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FIRE ALTERS VEGETATION AND SOIL MICROBIAL COMMUNITY IN ALPINE MEADOW

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ABSTRACT

Grassland fire, as an important ecological factor, is quite influential in determining the structural and functional stability of ecosystem. In this work, the fire-induced changes on the vegetation and soil microbial community were studied in alpine meadow. Microbial community composition was assessed by phospholipid fatty acid (PLFA) analysis, and functional diversity was determined by Biolog EcoPlate method. Our results showed that burning caused a significant increase in plant functional group coverage, biomass of grasses, soil bulk density and the ratio of roots to soils. Fire also caused an increase in soil pH and a decrease in total soil nutrient contents and soil moisture. The average well colour development of soil microorganism, the microbial functional diversity and the number of carbon source utilisation were also significantly affected by fire. Total bacteria PLFA, Gram-positive bacteria (G^+) PLFA, Gram-negative bacteria (G^-) PLFA and total PLFA of the burnt sites all increased significantly in burnt soil. The BACT/FUNG, SAT/MONO and G^+/G^- ratio also appeared to be higher in burnt sites. The total PLFA, G^+ PLFA and G^- PLFA are closely related to the plant community quantitative characteristics and soil nutrients. The total PLFA, bacteria and G^+ PLFA are significantly correlated with the soil total nutrients and available nutrients. These results suggest that the ability of soil microorganisms to use a single-carbon substrate was increased after a fire event. Grassland fire not only has direct impacts on plant community structure and function but also indirectly alters the soil microbial properties because of fire-induced changes in plant community. Copyright © 2015 John Wiley & Sons, Ltd.

KEY WORDS: fire; microbial communities; vegetation characteristics; PLFA; Biolog; alpine meadow

INTRODUCTION

Fire, as a natural process, is a widespread phenomenon, which happens almost in every ecosystem. Fire impacts the entire ecosystems including flora, fauna, the atmosphere and soil. Fire is also a political topic (Carreiras *et al*., 2014), and the perception of the society is very important (Pereira *et al*., 2015). Fire is especially important for soil properties of burnt areas in grasslands (Cerdà & Doerr, 2008; Dickie & Parsons, 2012). For example, burning and resulting post-fire environmental conditions can modify the functioning of soils physically, chemically and biologically (Doerr & Cerdà, 2005); burning caused a significant change in soil properties (Martín *et al*., 2012; Aznar *et al*., 2013). Guénon *et al*. (2013) found that frequent wildfires slowed down the restoration at short term of soil organic matter (SOM) and nutrient availability in Maures mountain range. However, burning the ecosystem with a certain frequency and intensity can be very helpful to maintain the biodiversity and ecological balance (Hart *et al*., 2005). For example, ash is an important factor for soil micro-environment protection and restoration after fire, because different fire intensity can change the soil environment by surface fuels to different particulates that are transported and leached into the soil profile (Pereira *et al*., 2013a). Grassland fire is an important ecological factor because of its direct and indirect impacts on the grassland communities (Zhou, 1995), and fire impacts soil properties because of the heat (Aznar *et al*., 2013); fire regime has attracted great attention of ecologists. Hence, understanding both the direct and indirect effects of fire on plant community and soil microbial properties is very important to explain the role of fire in maintenance ecosystem function of alpine meadow.

Soil is a complex and dynamic biological system; it determines the plant productivity of terrestrial ecosystems and maintains biogeochemical cycles (Nnnnipieri *et al*., 2003; Rafique *et al*., 2012). Microorganisms are crucial to soil function, particularly in organic matter decomposition and nutrient cycling (van der Heijden *et al*., 2008). Response of fire on soil microorganism populations and species composition depends on the severity of the fire and site conditions (Neary *et al*., 1999). In addition, physical and chemical changes induced by fire can alter soil microbial dynamics in several ways (Docherty *et al*., 2012), depending on the severity of the event (Boerner *et al*., 2005), and phylogenetically distinct microorganisms show varied physiological responses to stress and disturbance with different implications for biogeochemical cycling (Schimel *et al*., 2007). Fire can induce long-term changes in soil microbial community dynamics by altering the responses of

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microorganisms to available nutrients. Soil microorganisms being an essential source of soil fertility are quite sensitive to the microenvironment where they live. They give spontaneous response to any change in the soil ecological environment. Therefore, soil microbial biomass (total PLFA) and functional diversity may be considered as the determination index for maintaining the structure and function of the soil ecosystem (Campbell *et al*., 2008). Soil microbial communities are responsible for the cycling of carbon (C) and nutrients in ecosystems, and their activities are regulated by biotic and abiotic factors such as the quantity and quality of litter inputs, temperature and moisture (Castro *et al*., 2010). Furthermore, the effect of fire on soil microbial community structure and composition is determined by different timescales. DeBano *et al*. (1998) showed that the soil microbial composition and activity were changed by fire through heat-induced microbial mortality in the short term. Hart *et al*. (2005) suggested that the long-term responses of soil microbial community structure for firing may be modification in plant community composition and production.

Soil microbial community structure and composition measures are increasingly being used to determine the responses of soils to different disturbances (Banning *et al*., 2011) and to provide an indicator of ecosystem restoration (van Dijk *et al*., 2009). Currently, methodological advances such as analysis of DNA and PLFAs as well as cultivation on Biolog EcoPlates allow more detailed information on soil microbial activities and community structure (Guénon *et al*., 2013). Community-level PLFA profiles have been found to be useful in detecting the responses of soil microbial communities to a variety of land uses or disturbances in ecosystems (Harris, 2003).

The importance of fire to form the plant community structure and composition is well documented. For instance, fire can enhance plant and animal habitat by promoting the establishment of specific plant communities (Luke *et al*., 2000). In savanna grasslands, fire is an important force affecting plant species composition, richness, diversity and cover (Govender *et al*., 2006), as well as an important determinant of plant diversity and vegetation structure (DeBano *et al*., 1998). Therefore, understanding soil microbial processes is also of great relevance to assess plant species composition (or plant functional composition) and biomass in alpine meadow. The Qinghai–Tibetan plateau is the most important area for livestock grazing, especially for Tibetan sheep and yak grazing (Zhou *et al*., 2003). Alpine meadow and steppe cover about 1·5 million square kilometres, accounting for two-thirds of the total plateau area (Sun & Zheng, 1998). However, until now, most studies in alpine meadow have focused on the soil nutrients and plant community characteristics and their relationship under grazing disturbance and different degraded conditions (Wang *et al*., 2008; Cao *et al*., 2011), but a few have focused on the effects of fire on vegetation and soil microbial community dynamics on relationship between plants and microorganisms in alpine meadow, and it is still unclear whether soil microbial structure and composition are likewise influenced by plant community structure and function [e.g. aboveground biomass (AGB)] in the short term.

The main objectives of this study were as follows: (i) to assess the impacts of fire on plant community characteristics; (ii) to assess the impacts of fire on soil nutrients and microbial community structure and functional diversity, especially bacterial or fungal community composition; and (iii) to determine what link is existing on fire–plant–soil microbe in the short term. The special objective of the paper is to determine the indirect effects of fire on soil microbial community structure and composition due to changes in plant community composition and biomass in alpine meadow.

MATERIALS AND METHODS

Site Description

This study was conducted at the Ga-wu Mountain of Xian-shui township (31°00·262′N, 101°06·058′E with the elevation of 3,368 m), Dao-fu county, Gan-zi Tibetan autonomous prefecture, Si-chuan Province. The study area is characterised with cold climate with smaller temperature variation and strong ultraviolet radiation. The mean annual temperature is 8·0 °C with minimum −26·9 °C and maximum 27 °C. The annual precipitation is 608 mm with heavy rain in June and September. This area has no absolute frost-free season with the average temperature of 2·5 °C in January and 16 °C in July (Wang *et al*., 2009). Because of complex geomorphology, the grassland types can be characterised as alpine meadow, subalpine meadow, alpine scrub meadow, subalpine scrub meadow and alpine swamp meadow (Li, 2008). The soils at study site are classified as alpine meadow and alpine bush meadow. The *Kobresia humilis* Sergiev and *Kobresia capillifolia* Decne are two main summer-grazed and winter-grazed meadows, in which *K. humilis*, *Poa pratensis* L. and *Bromus inermis* Leyss are the dominant plant species. The plant community structure is characterised with an herbaceous layer. The *Koeleria cristata* Pers, *Potentilla anserine* L., *Aster alpinus* L. and *Galium aparine* L. are common species in the plant community.

Experimental Design and Vegetation Sampling

Three experimental plots (20 m × 20 m) were randomly selected in the burnt area of Ga-wu Mountain located at 5 km away from Dao-fu County. This area came under a severe fire event on December 2010 and ashed aboveground litter on huge scale. Three control plots with the same size were also selected in nearby unburnt area to investigate the effects of fire event. We used 10 1 m × 1 m observation quadrats for plant measurements and soil sampling. At the end of the growing seasons on August 2011, plant species composition, plant height, coverage and AGB were measured. A visually estimated method was used to measure the changes in the community coverage. Plant cover was measured annually in the quadrats (1 m × 1 m). A 1 m × 1 m frame with 100 equally distributed grids (10 cm × 10 cm) was put above the canopy in each quadrat.

The coverage of each species was visually estimated in all the grids and summed as the species coverage in the quadrat. All the species coverage was summed as the coverage of the whole community. The species richness was recorded as the occurrence of the number of plant species in the quadrat. Canopy height of each species within a quadrat was calculated as the average of ten random measurements of species' natural height. The AGB was harvested by clipping above the soil surface in subquadrat (50 cm × 50 cm). The clipped plants were sorted, dried at 65 °C for 48 h and weighed. The plant biomass was divided into four plant functional groups: grass, sedge, legume and forbs (Wang *et al*., 2004). Plant biomass was measured using oven-dry method. Species richness (species number per quadrat), Shannon–Wiener index (H') and Pielou index (J) were used to describe the ecological properties of plant community:

$$H' = -\sum_{i=1}^{s} P_i LnP_i$$

$$J = \left(-\sum_{i=1}^{s} P_i LnP_i\right)/LnS$$

Where: P_i stands for the relatively important value of species i in each quadrat [P_i = (relative cover + relative height + relative frequency) / 3] and S is for species richness. The belowground biomass at the end of the growing seasons on August 2011 was collected from the topsoil (0–10 and 10–20 cm) using cylindrical soil core (90 cm in depth and 5 cm in diameter) (Ren, 1998). The soil samples were carefully washed to retrieve fine roots. The roots of 0–10 and 10–20 cm soil layers were described as 0–10 and 10–20 cm belowground biomasses. The total roots of 0–20 cm soil layer were described as the total belowground biomass. Meanwhile, soils at 0–10 and 10–20 cm soil layers were described as soil contents. The roots and soil samples were dried at 60 °C for 48 h and weighed. Roots/soil contents (g/g) is the ratio of roots to soils.

Soil Analysis

After removing the roots and stones using the sieves of 2-mm mesh, the fresh soil samples were divided into two subsamples and transported to the molecular biology laboratory in Southwest University for Nationalities, PR China. One subsample was homogenised with 2-mm mesh-sized sieves and frozen at −70 °C for phospholipid fatty acid (PLFA) analysis, whereas the other subsample was air dried and sieved with 0·1-mm mesh to measure soil organic C, total N, total P, available N, available P and pH using the standard methods described in the soil analysis manual (Institute of Nanjing Soil Science, Chinese Academy of Sciences, 1983). Soil bulk density was measured by metal ring method, calculation formula: bulk density ($g\,cm^{-3}$) = dry soil weight (g) / soil volume (cm^3) (McKenzie *et al*., 2004). Soil moisture was measured by a gravimetric method.

Phospholipid Fatty Acids of Soil Microbes

Total lipids were extracted from the SOM using a single-phase extraction method (Zelles, 1999). Fatty acid methyl esters were suspended in 100-μL chloroform : hexane [1:4 (v/v)] containing 33 $\mu g\,mL^{-1}$ of nonadecanoic acid methyl ester (Sigma-Aldrich Co, MO, USA). Fatty acid separation was carried out using a Hewlett-Packard HP7890 gas chromatograph equipped with a Hewlett-Packard hp5 capillary column [60 m × 0·32 mm × 25 (micrometre film thickness)] (Hewlett-Packard, 8000 Foothills Blvd. Roseville, CA, USA). The injector was set at 230 °C, and the oven was held at 50 °C for 1 min after injection. The nomenclature of fatty acids was followed as described by Frostegård *et al*. (1993a). Fatty acids are designated in terms of the total number of C atoms with the number of double bonds given after a colon. The position of the double bond is defined by the symbol ω followed by the number of carbons from the methyl end of the fatty acid molecule. *Cis* and *trans* configurations are indicated by *c* and *t*, the *i* and *a* refer to *iso* and *anteiso* branching, *br* indicates an unknown branch position, and *cy* refers to cyclopropyl fatty acids. The 10*Me* indicates a methyl group on the tenth C atom from the carboxyl end of the molecule. The abundance of individual fatty acid methyl esters was expressed as mole percentage.

The fatty acids 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0 and 24:0 were chosen to represent the general bacterial PLFAs (Kimura & Asakawa, 2006; Johansen & Olsson, 2005), whereas the fatty acids a14:0, i15:0, a16:0, i16:0, i17:0 and 10Me18:0 were chosen to represent the Gram-positive bacteria (G^+) (Böhme *et al*., 2005; Frostegård & Bååth, 1996). Similarly, the Gram-negative bacteria (G^-) were identified as 16:1ω7c, 16:1ω9c, cy16:0, 18:lω10t, 18:lω11t and cy18:0 (Zelles *et al*., 1992); 18:lω9c and 18:2ω9 12t were used as indicators of fungal biomass (Zogg *et al.*, 1997), and actinomycetes were identified by the PLFA 10Me18:0 (Frostegård & Bååth, 1996). Monounsaturated fatty acids (MONO) were chosen to represent the fungi and G^-. The normal saturated fatty acids (SAT) were used as indicators of general bacterial and Gram-positive bacterial communities. The fatty acids 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 24:0, a14:0, i15:0, a16:0, i16:0, i17:0, 10Me18:0, 16:1ω7c, 16:1ω9c, cy16:0, 18:lω10t, 18:lω11t and cy18:0 were chosen to represent the bacterial PLFAs (Frostegård *et al.*, 1993b; Kimura & Asakawa, 2006).

Functional Diversity

The functional diversity of the microbial communities was measured using Biolog EcoPlates (Biolog Inc, CA, USA). The 96-well EcoPlate comprised three replicate wells of 31 C substrates and a no-substrate well without a C. The substrates were amines (n = 2), polymers (n = 4), amino acids (n = 6), carbohydrates (n = 9) and organic acids (n = 10). Soil samples (1 g) were diluted 10^3 times with sterile distilled water, and 150 μL of the mixture was inoculated into each well of EcoPlate with eight-channel repeating pipettor. Plates were incubated at 30 °C. The optical density

($\lambda = 590$ nm) of each well was determined at 0 time and every 24 h thereafter up to 96 h using a plate reader (BIO-RAD model 550; Laboratories Inc. Hercules, CA, USA). The rate of colour development on Biolog plates was determined by calculating an average well colour development (AWCD) (Garland, 1996). The AWCD for all C sources was calculated as a measure of total activity. The functional diversity is measured by the Shannon–Wiener index (H'), Pielou evenness index (J) and McIntosh index using the following equations:

$$H = -\sum P_i \ln P_i$$

$$J = (-\sum P_i \ln P_i)/\ln S$$

$$U = \sqrt{\sum n_i^2}$$

$$AWCD = \sum (Ci - R)/n$$

Where: Ci is the absorbance data of each well except for the control well, 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 the well that has taken on a colour change and n_i is the relative absorbance value of well i (Anna *et al.*, 2010). Biolog data incubated for 72 h were analysed to give 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 analysed the data from the EcoPlates by averaging the three values for each individual substrate used within a plate.

Statistical Analysis

For vegetation samples, we used the AGB values to characterise the community status of four different plant functional groups: grass, sedge, legume and forbs. The means obtained for the quadrats in each plot were used to calculate the treatment means. Treatment effects on AGB, Shannon–Wiener index, coverage and plant functional group biomass were examined using a one-way analysis of variance. Tests for significant differences among treatments were conducted by analysis of variance with Duncan's multiple range tests at the significance level of $p = 0{\cdot}05$. All the statistical analyses were conducted using SPSS 16·0 software (version 16·0; SPSS Inc, IL, USA). In addition, substrate utilisation patterns (Biolog data) and PLFA profiles were analysed using a principal component analysis (PCA) to identify the differences in soil microbial community structure induced by burning. Correlations were made using a linear Pearson's r coefficient. PCA was performed using CANOCO for Windows, version 4·02 (ter Braak, 1998).

RESULTS

Plant Community Quantitive Characteristics

The quantitive analysis of community characteristics showed that burning induced an obvious change for plant species composition, plant functional group and diversity index in burnt site of alpine meadow (Table I and Figure 1). The plant

Table I. Results of one-way analyses of variance showing the effect of fire on the quantitative properties in alpine meadow community

	Plant community coverage (%)			Plant community biomass (g m^{-2})			Shannon–Wiener index			Pielou index			Plant species richness			Height (cm)		
Factors	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Fire	1, 19	82·2	<0·001	1, 19	36·4	<0·001	1·19	3829·9	<0·001	1, 19	22·9	<0·001	1, 19	11·5	<0·001	1, 19	69·1	<0·001

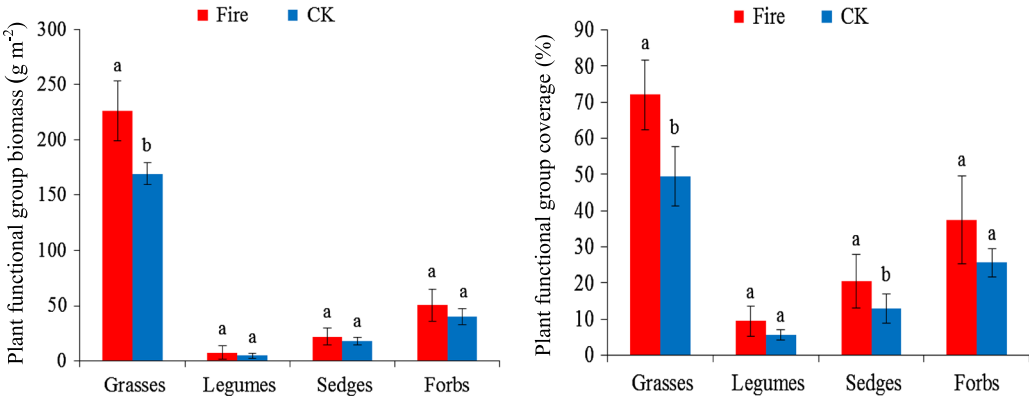

Figure 1. Effect of fire on plant functional group biomass and coverage in the alpine meadow; unburnt site soils (control) abbreviated as CK. This figure is available in colour online at wileyonlinelibrary.com/journal/ldr.

species richness of alpine meadow increased by 1·61 times compared with unburnt site. Similarly, the total coverage of plant community, plant height and biomass increased by 8·20%, 6·18 cm, and 73·76 g m^{-2} ($F_{1,\ 18}$ = 82·2, 69·0, 36·4, $p < 0·001$) (Table I), respectively. The coverage and AGB of grass increased significantly by 22·60% and 56·86 g m^{-2} ($p < 0·05$), respectively. However, the coverage and biomass of legume, sedge and forbs did not show significant differences (Figure 1). Plant species richness ($F_{1,\ 18}$ = 11·5, $p < 0·001$), Pielou index ($F_{1,\ 18}$ = 22·9, $p < 0·001$) and Shannon–Wiener index ($F_{1,\ 18}$ = 3829·9, $p < 0·001$) (Table I) increased by 1·61, 0·0156 and 0·3184, respectively.

Soil Physicochemical Characteristics

Fire significantly increased the soil bulk density and the ratio of roots to soils ($F_{1,\ 36}$ = 52·0, 19·4, $p < 0·001$; Table II) but decreased the soil moisture ($F_{1,\ 36}$ = 9·5, $p < 0·01$; Table II). Soil bulk density at 0–10 and 10–20 cm soil layers increased by 0·08 g cm^{-3} ($F_{1,\ 36}$ = 9·5, $p < 0·01$; Table I). Likewise, the ratio of roots to soils increased by 2·29% at 0–10 cm soil layer ($F_{1,\ 36}$ = 298·6, $p < 0·001$; Table II), and the soil moisture decreased by 4·76% ($F_{1,\ 36}$ = 13·4, $p < 0·001$; Table II) at 0–10 cm soil layer. Burnt site resulted in a significant decrease of SOM content, total N, total P and total K ($F_{1,\ 8}$ = 28·2, 8·0, 59·2, 12·4, $p < 0·05$) while the pH was increased ($F_{1,\ 8}$ = 35·8, $p < 0·001$; Table III). However, the total available N, P and K in soil were increased significantly in burnt sites ($F_{1,\ 8}$ = 16·6, 77·1, 23·2, $p < 0·01$; Table III). Particularly, the available N and K contents were increased by 20·75 and 20·10 mg kg^{-1}, respectively. The loss of organic matter also caused a reduction in soil water-holding capacity.

Soil Functional Diversity by Biolog EcoPlates

Functional diversity of the microbial communities was investigated by examining the potential of C utilisation out of 31 different C sources present in EcoPlates. The AWCD of the C sources for all the soil samples followed the same pattern during the incubation period of 240 h (Figure 2). The AWCD was observed significantly ($p < 0·05$) higher in the burnt site compared with the unburnt site. Fire has positive effects on the metabolic activity of soil microbial community and showed higher utilisation rate. Burning induced a significant increase in the soil microbial community diversity index and the number of C source utilisation in the burnt site ($p < 0·05$; Table IV).

The PCA of 31 C sources showed more obvious pattern of functional diversity of microbial community between burnt and unburnt sites (Figure 3). In PCA, the first principal component (PC1) contributed mainly 64·8%, and the second principal component (PC2) 15·4% (Figure 3). The PCA of the loading factor showed that variability in PC1 was explained by utilisation of carbohydrates, amino acids, phenolic acids, amines, carboxylic acids and polymers. A label for this principal component might be 'relatively low utilisation of B4, G4, C1, E3, H3, high A2, G1, B2, D3'. PC2, described by both positive and negative loadings, could be labelled as 'relative high utilisation of three carbohydrates (A3, D2 and F2), two amino acids (E4 and D4) and low polymer (F1)' (Table S1). This result shows that there exists

Table II. Results of two-way analyses of variance showing the effect of fire, soil layers (0–10 and 10–20 cm) and their interactions on the ratio of roots to soils, soil moisture and soil bulk density in alpine meadow community

	Ratio of roots to soils			Soil moisture (%)			Soil bulk density (g cm^{-3})		
Factors	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Fire	1, 36	19·4	<0·001	1, 36	9·531	<0·001	1, 36	52·0	<0·001
Depth	1, 36	298·6	<0·001	1, 36	13·402	<0·001	1, 36	584·7	<0·001
Fire × Depth	1, 36	22·9	<0·001	1, 36	1·867	>0·05	1, 36	1·2	>0·05

Table III. Analysis of variance for pH value, soil organic matter moisture, total nitrogen, total phosphorous, total potassium, available N, available phosphorous and available potassium in the 20-cm soil profile in the fire plot of alpine meadow

	pH value			Soil organic matter (g kg^{-1})			Total nitrogen (g kg^{-1})			Total phosphorous (g kg^{-1})			Total potassium (g kg^{-1})			Available N (mg kg^{-1})			Available phosphorous (mg kg^{-1})			Available potassium (mg kg^{-1})		
Factors	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Fire	1, 8	35·8	<0·001	1, 8	28·2	<0·001	1, 8	8·031	<0·05	1, 8	59·2	<0·001	1, 8	12·4	<0·01	1, 8	16·6	< 0·01	1, 8	77·1	<0·001	1, 8	23·2	<0·001

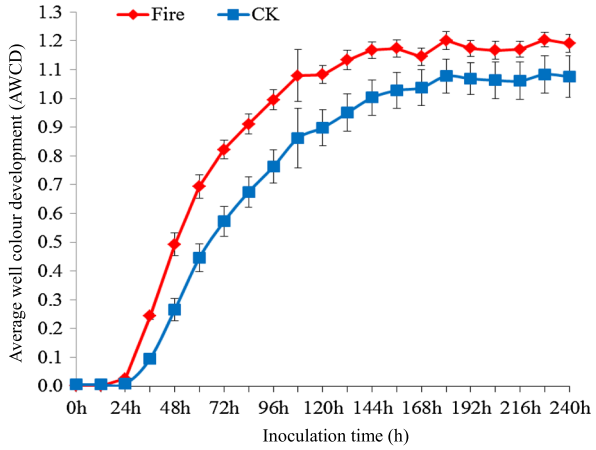

Figure 2. The average well colour development for all C sources for fire soils and unburnt site soils (CK) as a measure of total microbial activity. The values are means of triplicate analyses on six soil sample replicates. Error bars represent one standard error. The average well colour development rate in fire soils (240 h) versus CK (240 h) was statistically different ($p < 0{\cdot}05$). This figure is available in colour online at wileyonlinelibrary.com/journal/ldr.

obvious difference in the C source utilisation capacity between microbial community of burnt and unburnt soils.

Soil Microbial Community Composition and Structure Characteristics

Different treatment soils contained a variety of PLFA composed of saturated, unsaturated, methyl-branched and cyclopropane fatty acids (Figure 4). Twenty-three PLFA with chain lengths from C12 to C21 varied significantly in their relative abundance between soils (Figure 4), representing G^+ (a14:0, i15:0 and a16:0), G^- (16:1ω9c), fungal (18:1ω9c) and general bacteria (14:0, 15:0, 16:0 and 18:0). They occupy 73·50% and 75·33% of burnt and unburnt site soils, respectively. The content of monounsaturated fatty acids and bacteria PLFA in burnt site soils was significantly higher than that in unburnt site soils. However, the total number of microbial species was unchanged. The microbial communities can be classified into different groups (bacteria, G^+, G^-, fungi and actinomycetes) according to their PLFA contents. The contents of bacteria, G^+, G^- and total PLFA increased significantly by 41·19, 16·26, 6·18 and 44·88 nmol g^{-1}, respectively ($p < 0{\cdot}05$) in burnt site. The same trend was also found in actinomycetes and fungi, which increased by 0·74 and 3·69 nmol g^{-1} ($p > 0{\cdot}05$), respectively. The PLFA concentrations of total bacteria, G^+, G^-, fungi and actinomycetes at burnt and unburnt sites are shown in Table V.

We found that the ratio of bacterial to fungal PLFAs was slightly higher in burnt site (89·3% and 10·7%) compared with unburnt site (88·4% and 11·6%). We also found that the ratio of BACT/FUNG, G^+/G^- and SAT/MONO increased in burnt site (Figure 5). The proportion of the bacterial, G^+ and normal saturated fatty acids (SAT) PLFA in total PLFA was increased whereas that of the fungal, G^- and monounsaturated fatty acids (MONO) PLFA

Table IV. Results of one-way analyses of variance showing the effect of fire on the soil microbial community diversity index and number of carbon source utilisation in the Biolog EcoPlate in alpine meadow

	Shannon–Wiener index			Pielou index			McIntosh index			Number of carbon source utilisation		
Factors	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Fire	1, 10	33·3	<0·001	1, 10	9·9	<0·01	1, 10	27·2	<0·05	1, 10	10·4	<0·01

decreased, which indicates a change in soil microbial community structure in burnt site. The PCA of PLFA showed that fire induced significant dissimilarities in the soil microbial community structure in burnt site. PC1 and PC2 explain 87·5% and 10·5% of the overall variance in the data, respectively (Figure 6). The PCA of the loading factor on PC1 (Figure 6) found that the most influential individual PLFAs were 13:0, i15:0, i16:0, 17:0, 18:1ω9c, 18:1ω10t, 10Me18:0, 18:1ω11t, 20:0, 18:2ω9·12t and cy16:0. The individual PLFA with the highest loadings on PC2 were 12:0, 16:1ω7c, a16:0, 18:0, cy18:0 and i17:0.

Correlation Between Phospholipid Fatty Acid Contents and Plant and Soil Nutrients

The plant species richness and the ratio of roots to soils were found to be positively correlated with total PLFA, bacterial PLFA, G^+ bacterial PLFA and G^- bacterial PLFA ($p < 0{\cdot}05$; Table VII). The plant community coverage, height and biomasses exhibited negative correlation with total PLFA, bacterial PLFA, G^+ bacterial PLFA, and G^- bacterial PLFA ($p < 0{\cdot}05$; Table VI). Total PLFA, bacterial PLFA and G^+ bacterial PLFA showed a positive correlation with soil available nutrients and total nitrogen ($p < 0{\cdot}05$; Table VII) and a negative correlation with pH value, SOM, total phosphorus and total potassium. Similarly, G^- bacterial PLFA has positive correlation with available phosphorus and potassium and negative correlation with pH, SOM, total phosphorus and total potassium. A notable positive correlation also observed among G^+/G^-, available N and available potassium. However, there was negative correlation between G^+/G^- and total potassium and fungal PLFA and total potassium ($p < 0{\cdot}05$; Table VII). All other soil microbial PLFAs did not show significant correlation with soil nutrients ($p > 0{\cdot}05$; Table VII).

DISCUSSION

Fire as a key ecological factor can show long-term effects on microbial population dynamics, plant community composition and biodiversity characteristics, as well as plant growth and development (Bond & Wilgen, 1996). After a fire, plants can survive and renovate through morphological and physiological adaptive mechanisms (Schwilk, 2003). In this study, we found that short-term fire has a relatively greater effect on vegetation (e.g. coverage, height and biomass) largely because of a larger amount of organic matter removed by fire in alpine meadow, especially grass plant species that develop fire-adaptive traits to maximise their chances of survival and regenerate in a fire environment. On the other hand, the

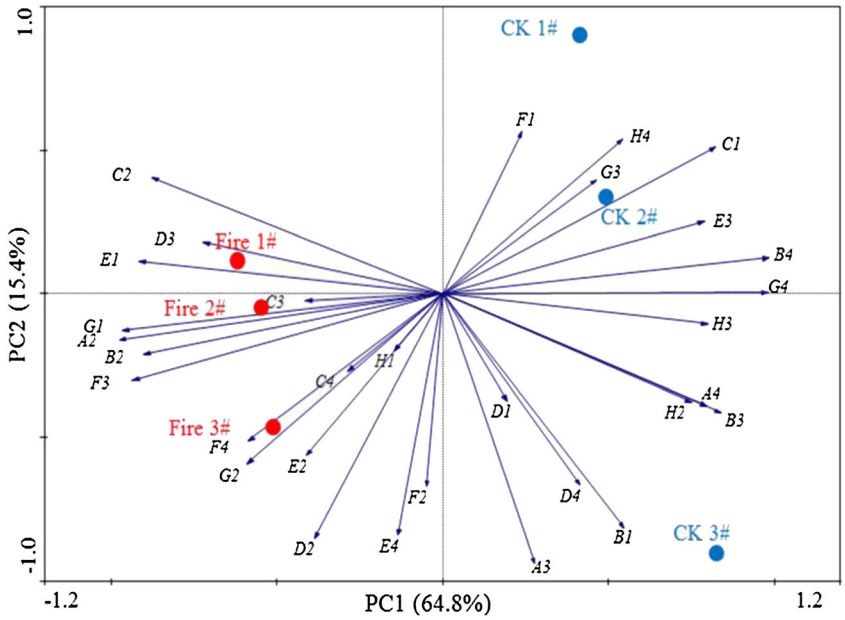

Figure 3. Principal component analysis plots of Biolog data in burnt site soils (fire 1#, fire 2# and fire 3#) and unburnt site soils (CK 1#, CK 2# and CK 3#). PC1 and PC2 explain 64·8% and 15·4% in alpine meadow, respectively. Unburnt site soils (control) abbreviated as CK; # meaning repeat. This figure is available in colour online at wileyonlinelibrary.com/journal/ldr.

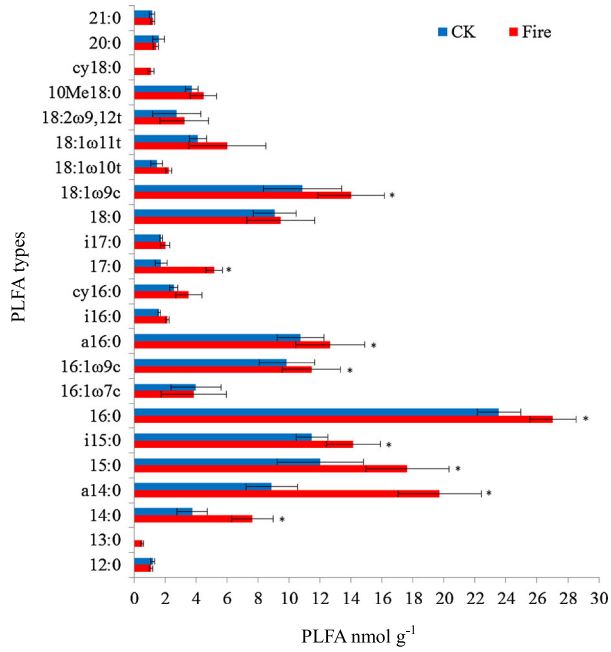

Figure 4. Phospholipid fatty acid (PLFA) composition (mean nmol g^{-1}, mean ± standard deviation, $n = 4$) of burnt and unburnt sites. This figure is available in colour online at wileyonlinelibrary.com/journal/ldr.

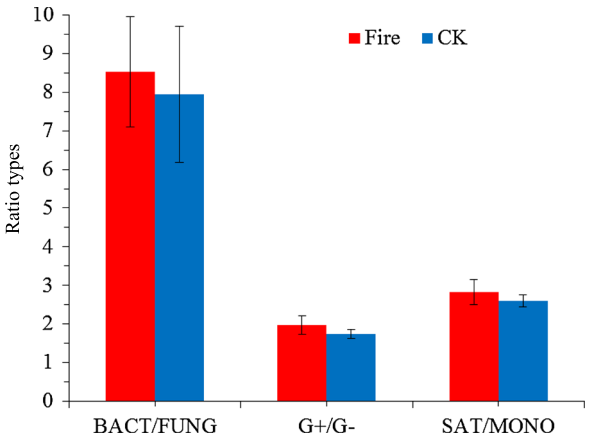

Figure 5. The ratios of each bacterial group of soil microorganisms in burnt site and unburnt site soils in alpine meadow. This figure is available in colour online at wileyonlinelibrary.com/journal/ldr.

response of grass plant biomass may depend on the degree of damage at the time burn. Guo (2003) indicated that in the early stage of fire, plant community diversity and AGB may increase but it would reduce in the later stage of succession. Our results also showed that both the plant species richness and the diversity index were increased. It is more likely that grass plant enables to survive following a fire or to adapt as aggressive invaders of burnt areas of alpine meadow. Moreover, grass plant species with higher ratio of roots to soils will be beneficial, recover quickly and increase in biomass (aboveground and belowground) following a short-term fire. If some plant species succeed in quickly recolonising the burnt area, most of soil properties can be recovered and even enhanced (Certini, 2005).

Ash can provide meritorious soil protection (Pereira *et al.*, 2013b). The aggregate stability plays a crucial role in the soil structure resilience and nutrients accumulation such as SOM (Mataix-Solera *et al.*, 2011; Novara *et al.*, 2010). The impact of the soil erosion and the vegetation recovery is definitive to understand the fire impact on soils (Cerdà & Doerr, 2005). After a fire, the soil properties and microclimate conditions change immediately because of heated soil and ash cover (Aznar *et al.*, 2013; Cerdà & Doerr, 2008). The increases or decreases of SOM and nutrients are related closely to fuel conditions (Fernández *et al.*, 1999; Certini, 2005), fire intensity and duration of the fire (Martín *et al.*, 2012; Certini, 2005) and the input nutrients from the burnt vegetation (Ulery & Graham, 1993). In this study, we found significant differences in nutrients between burnt and control plots. We observed a decrease in SOM content, total N, P and K following a fire. In contrast, the results from this study show an increase of total available N, P and K in burnt plots. After a fire, a significant decrease in SOM content can be related to rapid oxidation of the living and dead organic matter in the vegetation and litter layer. Likewise, changes in chemical properties after a fire can be attributed to alterations in the quantity and quality of the organic matter on the soil surface. The availability of nutrients is generally increased by the combustion of SOM (Kutiel & Shaviv, 1992). Therefore, organic matter acts as the primary nutrient pool for most of the available N, P and S in the soils. In addition, the soil pH value in alpine meadow showed a rising trend after a fire disturbance, largely because of denaturized organic acids due to heating (Certini, 2005).

Soil microorganisms as an important part of grassland ecosystem play a crucial role in material circulation, energy flow, SOM decomposition and nutrient transformation. Soil microbial biomass reflects the speed of ecosystem processes and productivity. They are very sensitive to soil conditions and act as index of ecosystem structure and function (Zou *et al.*, 2011). This study shows that the AWCD value of soil microbe and metabolic diversity of soil microbe community increased significantly in burnt site (Table IV). Fire changed the ability of C source utilisation and improved the nutrition condition and diversity of soil microorganism as well as changed the microbe metabolic characteristic (Figure 3).

Table V. Change of soil microbial quantity in the alpine meadow (mean ± standard deviation, $n = 4$)

Experimental group	Bacterial PLFA	G^+ PLFA	G^- PLFA	Actinomycetic PLFA	Fungal PLFA	Total PLFA
Fire	154·50 ± 2·87 a	50·77 ± 3·05 a	28·23 ± 1·88 a	4·49 ± 0·84 a	18·51 ± 3·17 a	173·01 ± 4·44 a
CK	113·31 ± 7·73 b	34·51 ± 1·97 b	22·05 ± 1·46 b	3·75 ± 0·40 a	14·82 ± 3·83 a	128·13 ± 10·06 b

Data of different treatment followed by the same letters were not significantly different at 0·05 levels. Duncan's multiple range tests.
PLFA, phospholipid fatty acid; G^+, Gram-positive bacteria; G^-, Gram-negative bacteria; CK, unburnt site soils.

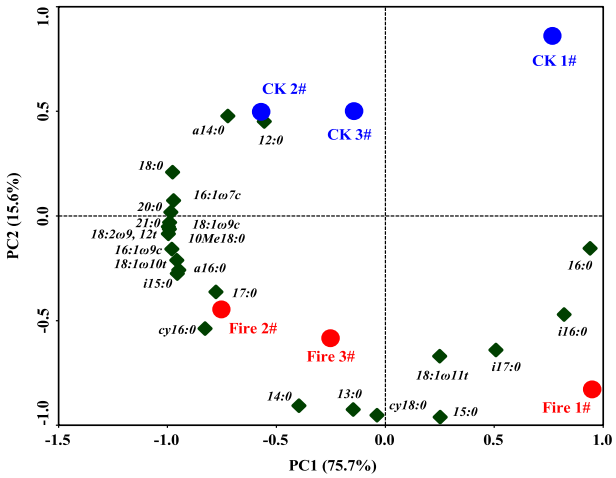

Figure 6. Principal component analysis plots of all phospholipid fatty acid signatures detected in burnt site soils (fire) and unburnt site soils (CK). PC1 and PC2 explain 75·7% and 15·6% in alpine meadow, respectively. This figure is available in colour online at wileyonlinelibrary.com/journal/ldr.

The pattern of Biolog metabolic diversity is related to microbial community composition and is sensitive to the change of functional microbial community (Rogers & Tate, 2001). The AWCD reflected the integrated capability of using C source of soil microbe and microbial activity (Zabinski & Gannon, 1997). Shannon–Wiener diversity index reflected the number of C source utilisation of microbe and its functional diversity (metabolic diversity) (Bending *et al.*, 2000). By analysing the main components of factor loading, we found that the C source related to PC1 differs from that connected with PC2, in which carbohydrate, carboxylic acids, amino acids and polymers are the main C sources. Our results suggest that there exists a clear difference between the soil microbial communities of burnt and unburnt sites in using carbohydrate, carboxylic acids, phenolic acids, amines, amino acids and polymers C sources. Also, metabolic profiles obtained by the Biolog plates reflect the potential of soil microbial community composition that responds to various C substrates.

Phospholipid fatty acids are essential membrane components of all living cells and are sensitive to environmental factor as they degrade rapidly. Our results showed that the monounsaturated fatty acids contents and fungal PLFA in burnt site soils are higher than those of unburnt site soils. The total PLFA, bacterial PLFA, G^+ bacterial PLFA and G^- bacterial PLFA in soil microbial community as well as the actinomyces and fungal PLFAs increased significantly. The proportion between fungi and bacteria can reflect its variation of relative content and the two population's relative richness degree (Frostegård *et al.*, 1993b). Shifts in the *iso*/*anteiso* and saturated/unsaturated PLFA ratios have been associated with nutrient stress, physical or chemical disturbance, or communities undergoing changes in composition (Fierer *et al.*, 2003).

Changes in plant diversity are known to affect aboveground ecosystem functioning (Tilman *et al.*, 1997), but it is increasingly recognised that changes in plant diversity also have an impact on belowground ecosystem functioning, including soil processes, soil structure and soil biota (Zak *et al.*, 2003). Fire disturbance leads to the change of soil environment, which can be attributed to an increase in grass functional group coverage and biomass, root-to-soil ratio and soil physical–chemical property. The fire disturbance also changes the soil organic quality and quantity of the utilisation of C and N. Changes in the physical and chemical soil properties were reflected by changes in the microbial communities and their activities (Chaerun *et al.*, 2011). In this study, we found that the total PLFA, G^+ bacterial PLFA and G^- bacterial PLFA are closely related to plant community characteristics. The total PLFA, bacterial PLFA and G^+ bacterial PLFA are significantly related to soil total and available nutrient. Therefore, our results suggest that the change of soil environment and vegetation is likely to increase of microbial type and biomass for those that are suitable to. Many responses that we observed can be explained by greater AGB associated with higher levels of plant diversity, which in turn influence the soil microbial community through plant AGBs. Fire disturbance is possible to cause the consecutive effect of soil biochemical process, to change soil microbial biomass, microbial catabolic activity and microbial community structure and to speed up the fixing and mineralization of microbial nutrition so as to form new models of rhizosphere nutrition. Although our results showed that fire may act as a short-term disturbance to alpine meadow ecosystems, change in soil resources may also affect microbial communities and

Table VI. Correlation between plant community characteristics and soil microbial PLFA contents

Plant community characteristic	Total PLFA	Bacterial PLFA	Fungal PLFA	G^+ PLFA	G^- PLFA	Actinomycetic PLFA	BACT/FUNG	G^+/G^-	SAT/MONO
Richness	0·886*	0·904*	0·521	0·952**	0·757	0·541	−0·093	0·742	0·384
Coverage	−0·977**	−0·976**	−0·557	−0·940**	−0·975**	−0·407	−0·317	−0·764	−0·558
Height	−0·986**	−0·991**	−0·574	−0·974**	−0·951**	−0·485	−0·226	−0·782	−0·537
Biomass	−0·935**	−0·950**	−0·527	−0·961**	−0·862*	−0·311	−0·052	−0·741	−0·38
Ratio of roots to soils	0·927**	0·934**	0·53	0·857*	0·996**	0·438	0·465	0·658	0·556

PLFA, phospholipid fatty acid; G^+, Gram-positive bacteria; G^-, Gram-negative bacteria.
**Significant at $p < 0·01$; *significant at $p < 0·05$.

Table VII. Correlation between soil microbial PLFA contents and soil nutrients

Item	pH value	Soil organic matter	Total N	Total P	Total K	Available N	Available P	Available K
Total PLFA	−0·953**	−0·873*	0·833*	−0·895*	−0·974**	0·910*	0·928**	0·970**
Bacterial PLFA	−0·937**	−0·899*	0·849*	−0·927**	−0·971**	0·899*	0·924**	0·964**
Fungal PLFA	−0·503	−0·591	0·442	−0·521	−0·827*	0·578	0·406	0·604
G^+ PLFA	−0·917*	−0·882*	0·923**	−0·878*	−0·938**	0·958**	0·966**	0·998**
G^- PLFA	−0·938**	−0·830*	0·675	−0·925**	−0·887*	0·739	0·840*	0·841*
Actinomycetic PLFA	−0·551	−0·648	0·329	−0·455	−0·646	0·431	0·37	0·562
BACT/FUNG	−0·295	−0·185	−0·237	−0·269	−0·393	−0·051	−0·046	0·057
G^+/G^-	−0·783	−0·678	0·71	−0·602	−0·909*	0·873*	0·756	0·876*
SAT/MONO	−0·636	−0·478	0·258	−0·397	−0·751	0·507	0·401	0·57

PLFA, phospholipid fatty acid; G^+, Gram-positive bacteria; G^-, Gram-negative bacteria.
**Significant at $p < 0·01$; *significant at $p < 0·05$.

aboveground–belowground interactions. However, it is difficult to predict whether resilience will continue and influence plant community composition, primary productivity, and soil moisture and nutrition effectiveness. We believe that this study will help the scientific and modelling research in understanding better the effect of fire on grassland ecosystem in terms of plant and microbial communities' response.

CONCLUSION

Many physical, chemical and biological soil characteristics can be affected by grassland fire. We have examined the short-term effect of fire on vegetation (direct) and soil microbial community structure (indirect) in burnt and unburnt plots. Our results show that plant community coverage, height and diversity changed significantly after fire, with higher values of plant AGB, ratio of roots to soils and lower values of soil moisture in burnt soils. Nitrogen, phosphorous and potassium became more available following a fire, whereas SOM contents were found to be lower in the burnt alpine meadow. Changes in soil properties were more likely a result of removal, combustion and oxidation of the litter layer and vegetation during the fire. Burning also alters the specific composition of soil microbial community. Data from PLFA analysis suggest that the ratio of bacterial to fungal PLFAs was slightly higher in burnt site compared with unburnt site. Fire changes soil microbial community structure and diversity through altering plant community composition and AGB.

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