EFFECTS OF ANT MOUNDS ON THE PLANT AND SOIL MICROBIAL COMMUNITY IN AN ALPINE MEADOW OF QINGHAI–TIBET PLATEAU

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ABSTRACT

Ants are important soil engineers, affecting the structure and function of ecosystems. To address the impacts of ants (*Camponotus herculeanus*) on the properties of an alpine meadow ecosystem of Qinghai–Tibet Plateau, we investigated the effects of ant mounds on plant biomass, soil physicochemical properties, microbial diversity, and functions. We found that the total biomass of plant community was significantly greater in ant mound periphery. Plant species richness in ant mounds was reduced compared with that of control plots without ant mounds. Significant changes in physicochemical properties of soil were also observed. Soil organic matter, total nitrogen, available phosphorous, total potassium, and available potassium increased in ant mound soil due to the excavation activities by ants as well as the accumulation of organic matter and other nutrients during mound construction. For example, roots/soil contents (g/g) and soil moisture in ant mound soils were lower than those in controls. Microbial community composition and microbial biomass were clearly changed in ant mound soils. BIOLOG analysis further indicated that the functional diversity of the microbial community of ant mound soil increased and differed from that of controls. This study indicates that ant-induced modification of soil properties indirectly influences plant biomass and species composition, and ant mounds have different microbial communities from those of control soil. Copyright © 2016 John Wiley & Sons, Ltd.

KEY WORDS: ant mounds; soil microbial community; phospholipid fatty acid (PLFA); BIOLOG; Qinghai–Tibet plateau

INTRODUCTION

Ants are social, mound-building creatures (Hölldobler & Wilson, 1990). During mound construction and food harvest, they cause disturbances to grassland ecosystems that modify the structure and functioning of the plant community (King, 1977a; Sousa, 2004; Zhao *et al.*, 2008). The frequency and intensity of disturbances are the keys for the determination of the degree of such impacts (Zhou & Zheng, 1993). Appropriate disturbances can result in heterogeneous patches that enhance biodiversity, ecological niche, and complexity (Zhang *et al.*, 1995; Li, 2000). Ants can alter the assemblages in plant communities (Brown *et al.*, 2012). For example, harvester ants form underground colonies leading to the establishment of patches with different plant species composition compared with those in the surrounding areas (Jones *et al.*, 1997; Cammeraat *et al.*, 2002). Ant-induced soil changes [e.g., reduction in soil bulk density (BD) and increase in soil organic matter (SOM)]

increased water infiltration rates and runoff discharge, and under wet soil conditions, the presence of macropores (i.e., nest entrances) becomes important, reducing overland flow in mound plots (Cerdà & Jurgensen, 2008, 2011; Cerdà *et al.*, 2009; Cerdà & Doerr, 2010).

It is recognized that ants affect ecosystem dynamics through alteration of environmental conditions (Chen, 2000). They affect soil nutrient contents and availability, pH, microbial activity and biomass in their mounding sites (Frouz & Jilková, 2008), and the diversity of soil biota (Wagner & Jones, 2004; Whitford *et al.*, 2008). Ants increase heterogeneity within the microbial community by building mounds, which can change soil properties, for example, soil moisture, SOM, and concentrations of N, P, K, Na, Zn, Cu, and Mn (Dean *et al.*, 1997; Folgarait *et al.*, 2002).

The Qinghai–Tibetan Plateau, recognized as the third "pole" of the world, is characterized by high elevation, high solar radiation, low temperature, and hypoxic conditions (Qiu, 2008). Alpine meadow and steppe landscapes here cover about 1·5 million km^2, which are about two thirds of the total area of the plateau. Most studies in alpine meadows have focused on soil nutrients and characteristics of the plant community and their relationship under grazing disturbance (Wang *et al.*, 2008). *Camponotus herculeanus* is one important component because it extensively distributes in the alpine meadow (Yu *et al.*, 2010; Meng *et al.*, 2011).

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Although the impacts of ant activities (e.g., *C. herculeanus*) on plant community composition and soil nutrients have been intensively investigated in alpine meadow ecosystems of the Qinghai–Tibet Plateau (Chen *et al.*, 2007; Yu *et al.*, 2010; Meng *et al.*, 2011), and aboveground plant communities have long been considered to alter belowground microbial diversity (George *et al.*, 2002), to date, little is known as to how plant species composition, biomass, and diversity influence the soil microbial community composition and functional diversity in the alpine meadow. Soil microbial community composition and functional diversity in ant mound of alpine meadow were first measured by using phospholipid fatty acid (PLFA) and BIOLOG methods.

In this study, we aimed to assess the effects of ant mounds on soil microbial composition and functional capabilities and plant community properties. Especially, we were interested in the following questions: (i) whether *C. herculeanus* ant mounds alter soil physicochemical properties, which lead to increase aboveground plant community biomasses and decrease plant species richness and (ii) how ant mounds affect soil microbial biomass, community composition, and functional diversity; (iii) the relationships among ant, plant community characteristics, and soil microbial community composition were investigated.

MATERIALS AND METHODS

Study Site

Fieldwork was carried out in an alpine meadow ecosystem located in the eastern Qinghai–Tibetan Plateau (32°48′N, 102°33′E; Figure S1). The elevation of the study site is 3,500 m asl with an annual mean temperature of 0·9 °C and an annual mean precipitation of 690 mm. The dominant plant species in alpine meadow include sedges (*Kobresia setchwanensis* and *Kobresia pygmaea*), grasses (*Agrostis clavata* and *Elymus nutans*), and forbs (*Anemone trullifolia*, *Potentilla anserine*, and *Saussurea nigrescens*). The grass community typically grows in 1–2 layers, with a maximum height of 45–60 cm. Grasses cover an overall 60–95% of ground. Soils at the study site are described as Cryosol, according to the World Reference Base for Soil Resources (Li *et al.*, 2011).

Camponotus herculeanus Ant Mounds

Mounds of *C. herculeanus* are widely spread throughout the study area, occurring at a density of 6 to 9 mounds ha^{-1} in native vegetation (no degraded) conditions. A typical mound appears as large, bare mound with an approximate diameter of 30–50 cm. In these mounds, ants are usually active above ground from approximately April to October. Ants keep the mound surface clear in the center of the deposit debris area and cover the disk at the edge of each ant mound ("ant mound plot") with collected debris: for example, seed husks, plant litters, and insect carcasses and feces. In this study, all sampled ant mounds were active and inhabited by *C. herculeanus*, having a minimum height of 10 cm with 30 to 50 cm in diameter. A control plot was selected in the surrounding vegetation unaffected by ant nesting activity, at least 4 m from the edge of each *C. herculeanus* mound in a random direction because ants may affect surrounding soil 1–2 m away from the nest (Dean *et al.*, 1997).

Vegetation Property Measurement

The properties of vegetation were investigated at the end of the growing season in August 2011 (at the time of peak aboveground plant biomass). At the experimental site, perennial plants were sampled at the edges of ten ant mounds and at ten control plots, 4–5 m from the edge of the mound disk, using a 0·25-m^2 frame in a randomly selected direction from each mound (divided into 10 × 10 cm square grids) designed to estimate density and cover (Whitford, 1988). Within each 0·25-m^2 sampling quadrat, the coverage of each species was estimated visually to calculate total species coverage. Species richness was recorded as the occurrence of the number of plant species in each 0·25-m^2 sampling quadrant. Canopy height of each species within a quadrant was measured as the average of ten random measurements of the species' natural height. The aboveground biomass was determined by clipping plants above the soil surface at each 0·25-m^2 sampling quadrant. The clipped plants were sorted, dried at 65 °C for 48 h, and weighed. The plants were divided into four plant functional groups: grass, sedge, legume, and forbs (Wang *et al.*, 2008).

The important value (IV) of individual plant species was calculated by the following equation to value the dominance of one species in the community:

$$IV = (Cr + Hr + Fr)/3$$

Where: *Cr*, *Hr*, and *Fr* are the relative coverage, relative height, and relative frequency, respectively (Tan *et al.*, 2008; Table S1).

Soil Property Measurements

Ten active ant mounds where the aboveground biomass had just been removed were selected for the soil study. The mound was about 10 cm higher than original soil surface. Mounds and control soils were sampled in a randomly selected direction from each mound. To collect mound soil, the sample of the top 10 cm from the mound surface was discarded, so that soil from 10 cm below the mound surface was collected at the depth of 0–10 cm and 10–20 cm (Zhou, 2001; Yu *et al.*, 2010). Control soils were sampled 4–5 m from the edge of the ant mounds. Soil was sampled by using a 5-cm-diameter stainless steel hand corer. The soil was carefully sieved through a 2-mm mesh. The soil above the 2-mm sieve was washed to retrieve fine roots, and the fraction below 2 mm was used for further analysis. The roots collected from the 0–10-cm and 10–20-cm soil layers were described as 0–10-cm or 10–20-cm belowground biomass. The total roots collected from the 0–20-cm soil layer were described as total belowground biomass. The roots and soil samples were dried at 60 °C for 48 h and weighed. Root/soil content (g/g) is the ratio of roots to soil. Soil

sampling for measurements of soil microbial and physico-chemical properties.

Soil used for the PLFA measurement was conducted by the following ways. Among the ten selected active mounds, soil cores from every three adjacent mounds were obtained and thoroughly mixed as a replicate, and total three replicates were obtained for PLFA analysis. The soil samples were passed through a 2-mm mesh sieve and kept at −70 °C for subsequent PLFA and BIOLOG analyses. These soil samples were also used for soil physicochemical property measurements as described in the succeeding texts. Second, every two adjacent mounds of soil cores were obtained and thoroughly mixed as a replicate, and total five replicates were obtained. The soil samples (five replicates here and above triplicate samples) were air-dried, finely ground, and sieved with 0·1-mm mesh for subsequent soil physicochemical property analysis. Soil pH was measured by a combination glass electrode (soil : water = 1:2·5; Chu *et al.*, 2007), SOM was determined by dichromate oxidation method (Brady & Weil, 1999), and total N (TN) was determined by Kjeldahl digestion (Bremner & Mulvaney, 1982). Total P (TP) and total K (TK) were digested by HF–HClO$_4$ and determined by molybdenum blue colorimetry and flame photometry (Olsen & Sommers, 1982; Jackson & Barak, 2005), respectively. Available P (AP) was determined by using the molybdenum blue method (Olsen *et al.*, 1954); available potassium (AK) and available N (AN) were detected by NH$_4$OAC extraction-flame photometry method and by alkaline hydrolyzation diffusion method (Cong *et al.*, 2012), respectively. Five-gram subsamples were weighed, dried at 105 °C, and re-weighed to obtain gravimetric soil moisture (Whitford *et al.*, 2008). Soil BD was calculated by using the following formula: BD (g cm^{-3}) = dry soil weight (g) / soil volume (cm^3) (McKenzie *et al.*, 2004).

Microbial Functional Diversity

The functional diversity of the microbial community was measured by using BIOLOG ECO plates (Biolog Inc., Hayward, CA, USA). The 96-well ECO plate comprised 31 carbon substrates in triplicate for each substrate and control well without any carbon substrate. The substrates were carbohydrates, amino acids, carboxylic acids, polymer, phenolic acids, and amine. Soil sample (1 g) was diluted 10^3 times with sterile distilled water, and a 150-µl soil suspension was inoculated into each well of ECO plate with an eight-channel repeating pipette. The plates were incubated at 30 °C. The optical density (λ = 590 nm) was determined at time 0 and every 24 h thereafter, up to 240 h, using a plate reader (BIO-RAD Model 550). The rate of color development on the BIOLOG plate was determined by calculating an average well color development (AWCD; Garland, 1996). The AWCD for all carbon sources was calculated as a measure of total activity. In addition, the α diversity indices of the Shannon–Wiener index (H'), the Pielou evenness index (J), and the McIntosh index (U) are calculated to describe

characteristics of soil microbial community, respectively (Li *et al.*, 2010).

$$AWCD = \sum(C_i - R)/n$$
$$H' = -\sum P_i \ln P_i$$
$$J = (-\sum P_i \ln P_i)/\ln S$$
$$U = \sqrt{\sum n_i^2}$$

Where: C_i is the absorbance data of each well, apart from the control well, and R is the absorbance data of the control well. P_i is the proportion of the relative absorbance of well i to the sum of the absorbance of all wells, S is the number of wells that exhibits a color change, and n_i is the relative absorbance of well i (Stefanowicz *et al.*, 2010). BIOLOG data collected from a plate incubated for 72 h were analyzed to calculate the microbial community diversity index (Begon *et al.*, 1990). Well optical density values that were negative or under the detection limit (0·06) were set to zero (Classen *et al.*, 2003). We analyzed the data from the ECO plates by averaging the three values for each individual substrate used within a plate.

Phospholipid Fatty Acid Analysis

The single-phase extraction method (Malosse *et al.*, 2004) was used to isolate the total lipids from the SOM. PLFAs have been extracted according to the method of Wilkinson (Wilkinson & Anderson, 2001), and nonadecanoic acid methyl ester (Sigma Aldrich, St. Louis, MO, USA) has been used as internal standard (c = 33 mg mL^{-1}). A 1·5-mL internal standard was added at the end.

Full process of fatty acid methyl esters was quantified by gas chromatography–mass spectrometry. Fatty-acid separation was carried out by using a Hewlett Packard HP7890 gas chromatograph equipped with a capillary column [60 m × 0·32 mm × 25 µm (film thickness)]. The injector was set at 230 °C, and the oven was held at 50 °C for 1 min after injection. Oven temperature was then increased to 180 °C at 12 °C · min^{-1} and held for 2 min, increased to 220 °C at 6 °C · min^{-1} and held for 2 min, and increased to 240 °C at 15 °C · min^{-1} and held for 1 min. Finally, the oven temperature was increased to 260 °C at 15 °C · min^{-1} and held for 15 min. The transfer line was held at 280 °C throughout. Electron ionization mass spectrometry (Hewlett Packard HP5975 Mass Selective Detector) with helium as carrier gas was used to identify the full process of fatty acid methyl esters. Electron impact energy was set at 70 eV. Definitive peak identification was made for representative samples by gas chromatography–mass spectrometry and was applied to the nmole per gram PLFA contents.

The abundance of individual fatty acid methyl ester was expressed in relative nmole per gram of dry soil. The nomenclature of fatty acids followed the description by Frostegård *et al.* (1993a, 1993b). The fatty acids 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, and 24:0 were chosen to

represent general bacterial PLFAs (Johansen & Olsson, 2005; Kimura & Asakawa, 2006). Gram-positive (G$^+$) bacteria were identified by the PLFAs: i11:0, a14:0, i15:0, a16:0, and 2Me18:0 (Frostegård & Bååth, 1996; Böhme *et al.*, 2005). Gram-negative (G$^-$) bacteria was identified by the PLFAs: 16:1ω7t, 16:1ω9c, 16:1ω11t, i16:1ω11t, cy16:0, 18:lω11t, and cy18:0 (Zelles *et al.*, 1995; Johansen & Olsson, 2005). Similarly, 18:lω9t, 18:2ω9, and 12c were used as indicators of fungal biomass (Zog *et al.*, 1997); actinomycetes were identified by the PLFAs 10Me17:0 and 10Me18:0.

Statistical Analysis

Aboveground biomass values were used to characterize the community status of four different plant functional groups: grass, sedge, legume, and forbs. The means obtained for the quadrants in ten mound plots were recognized as the treatment means. Student's *t*-test was used to find the effects of ant mounds on the aboveground biomass, the species richness, coverage, and plant biomass by functional group. Two-way analyses of variance (ANOVAs) were used to find the effects of ant mounds on the ratio of roots to soil, soil moisture, and soil BD the pH, SOM, TN, TP, TK, AN, AP, and AK. One-way ANOVA was used to show the effects of ant mounds on the microbial group PLFA contents and the IV. Significant differences (at $p < 0.05$) among ant mounds and control were determined by the ANOVA with the least significant difference tests. In addition, substrate utilization patterns (BIOLOG data) were examined by using principal component analysis (PCA) to identify the differences in functional diversity of microbial community induced by ant activities. PLFA profiles (three replicates) were analyzed by using PCA to identify differences in soil microbial community composition induced by ant mounds. The linear model redundancy analysis (RDA) was used to examine the relationships between soil properties and soil microbial characteristics (Wang *et al.*, 2013). PCA and RDA were performed by using CANOCO for Windows, version 4·5 (ter Braak, 1998). Statistical analyses were conducted by using SPSS 16·0 software (SPSS Inc., version 16·0).

RESULTS

Plant Community Properties

The plant communities on the periphery of ant mounds were observed to be significantly different from those of control plots. The ant mounds were dominated by species of Cyperaceae (e.g., *Kobresia capillifolia* and *K. setchwanensis*) and Gramineae (e.g., *E. nutans* and *Festuca ovina*), while the control plots were mainly Compositae (*S. nigrescens*) and Ranunculaceae (*Thalictrum alpinum* and *Anemone rivularis*; Table S1). Compared with controls, plant coverage, height, and biomass of grass, and sedge were greater in the ant mounds plots. However, legumes and forb coverage, height, and biomass were significantly less in the ant mounds plots than in the control plots except for forb height ($p < 0.01$). Total plant biomass was significantly greater in ant mounds than that in the control. Plant species richness in ant mound plots reduced compared with control plots ($p < 0.01$; Table I).

Soil Properties

Soil BD significantly increased, while soil moisture and ratio of roots to soil decreased with sampling depth ($p < 0.001$). The ant mound soil had a significantly lower ratio of roots to soil at a depth of 0–10 cm compared with those of controls ($p < 0.05$), but soil BD was not significantly different between ant mounds and controls ($p > 0.05$). The interactions between ant mounds and soil depths were not significantly changed, except for the ratio of roots to soil (Table II, Figure 1).

Ant mound soil contained higher TN, TK, AP, and AK than those in controls. There were no significantly differences in SOM, TP, and AN contents between ant mounds and controls. In addition, ant mound soil was slightly more acidic than control soil. The interactions between ant mounds and soil depth were not significantly changed in soil nutrients (Table III).

Microbial Community Composition Assessed by Phospholipid Fatty Acid

The PCA analysis of the PLFA indicated a major split in microbial-community composition between ant mounds

Table I. Plant community quantitative properties ($n = 10$, mean ± SE) in ant mound and control plots in an alpine meadow

Component	Ant mounds	Control	Paired *t*-test	p
Grass plant coverage (%)	78·70 ± 3·61	17·25 ± 1·02	16·580	0·001
Legume plant coverage (%)	3·10 ± 0·91	23·60 ± 1·34	−11·540	0·001
Sedge plant coverage (%)	31·70 ± 2·01	20·55 ± 1·47	4·504	0·001
Forb plant coverage (%)	27·70 ± 4·80	83·08 ± 2·57	−9·416	0·001
Grass plant height (cm)	32·28 ± 1·94	22·84 ± 0·57	5·475	0·001
Legume plant height (cm)	5·25 ± 0·25	6·13 ± 0·22	−2·711	0·024
Sedge plant height (cm)	19·33 ± 0·86	16·00 ± 0·50	2·783	0·021
Forb plant height (cm)	12·90 ± 0·28	13·66 ± 0·29	−1·663	0·131
Grass plant biomass (g m^{-2})	137·38 ± 7·63	84·06 ± 4·04	7·078	0·001
Legume plant biomass (g m^{-2})	16·10 ± 1·98	28·30 ± 1·52	−6·377	0·001
Sedge plant biomass (g m^{-2})	109·09 ± 4·79	39·77 ± 2·26	10·931	0·001
Forb plant biomass (g m^{-2})	44·66 ± 3·04	130·73 ± 9·25	−9·933	0·001
Total biomass (g m^{-2})	307·22 ± 8·12	282·86 ± 6·52	3·242	0·010
Species richness	9·00 ± 0·75	20·10 ± 0·71	−14·478	0·001

Table II. Effects of ant mounds, soil depth (0–10 cm and 10–20 cm), and their interactions on the ratio of root to soil, soil moisture, and soil bulk density indicated by two-way analyses of variance (means ± standard error, $n = 10$)

Factors	Ratio of root to soil		Soil moisture (%)		Soil bulk density (g cm^{-3})	
	F	p	F	p	F	p
Ant mounds	5·433	0·034	9·037	0·009	0·017	0·897
Depth	743·089	<0·001	180·388	<0·001	30·930	<0·001
Ant mounds × depth	5·310	0·036	4·111	0·061	0·052	0·822

and the control soil along the first principal component axis (PC1; Figure 2). PC1 and PC2 explained 96·7% and 3·1% of the overall variation in the PLFA signatures. Total PLFA and bacterial PLFA contents in the ant mound soil were
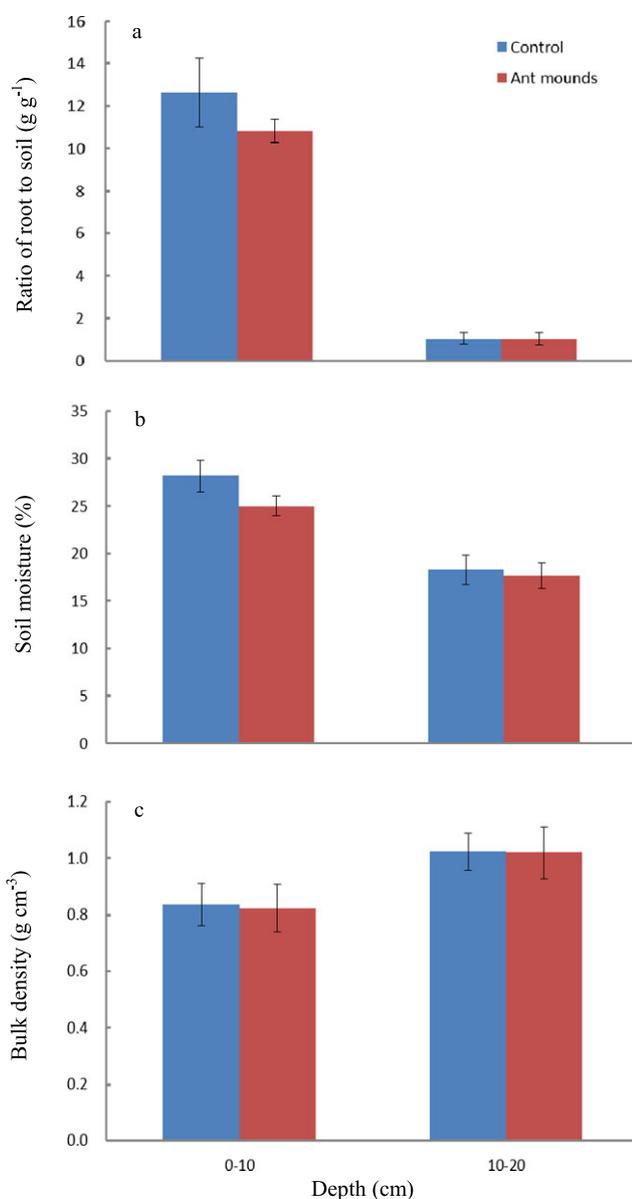

Figure 1. Ratio of roots to soil a, soil moisture b, and bulk density c ($n = 10$, mean ± SE) in ant mounds and controls sampled at two depths, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

significantly greater than those in the controls ($F = 10·283$, $p < 0·05$; $F = 289·975$, $p < 0·01$; Figure 3). The G$^-$ bacterial PLFA content in the control soil was greater than those in the ant mounds ($F = 29·438$, $p < 0·05$). The control soil had greater contents of PLFA 18:2ω9, 12c, a16:0, 10Me17:0, 16:1ω11t, and 18:1ω11t (Figure S2). The soil of ant mounds had significantly greater fungal PLFA contents ($F = 330·541$, $p < 0·01$). It contained greater contents of PLFA 16:1ω9c, 10Me18:0, 20:0, 16:0, 16:1ω7t, 18:1ω9t, cy16:0, 17:0, 18:0, and 24:0 (Figure S2).

Soil Microbial Functional Diversity

Average well color development generally followed a sigmoidal shape by incubation time, but the increasing rate varied between ant mounds and controls (Figure 4). After 240 h, AWCD was significantly lower in the controls than in ant mounds. Ant mounds had an increase trend in the microbial diversity indices (H', J, and U) compared with controls, although a significant difference is not shown in the statistics (Table IV). The PCA of the Biolog data indicated a distinct differentiation in microbial community structure between ant mound and control soil (Figure 5). PC1 explains 47·5%, while PC2 explains 27·1% of total variations. Furthermore, carbon substrate utilization (metabolic activity) was different between ant mound soil and controls. Only one type of carbon substrate utilization (carbohydrates) was observed in controls, while the ant mound soil had six types of carbon substrate utilization (carbohydrates, amino acids, carboxylic acids, polymer, phenolic acids, and amine; Figure 5).

Relationships of Phospholipid Fatty Acid with Soil pH and Nutrients

Redundancy ordination analysis showed the correlations of PLFA data and soil environmental properties (Figure 6). Axes 1 and 2 explained 61·0% and 33·9% of the total variations, respectively. Soil properties having significant effects on soil microbial community structure were SOM ($p = 0·04^*$, $F = 5·81$) and AK ($p = 0·02^*$, $F = 7·61$). Total PLFA content was affected by SOM and AK. The PLFAs from bacteria and fungi were significantly correlated with AK. The *Actinomyces*, G$^+$ bacteria, and G$^-$ bacteria were significantly correlated with SOM. The correlations among the pH value ($p = 0·46$, $F = 0·84$), TN ($p = 0·11$, $F = 3·36$), and microbial community structure were not significant.

Table III. Effects of ant mounds, soil depth (0–10 cm and 10–20 cm), and their interactions on the soil chemical properties indicated by two-way analyses of variance (means ± standard error, $n = 5$)

Factors	pH		SOM (g kg^{-1})		TN (g kg^{-1})	
	F	p	F	p	F	p
Ant mounds	1·631	0·221	3·870	0·058	4·499	0·051
Depth	1·011	0·331	21·489	0·001	20·009	0·001
Ant mounds × depth	0·002	0·988	0·080	0·781	0·225	0·642
Factors	TP (g kg^{-1})		TK (g kg^{-1})		AN (mg kg^{-1})	
	F	p	F	p	F	p
Ant mounds	0·997	0·339	9·686	0·007	0·061	0·808
Depth	0·180	0·678	0·123	0·731	5·507	0·033
Ant mounds × depth	0·103	0·753	1·085	0·314	0·012	0·914
Factors	AP (mg kg^{-1})		AK (mg kg^{-1})			
	F	p	F	p		
Ant mounds	11·742	0·004	36·724	0·001		
Depth	17·915	0·001	20·230	0·001		
Ant mounds × depth	0·084	0·776	1·035	0·325		

SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; AK, available potassium.
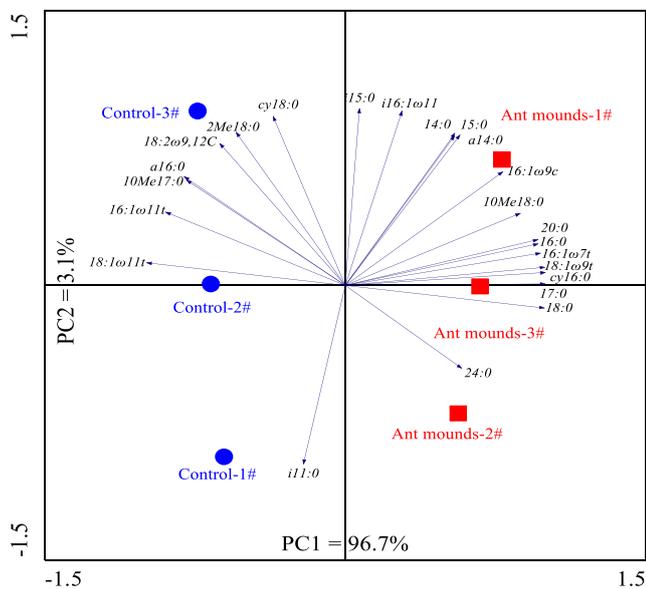

Figure 2. Principal component analysis for all phospholipid fatty acid signatures detected in ant mounds and controls. [Colour figure can be viewed at wileyonlinelibrary.com]

Figure 3. Phospholipid fatty acid biomass of soil microorganisms in the ant mounds and control soils. Values are means of triplicate ± standard errors. Different letters above the bar indicate significant difference at 0·05 levels between treatments. [Colour figure can be viewed at wileyonlinelibrary.com]

DISCUSSION

Vegetation Property Changes

Ants can disperse plant propagules (Wilson, 1992), and ant mounds accelerate the appearance of invasive plant species (Farji-Brener & Margutti, 1997). Grubb *et al.* (1969) found that most stunted forb plants disappear in the smothering phase, but the high grass plant survives and becomes dominant in subsequent competition. Some forbs vanish due to the building activity of ants in the invasion phase. Constant change in physical environment, interactions between the ant and the plant, and competition among plants in ant mounds together promote successional process of plant communities. Some plan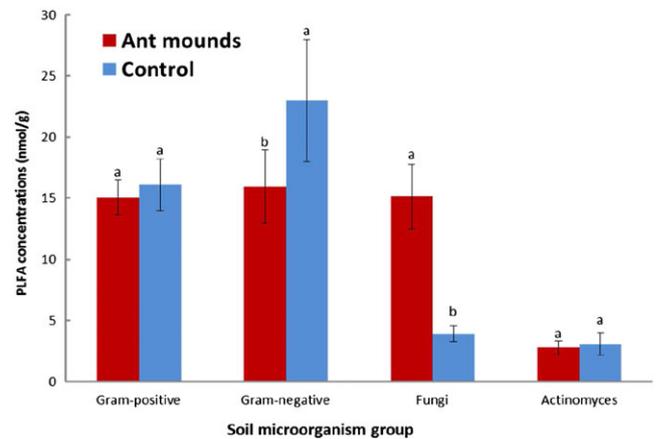ts may disappear on the surface of the mounds by ants' burial activity because the seedlings, seeds, and stunted plants (e.g., forbs) may be completely covered by heaped soil (King, 1977b). The effect of the *Messor aciculants* foraging on the density of seed in the soil seed bank was different with the different kinds of plants, especially the distribution of preferred seeds, which further effected the composition and space distribution of plant community (He *et al.*, 2003). The plant species composition and diversity variation of community on the ant hills correlated with the improvement of the soil physical–chemical properties, and the seed flows from the plant communities around the ant hills (Hou *et al.*, 2002). In addition, selective predation upon plant seeds can result in qualitative changes in plant community structure by increasing plant species richness (Samson *et al.*, 1992). Ants can provide nutrients for plants; the actual ecological niche of plants was enlarged and increased their competitive ability (Treseder *et al.*, 1995; Wagner, 1997).
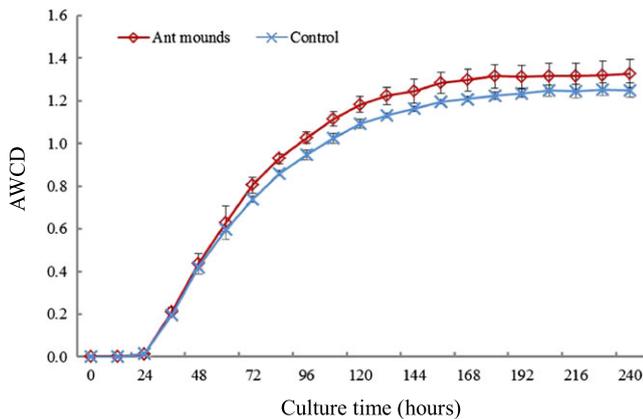
Figure 4. Average well color development profiles for the microbial community in the ant mound soil and controls. The values are means of triplicate ± standard errors. [Colour figure can be viewed at wileyonlinelibrary.com]

Soil Physical–Chemical Property Changes

Ants are recognized as ecosystem engineers (Frouz & Jilková, 2008). They may greatly impact soil physical, chemical, and biological properties through building corridors, galleries, and changing nutrient contents and soil microbes in ant mound-affected soils (Karlen *et al.*, 2003). We observed that soil in *C. herculeanus* ant mounds contained more TN, TK, AP, and AK than those in controls. The higher nutrient levels in ant mound soil are attributed to the accumulation of leaves, seeds, honeydew, feces, and corpses (Yu *et al.*, 2010) in ant mound areas during ant mound building. Many studies have shown that nutrient enrichment can positively influence seedling establishment (King, 1977a; Jones & Wagner, 2006; Sebastià & Puig, 2008). As a consequence, ant mounds may create nutrient-rich patches and increase soil heterogeneity that further affects plant community structure and dynamics.

Ants can change plant density, surface morphology (the always appearing bare patch in the center), and light condition, thus exerting important effects on soil moisture (Ohashi *et al.*, 2007). It is well known that the burrowing activity of ants increases the porosity of the soil (Cammeraat & Risch, 2008) and leads to lower soil BD (Cammeraat & Risch, 2008). We found lower ratio of roots to soil at a depth of 0–10 cm, and total plant biomass was significantly greater in ant mounds than that in the control. Our results were not in agreement with those of Karhu & Neuvonen (1998). There are a number of possible reasons for this finding. First, the control has higher forb plant biomass (130.73 g m^{-2}) than that (44.66 g m^{-2}) of the ant mounds (Table I), and forb plant biomass accounts for 46.22% of total plant biomasses in the control but only 14.54% of total plant biomasses in the
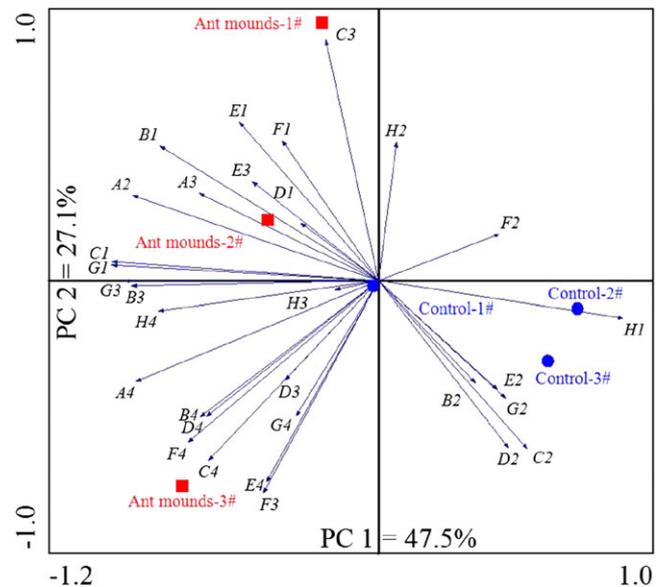

Figure 5. Principal component analysis for BIOLOG data in ant mound soil (ant mounds 1#–3#) and control soil (controls 1#–3#). A2: *β*-methyl-D-glucoside; A: D-galactonic acid lactone; A4: L-arginine; B1: pyruvic acid methyl ester; B2: D-xylose; B3: D-galacturonic acid; B4: L-asparagine; C1: Tween 40; C2: i-erythritol; C3: 2-hydroxybenzoic acid; C4: L-phenylalanine; D1: Tween 80; D2: D-mannitol; D3: 4-hydroxybenzoic acid; D4: L-serine; E1: *α*-cyclodextrin; E2: *N*-acetyl-D-glucosamine; E3: *γ*-hydroxybutyric acid; E4: L-threonine; F1: glycogen; F2: D-glucosaminic acid; F3: itaconic acid; F4: glycyl-L-glutamic acid; G1: D-cellobiose; G2: *α*-D-glucose-1-phosphate; G3: *α*-ketobutyric acid; G4: phenylethylamine; H1:*α*-D-lactose; H2: D,L-*α*-glycerol phosphate; H3: D-malic acid; H4: putrescine. [Colour figure can be viewed at wileyonlinelibrary.com]

ant mounds; the existence of forb plants in the control may be related with their contain nutrient availability. Therefore, less forb root density and root biomass in ant mounds are expected, resulting in lower root biomass. Second, ant-driven changes in moisture may directly influence root distribution in the surface soil of alpine meadow (Figure 1). Third, after plant community structure has changed, the root distributional depth of different plant species can alter in the processes of interspecific between plant species (Bonin *et al.*, 2013). Finally, underground biomass moved to deeper soil layers in alpine meadows under a background of soil environmental perturbation (e.g., simulated warming increased the soil temperature by 0–2 °C; Klein *et al.*, 2005; Yu *et al.*, 2015), and our conclusion is consistent with the findings of Dostál *et al.* (2005); they found that root biomass were smaller in the mounds than in the control plots. This can be a direct effect of soil disturbance that prevents surface layer root development. It is also possible that plants in ant mounds have very different allocation patterns. For example, due to greater nutrient availability in ant mounds, plants

Table IV. Metabolic diversity indices of soil microbial communities in ant mounds and control soil

	Shannon-Wiener index	Pielou index	McIntosh index	Soil carbon substrate utilization
Ant mound plots	3·26 ± 0·01	0·95 ± 0·00	5·11 ± 0·22	30·67 ± 0·58
Control plots	3·21 ± 0·03	0·94 ± 0·01	4·83 ± 0·07	30·00 ± 0·00

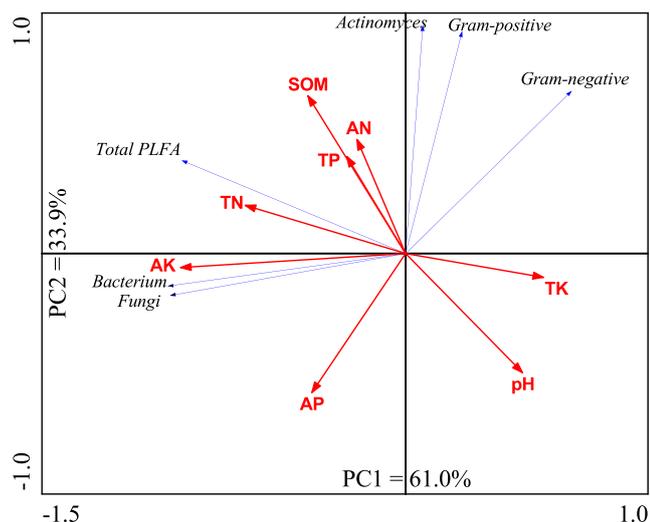Values are means of three triplicates ± standard errors.

Figure 6. Results of redundancy analysis for soil physicochemical properties and soil microbial community. [Colour figure can be viewed at wileyonlinelibrary.com]

may allocate more carbon to aboveground biomass (Wang *et al.*, 2010). Ant activity reduces pH in ant mound soil compared with controls. The decrease in pH may result from an accumulation of organic matter (Frouz *et al.*, 2003), which may produce more organic acids (Cammeraat & Risch, 2008).

Soil Microbial Community

Increased nutrient cycling in ant mound soil compared with controls was reported (Cammeraat & Risch, 2008). Our results further showed that the high nutrient amounts (e.g., TN, TK, and AP) in ant mounds, together with the lower root density and ratio of ratio of roots to soils, could increase nutrient availability in ant mounds. Further microbial community composition and activity were altered. PLFA analysis confirmed that total soil microbial biomass, fungal biomass, and bacterial biomass were greater in ant mound soil than in controls. In addition, there exists a clear difference between the soil microbial community of ant mounds and control soil in using carbohydrate, carboxylic acids, phenolic acids, amines, amino acids, and polymers C-substrate, carbohydrate, and amino acids and was the major carbon source type (Table S2). It suggests that microbial metabolic diversity is different in the ant mounds and control soil. The increase in microbial functional diversity in ant mounds is likely attributed to more diversified substrates introduced by ant activity. Also, SOM accumulates by around 10% in the ant mound, whereas nitrogen only by 0·3%, P by 0·06%, and K by around 2%. It means that in ant mounds, a surplus of electron donators (organic matter) compared with electron acceptors (nitrate) is available and a matter runaway is limited (see also BD). Ants process these substrates, for example, leaves, seeds, feces, and corpses, which may contribute more proportion of labile carbon in the ant mound soil. Also, honeydew may be transuded into the mound environment. Fungal and bacterial communities play important roles in soil carbon dynamics

(Zhou *et al.*, 2012). Even small changes in belowground microbial diversity can have critical effects on ecosystem processes (Fierer *et al.*, 2013). Ants maintain fungi as a food source for the scleroproteinous brood and carry out food preservation, infection control, and waste management (Benckiser, 2010). Thus, ants may significantly modify the soil microbial activity, soil quality, and carbon dynamics of alpine meadow ecosystem through mound construction activity.

Ant–Plant–Microbe Interactions

Ant mounds constitute patches widely distributed in alpine meadow of the Qinghai–Tibet Plateau. It appears that *C. herculeanus* enriches more nutrients (e.g., TN, AP, and AK) through their activities and impact the composition and activity of the soil microbial community. This suggests that these ant mounds indirectly affect the biomass of grasses and sedges via bottom-up processes. In addition, temperature is a key limiting factor for plant productivity in the alpine meadow ecosystem of Qinghai–Tibet Plateau. The bare surface of ant mound may absorb more heat, which facilitates the growth of heterotrophic decomposer (Laakso & Setälä, 1998; Schmid-Hempel, 1998; Christe *et al.*, 2003). Through ant mound construction, biogenic structures can constitute patches in the landscape where the availability of soil nutrients for plants is improved and microbial composition and activity are altered. For example, the actinomycetes PLFA 10Me18:0 and 10Me16:0 and G^+ bacteria PLFA cy17:0 and cy19:0 were abundant under N treatments, especially the NPK treatment (Zhang *et al.*, 2007). Erica *et al.* (2005) found that the actinomycetes PLFA 10Me18:0 and 10Me16:0 were significantly correlated with gross NH_4^+ mineralization. G^+ bacteria expressed opportunistic substrate use, representing organisms with the strategy to quickly colonize new substrates (Begon *et al.*, 1996). The present study suggested that soil nutrients (e.g., SOM) had an impact on the PLFA of actinomycetes and bacteria in ant mound soils. Therefore, soil microbial community structure and function are influenced by plant species with different physiological traits (Singh *et al.*, 2009), land-use practices (Bissett *et al.*, 2011), SOM (Dunaj *et al.*, 2012), and TN (Li *et al.*, 2014). The various direct and indirect interactions among ants, plants, and microbiota are powerful factors in creating spatial and temporal heterogeneities, which are thought to be positively correlated with floristic diversity (Vivian-Smith, 1997). Thus, ants may play a key role in altering plant species richness or modifying the plant–community structure of ecosystems (Lavelle *et al.*, 1997).

Nutrient-rich patches may change the plant community through increasing the growth rates and the reproductive success of particular plant species (Rissing, 1986; Brown & Human, 1997). Breton *et al.* (2016) suggested that the gain in terms of plant survival and growth can be possibly decisive in revegetation work contexts, especially when extreme climatic events occur under wood chip amendments. Some tree species are the most suitable for restoration of

oil fields in Amazon Basin (Villacís *et al.*, 2016). Moreover, soil water repellency can stimulate land degradation processes by reducing the affinity of soil and water, thereby changing soil substrate contents and physical property (Keesstra *et al.*, 2016). The increase in plant biomass in the ant mound may result in more abundant sources of carbon through plant root exudation and litter. As a consequence, synergy and co-evolution processes between plant and microbial community, as well as high microbial diversity, are maintained. Microbial functional diversity also increased in the ant mound. Bacteria may be inclined to use plant litter rich in carbohydrates and saccharides, but fungi may prefer plant litter rich in phenols (Bardgett *et al.*, 1996). The interactions of the microbial community and plants may create positive feedbacks to nutrient cycling through enhancing the biomass and diversity of the plant and microbial community.

CONCLUSIONS

This study demonstrated that *C. herculeanus* ant mounds constitute fertile islands in the alpine meadow of the Qinghai–Tibet Plateau. Nutrient-rich patches increase soil heterogeneity and affect plant community structure and dynamics. Plant biomass increased in the ant mounds, especially grasses, sedges plant biomass, and coverage. Ant mounds significantly impact belowground processes through the modification of soil physical and chemical environments, as well as increasing microbial biomass and functional diversity. The interactions of microbial community and plants may create positive feedbacks to nutrient cycling by changing biomass and diversity of plant and microbial community. Thus, ant–plant–microbe interactions may play a fundamental role in maintaining the function and stability of alpine meadow ecosystems. Through modifying soil nutrients, soil microbial composition, and the plant community, ants may influence ecosystem services such as maintenance of biodiversity, stability, nutrient cycling, and biomass production in the fragile alpine meadow ecosystem of Qinghai–Tibet Plateau.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web site:

Table S1. Importance value of common species in plant communities.

Table S2. Change of carbon utilization profiles by soil microbial in ant mound and control plots in an alpine meadow.

Figure S1. Map showing the location of the long-term sampling site in the alpine meadow.

Figure S2. Phospholipid fatty acids (PLFAs) contents (nmol g^{-1}, mean $\pm$ SE, $n = 3$) in ant mounds and control soil in alpine meadow.