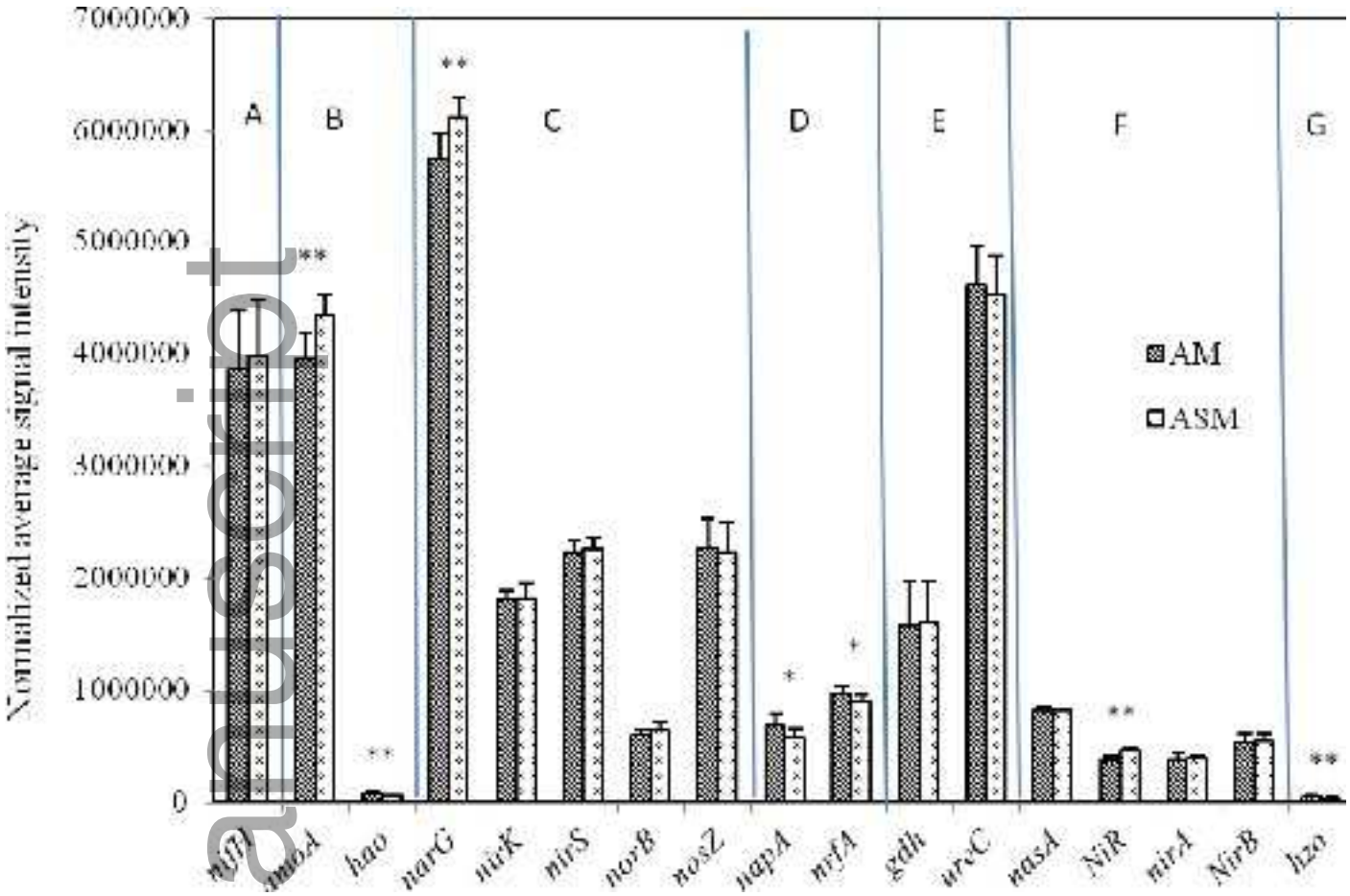

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