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FLEXIBLE SOIL MICROBIAL CARBON METABOLISM ACROSS AN ASIAN ELEVATION GRADIENT

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ABSTRACT. The function and change of global soil carbon (C) reserves in natural ecosystems are key regulators of future carbon-climate coupling. Microbes play a critical role in soil carbon cycling and yet there is poor understanding of their roles and C metabolism flexibility in many ecosystems. We wanted to determine whether vegetation type and climate zone influence soil microbial community composition (fungi and bacteria) and carbon resource preference. We used a biomarker (phospholipid fatty acids, PLFAs), natural abundance ^{13}C and ^{14}C probing approach to measure soil microbial composition and C resource use, along a 1900–4167-m elevation gradient on Mount Gongga (7556 m asl), China. Mount Gongga has a vertical mean annual temperature gradient of 1.2–10.1°C and a diversity of typical vegetation zones in the Tibetan Plateau. Soils were sampled at 10 locations along the gradient capturing distinct vegetation types and climate zones from lowland subtropical-forest to alpine-meadow. PLFA results showed that microbial communities were composed of 2.1–51.7% bacteria and 2.0–23.2% fungi across the elevation gradient. Microbial biomass was higher and the ratio of soil fungi to bacteria (F/B) was lower in forest soils compared to meadow soils. $\delta^{13}C$ varied between −33‰ to −17‰ with C_3 plant carbon sources dominant across the gradient. Soil organic carbon (SOC) turnover did not vary among three soils we measured from three forest types (i.e., evergreen broadleaved subtropical, mixed temperate, coniferous alpine) and dissolved organic carbon (DOC) turnover decreased with soil elevation. Forest soil microbial PLFA ^{14}C and $\delta^{13}C$ measurements showed that forest type and climate were related to different microbial C use. The ^{14}C values of microbial PLFAs i15, a15, 16:1, br17 decreased with elevation while those of C16:0, cyC17, and cyC19 did not show much difference among three forest ecosystems. Bacteria and bacillus represented by C16:1 and brC17 showed considerable microbial C metabolism flexibility and tended to use ancient carbon at higher altitudes. Anaerobes represented by cyC17 and cyC19 showed stronger C metabolism selectivity. Our findings reveal specific C source differences between and within soil microbial groups along elevation gradients.

KEYWORDS: compound-specific ^{13}C, compound-specific ^{14}C, individual PLFA, natural ecosystem, soil C dynamic.

INTRODUCTION

The activity of soil microorganisms is critical to decomposition processes and the accumulation and/or loss of soil organic matters (SOM) (Fierer and Schimel 2003; Filip et al. 1998). Recent research has demonstrated the roles of soil microbial diversity and composition as a predictor of soil properties and functions across non-agricultural biomes (Smith et al. 2014). The C source specificity and resource flexibility of soil microbial communities are important determinants of their ability to respond to perturbation including climate change since their effects on soil C reserve are underestimated in many predictive models (Liang and Balser 2011; Mambelli et al. 2011; Ziegler et al. 2013). Findings from an elevation gradient in the Peruvian Andes showed a strong relationship between both climate-vegetation and soil microbial activity (Nottingham et al. 2018; Whitaker et al. 2014). Many studies have

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shown that the soil bacterial/fungal diversity/abundance and soil biogeochemical functioning is closely associated (Schaeffer et al. 2012; Strauss et al. 2012; Wang et al. 2017). However, none of these studies combine direct measures of soil microbial function with their C resource uses matrices. Improved understanding of microbial C metabolism source is needed to predict storage and cycling of SOM in global soils (Trumbore 2009).

Phospholipid fatty acids (PLFAs) are biomarkers found in viable microbial cells (White et al. 1979) that can be used to indicate the structure and C metabolism of soil microbial communities in different ecosystems (Zelles et al. 1995; Frostegård and Bååth 1996). Individual PLFA determinations can be combined with isotopic analyses to identify compound specific microbial carbon cycling (Rethemeyer et al. 2004; Mendez-Millan et al. 2014). For example, PLFA carbon stable isotope (^{13}C) signatures can be used to determine carbon resources recently assimilated by soil microbes (Boschker and Middelburg 2002). This approach has been widely used to determine carbon sources of soil microorganisms using ^{13}C-labeled substrates in laboratory experiments (Abraham et al. 1998) or using natural variation in C_3-C_4 transition studies in the field (Balesdent et al. 1987; Boschker et al. 1998; Kramer and Gleixner 2006). However, natural abundance ^{13}C tracer approaches are constrained by relatively small differences in ^{13}C signatures observed between microbial and soil C pools (Faure 1978).

In recent years, the development and application of compound specific radiocarbon analysis (CSRA) using natural abundance radiocarbon (^{14}C) as a tracer offers new perspectives for the understanding of soil C resource use at the molecular scale (Trumbore 2000). The nuclear weapons testing in the early 1960s nearly doubled the $^{14}C/^{12}C$ ratio in the atmosphere, producing a "bomb-peak" of ^{14}C that now serves as a global tracer of biospheric C exchange (Levin and Hesshaimer 2000). Researchers have measured ^{14}C signatures of PLFAs (PLFA-CSRA) to identify microbial carbon sources in contaminated (e.g., petroleum) soils (Slater et al. 2005, 2006; Cowie et al. 2010; Ahad and Pakdel 2013; Mahmoudi et al. 2013; Mills et al. 2013), C_3-C_4 vegetational successions (Kramer and Gleixner 2006; Yevdokimov et al. 2013), and long-term experimental sites with leaf litter replacement or field trails of continuously cultivated soils (Rethemeyer et al. 2005a; Kramer et al. 2010). Another recent PLFA-CSRA application is the study of active microbial communities in extreme environments (Ziolkowski et al. 2013).

We hypothesized that the C metabolism of different soil microbes would vary differently with the change of vegetation and climate. Vegetation and climate determine soil microbial C reserve dynamics through their impacts on microbial community composition (fungi, bacteria, and constituents) and carbon resource use of different microbes (carbon resource flexibility). To test this hypothesis, we combined compound-specific ^{13}C and ^{14}C probing approaches of PLFAs to measure soil microbial composition and C resource use along a unique 2267-m elevation gradient on the Tibetan Plateau, China. C_3 and C_4 plants can provide different microbial carbon source and represented by different microbial ^{13}C signatures. The ^{14}C signatures of microbes are an indicator for their ancient or modern C source.

The Tibetan Plateau, the largest and highest plateau on earth, is one of the regions that has suffered the least anthropogenic perturbation in the world (Xu et al. 2014). Great uplift with an average elevation (alt.) of 4000 m above sea level (asl) creates a range of climates from tropical to arctic and associated vegetation types. However, this unique geographical environment makes the plateau particularly vulnerable to climate change

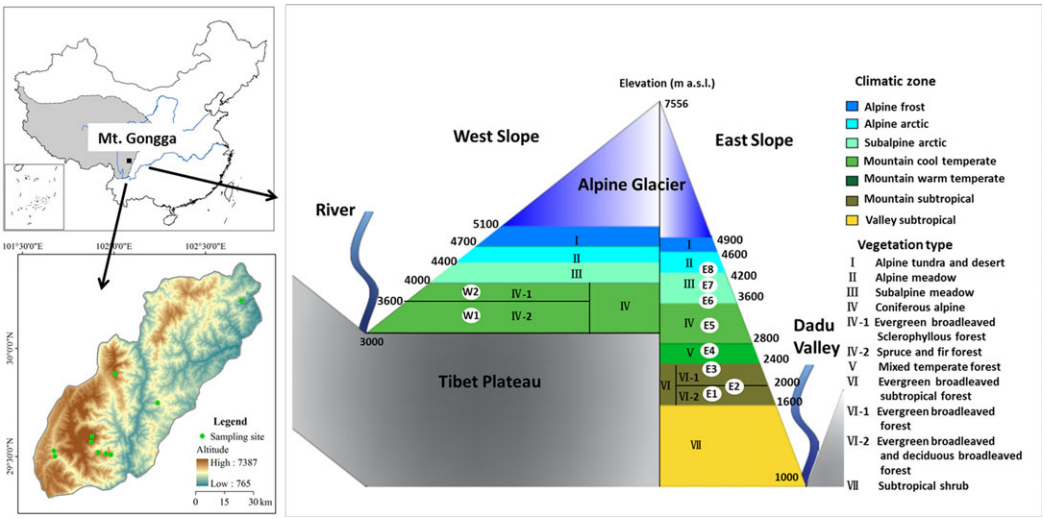

Figure 1 Elevation gradient sampling sites across Mount Gongga climatic zones and vegetation types.

(Wang et al. 2005b; Yang et al. 2006) and an unmatched region for scientific research about the adaptation of natural ecosystems to climate. For this reason, we selected Mount Gongga (7556 m asl), with an integrated elevation gradient of climatic zones and vegetation types, as the location for our study. The objectives of this research were (1) to investigate the difference in soil microbial community structures with the climatic parameters and soil properties in the vertical gradient, and (2) to identify the soil microbial C resource use in different climatic zones and forest ecosystems.

METHODS

Study Area and Soil Sampling

Mount Gongga (29.30–30.30°N, 101.50–102.25°E, 7556 m asl) is the third-highest mountain in China, located on the eastern side of the Tibetan Plateau (Wu et al. 2013) (Figure 1 and Table 1). The eastern slopes reach down into the Dadu Valley (1100 m asl) and are strongly impacted by the southwesterly monsoon. It is colder and more humid at comparable elevations than on western slopes which connect into the Tibetan Plateau (3000–3500 m asl) (Thomas 1997) (Figure 1). Climatic differences between east and west slopes result in different distributions of vegetation zones. On the east slope, the elevation gradient (i.e., 6400 m) between the Dadu Valley and the peak of Mount Gongga drives more diverse climatic and vegetation zones than the west slope. Three distinct forest types occur below the tree line on the east slope: evergreen broadleaved forest (1600−2400 m asl), mixed coniferous and broadleaved forest (2400−2800 m asl), and coniferous forest (2800−3600 m asl) (Song 1987) (Figure 1).

Climate information, mean annual temperature (MAT) and mean annual precipitation (MAP) were obtained from seven meteorological stations set up along an altitudinal gradient by the Alpine Ecosystem Observation and Experiment Station of Mount Gongga. For sites without a meteorological station, we calculated climatic conditions by fitting

Table 1 Environment, vegetation, and soil properties with microbial parameters.

	Site	E1	E2	E3	E4	E5	E6	E7	E8	W1	W2
Environ. prop.*	Longtitude (°N)	29° 36'12''	29° 36'12''	29°35' 44''	29°35' 43''	29°34' 21''	29° 32'59''	29° 32'46''	29° 32'39''	29° 31'48''	29° 32'22''
	Latitude (°E)	102° 04'14''	101° 57'39''	101° 57'28''	102° 02'41''	102° 00'03''	102° 00'03''	102° 02'39''	102° 04'15''	101° 45'53''	101° 45'40''
	Elev. (m asl)	1900	2060	2369	2500	2900	3614	3966	4167	3360	3800
	MAT[a] (°C)	10	10	9	8	7	4	2	1	5	3
	MAP[b] (mm)	1200	1353	1600	1613	1873	3200	1900	1400	2133	2393
	Climate zone[c]	Subtro	Subtro	Subtro	Warmt	Coolte	Subalp	Subalp	Alpine	Coolte	Coolte
	Vegetation[d]	VI-2	VI	VI-1	V	IV	III	III	II	IV-1	IV-2
Soil prop.**	Density (g/cm^3)	1.1	0.6	0.6	0.6	1.2	0.6	0.6	0.6	0.6	0.6
	SOC[e] (g/cm^2)	229	154	162	180	539	328	71	56	78	148
	TN[f] (g/cm^2)	16	11	12	10	25	15	5	3	3	8
	C/N[g]	15	14	14	18	22	22	14	20	25	19
	pH	6.5	3.4	4.1	6.3	4.2	4.2	3.4	3.6	3.7	3.5
	$\delta^{13}C$[h] (‰)	–27	–27	–27	–27	–26	–24	–25	–24	–25	–24
Micro. para.***	B[1] (μg/g)	54	13	18	105	337	0.2	3	1	16	23
	F[2] (μg/g)	12	11	15	22	36	0.2	3	1	19	19
	GN[3] (μg/g)	4	3	4	8	25	0.1	1	0.3	4	5
	GP[4] (μg/g)	31	7	10	67	248	0.1	0.5	0.2	3	4
	GP/GN	9	3	3	9	10	1	1	1	1	1
	F/B	0.2	1	1	0.2	0.1	1	1	1	1	1
	Biomass (μg/g)	114	54	67	204	337	9	17	9	82	116

*Environ. Info.: environment properties.
**Soil prop.: soil information.
***Micro. para.: microbial parameters.
[a]MAT: mean annual temperature.
[b]MAP: mean annual precipitation.
[c]Climate zone: subtro: subtropical; warmt: warm temperate; coolte: cool temperate; subalp: subalpine; alpine.
[d]Vegetation: III Subalpine shrub meadow, IV Mountain dark coniferous forest, IV-1 Sclerophyllous evergreen broad-leaved forest, IV-2 Spruce and fir forest, V mixed coniferous and broad-leaved forest, VI Subtropical evergreen broad-leaved forest, VI-1 Evergreen broadleaved forest, VI-2 Evergreen broadleaved and deciduous broadleaved forest.
[e]SOC: soil organic carbon inventory.
[f]TN: total nitrogen inventory (upper 30-cm soil layer, method from Torn et al. 2009).
[g]C/N: ratio of soil carbon to nitrogen.
[h]$\delta^{13}C$: $\delta^{13}C$ of soil organic C.
[1]B: common bacteria.
[2]F: fungi.
[3]GN: gram-negative bacteria.
[4]GP: gram-positive bacteria.

equations based on yearly climatic data using software New Loc_Clim designed by the Food and Agriculture Organization of the United Nations organization.

Soil sampling was conducted in May 2012 and August 2013. Ten elevations (8 from the east slope and 2 from the west slope) were selected between 2060 and 4167 m asl, each characterized by distinct vegetation zones from lowland subtropical forest to alpine meadow (Figure 1 and Table 1). The classification is based on Chinese government document (GB-T 14721-2010) and organized by Institute of Mountain Hazards and Environment, Chinese Academy of Sciences (Zhong et al. 1999) (Figure 1). Three zones of one evergreen broadleaved subtropical forest (E1, E2, E3), one mixed temperate forest (E4), one coniferous alpine forest (E5), and three alpine meadows (E6, E7, E8) were sampled on the east slope; two subzones of mountain dark coniferous alpine forest (W1, W2) were sampled from the west slope. The west slope has fewer MAP, higher MAT and more C_4 plants than the same elevation on the east slope. At each site, after removing the top layer of litter, three soil pits (>5 m apart) were dug using a cleansed stainless-steel spade. We collected soils from the most microbially active soil horizons to extract soil PLFAs (O and A horizons, 0–30 cm). The depth of humus horizon (O horizon) was less than 5 cm in the sampling sites above 3000 m asl, and less than 15 cm in all sampling sites.

An additional kilogram of soil sample for CSRA was also collected from each of the three forest types (evergreen broadleaved subtropical forest with dominant species *Liethocarpus cleistocarpus*; mixed temperate forest with dominant species *Picea brachytyla*; coniferous alpine forest with dominant species *Abies fabri*) on the east slope at the elevations of 1900 (E1), 2500 (E4), and 2900 (E5) m asl

Soil Preparation and Physicochemical Properties Analysis

Each soil sample was a composite of 3 subsamples. The detailed procedures of pretreatment, physical and chemical analysis of soil samples are listed in SI 1.

SOC, DOC Extraction, and Soil PLFA Extraction and Identification

The tube was vibrated for 12 hr and then centrifuged. The upper layer was transferred to a fresh tube to measure the DOC concentration. The analyzed result was used to calculate the soil amount to extract enough DOC content for ^{14}C analysis. A previous methodological experiment demonstrated DOC extraction was not influenced by using water below 80°C (Li2017). The extraction steps above were repeated and the upper layer solution was dried at 60°C to constant weight. The extraction of PLFAs followed the Bligh-Dyer method (Bligh and Dyer1959) and was slightly modified for extracting and collecting individual PLFAs for CSRA from larger sized forest soil samples (SI 2). This included improvement of solution amounts and purification steps suitable for least 500 g of forest soils. Qualitative analysis of resulting PLFA-fatty acid methyl ester fractions (FAMEs) is listed in SI 2.

Compound-Specific ^{13}C Analysis of PLFAs and ^{13}C Analysis of SOC

Compound-specific ^{13}C measurements on FAMEs and ^{13}C analysis of SOC were performed using a GC Agilent 7890 N coupled with an isotopic ratio mass spectrometer (Delta+XL, Finnigan MAT, Germany). The GC oven temperature was programmed from 50°C for 4 min to 155°C at a rate of 15°C/min, and to 230°C at a rate of 1.5°C/min. Subsequently,

the oven was heated to 290°C for 2 min at a rate of 15°C /min. The results are expressed in $\delta^{13}C$ per mil (‰) against the international standard V-PDB (Werner and Brand 2001).

Isolation and Harvesting of Individual FAMEs

Isolation of individual FAMEs for accelerator mass spectrometry (AMS) ^{14}C measurements was achieved using an Agilent 7890A GC equipped with an Agilent 7693 auto sampler and a flame ionization detector (FID), integrated with a Gerstel CIS 1. Harvesting of individual FAMEs using Agilent gas chromatograph 7890A and a Gerstel PFC (PCGC system), connected with a 30-m-long deactivate capillary (0.32 mm ID × 0.25 μm film). The GC operating conditions were the same as described above. PLFA C14-C18 were abundant in forest soils and thus selected as target FAMEs reaching an orthogonal experimental design for 7 crucial operation parameters in PCGC system (SI 3) (Eglinton et al. 1996, 1997; Uchida et al. 2000; Mandalakis and Gustafsson 2003; Zhang et al. 2013). The optimum PCGC conditions were as follows: CIS initial temperature (40°C), CIS end temperature (300°C), CIS solvent venting time (0.1 min), CIS heating rate (12°C/min), CIS injection mode (fast mode), PFC trapping temperature (room temperature 30°C), and injection volume (3 μL) (SI 3).

Six types of representative individual PLFAs were isolated from sites E1, E4 and E5. Among them, iC15:0+aC15:0 and C16:1w9 represent common bacteria; C16:0 represents common microbes; brC17 represents related bacillus; cyC17 and cyC19 represent anaerobes (SI Table_2_Supp.). The prefixes i, a, and cy indicate different branching, referring to iso, anteiso, and cyclopropyl, respectively. With the optimum PCGC conditions, the amount of all the six selected individual PLFAs exceeded 50 μg from 500 g soil,satisfied the detection amount limit of ^{14}C analysis (see supplemental materials for Table_4_Supp.).

^{14}C Analysis of Microbial PLFAs, SOC, and DOC

Because the extremely high cost and the difficulty of harvesting a sufficient amount of pure chemicals to meet the detection limit of ^{14}C analysis, we only undertook ^{14}C analysis on 6 kinds of relatively abundant microbial PLFAs, SOC, and DOC for soil samples from three sampling sites representing three main forest types on the east slope, to provide an original exploration of natural microbial carbon signatures in this natural elevation-forest gradient. Combustion and graphitization of lipid fractions, isolated FAMEs, SOC, and DOC were performed at State Key Laboratory of Isotope Geochemistry, Guangzhou Institute of Geochemistry (Chinese Academy of Sciences) using a published procedure (Ding et al. 2010). The graphitized samples were measured by an AMS facility in the State Key Laboratory of Nuclear Physics and Nuclear Technology (Peking University) with a precision better than 0.5‰. All the analyses were performed in triplicate.

^{14}C data were reported as $\Delta^{14}C$ values per mil relative to the ^{14}C activity of the oxalic acid standard with 1-σ measurement uncertainty (Reimer et al. 2004; Stuiver and Polach 2006). Negative $\Delta^{14}C$ values correspond to ^{14}C levels before 1950 whereas positive $\Delta^{14}C$ values indicate the contribution of ^{14}C derived from atmospheric nuclear weapons testing in the 1950s and 1960s to the samples. An additional blank sample was strictly proceeded with the same PLFA extraction steps, followed by the isolation steps on PCGC with same retention time and radiocarbon analytical steps on AMS with the soil samples. The background ^{14}C and the added methyl ^{14}C during methylation need to be removed from the ^{14}C content of the FAMEs to obtain the $\Delta^{14}C$ value of the PLFAs by Equation (1):

$$\Delta^{14}C_{PLFA} = \frac{N*\left(\Delta^{14}C_{FAME} - \Delta^{14}C_{blank} - \Delta^{14}C_{MeOH}\right)}{N-1} \quad (1)$$

Here, $\Delta^{14}C_{PLFA}$ is the ^{14}C content of the PLFAs, and $\Delta^{14}C_{FAME}$ is the ^{14}C content of the FAMEs. $\Delta^{14}C_{MeOH}$ is the ^{14}C content of methanol used for derivatization and N is the number of carbon atoms of the FAMEs. During the extraction, methylation of PLFAs, and separation/isolation of FAMEs, neither isotope fractionation for ^{13}C and ^{14}C nor background contamination of the samples was observed (Kramer and Gleixner 2006).

C Age and Turnover Rates of SOC and DOC

The C age of SOC and DOC was calculated using the CALIBomb program with the NH_zone2 dataset (Hua et al. 2013) bounded in the north by latitude ~40°N and in the south by the mean summer intertropical convergence zone.

SOC separated by density fractionation generally are found to display a similar isotope pattern as the total SOM (Rethemeyer et al. 2005), illustrating that the soil C pool could be considered as a steady C pool. Plant C including plant litter C, plant rhizospheric C, and some relatively modern C has heavier ^{14}C isotope signatures due to access to atmospheric CO_2 (Kramer et al. 2010; Mendez-Millan et al. 2014). Accordingly, we calculated the SOC and DOC turnover rates based on ^{14}C values and Cherkinsky and Brovkin's model (Stuiver and Polach 1977). The atmospheric radioactivity of every simulated year and SOC is obtained from published references (Burchuladze et al. 1989; Levin and Kromer 1997; Levin and Hesshaimer 2000) and calculated from SOC $\Delta^{14}C$ (Chen et al. 2002; Wang et al. 2005b).

Statistical Analyses

Correlation analysis was conducted using SPSS for Windows (ver. 20.0; SPSS Inc., Chicago, IL, USA). One-way ANOVA and multivariate analysis of variance (MANOVA) were used to examine the significance of differences and variability at the 95% confidence level ($P < 0.05$), respectively. Redundancy analysis (RDA) was performed by the "Bioenv" function in the "vegan" package (vegan v2.4-4) of R software (R Development Core Team, Vienna, Austria), using UniFrac weighting and the normalized distance matrix with default parameters.

RESULTS

Environmental Parameters and Soil Physicochemical Properties

As elevation increased, MAT declined (from 10°C to 1°C, Table_1_supp.) and MAP increased (from 1200 mm to 3200 mm; Table 1 and Figure 1). In general, SOC and total N (TN) were higher in forests (148–539 g/cm^2 and 3–25 g/cm^2, respectively) than in alpine meadows (56–328 g/cm^2 and 3–15 g/cm^2, respectively) (Table 1). The highest SOC and TN content was found in the forest-meadow transition area (coniferous forest, E5:539 g/cm^2 and 25 g/cm^2, respectively; subalpine meadow E6: 328 g/cm^2 and15 g/cm^2, respectively) on the east slope. The lowest SOC and TN values were found in the highest elevation alpine meadow (E8: 56 g/cm^2 and 3g/cm^2, respectively) on the east slope. This distribution was consistent with the MAP pattern (Table 1). SOC and TN results from the west slope (W1: 78 g/cm^2 and 3 g/cm^2, respectively; W2: 148 g/cm^2 and 8 g/cm^2, respectively) were lower than that in similar elevation in the east slope (E5: 539 g/cm^2 and25 g/cm^2,

respectively; E6: 328 g/cm^2 and15 g/cm^2, respectively). The average SOC and TN inventory of coniferous forest (E5, W1, and W2) and broad-leaved forest soils (E1, E2, and E3) was close (Table 1). The highest ratio of C and N (C/N) was found in the coniferous forests (W1: 25, E5: 22) and subalpine meadow (E6: 22), while the alpine meadow had the lowest C/N (E7: 14). All soil samples in Mount Gongga were acid (pH=3.4–6.5) across the elevation gradient (Table 1).

Soil Microbial Biomass and Structure

Overall, PLFA results indicated varying amounts of bacteria (2–52%) and fungi (2–23%) across the elevation gradients (Table 1). Microbial biomass was highest in the coniferous forests (82–116 μg/g), followed by the mixed forest (93 μg/g) and the broadleaved forest (54–76 μg/g), all significantly higher than that in the meadow (9–12 μg/g) (Table 1). The ratios of soil fungi to bacteria (F/B) were 0.1–1.3 across the gradient (Table 1), higher in dark coniferous forest (1.0) and meadow soils (1.0) than in other forests.

RDA attempted to explore the potential environmental factors (longitude, latitude, MAT, MAP, and soil abiotic parameters including soil density, moisture, pH, SOC, TN, C/N, and TP) driving microbial change (Table 2). The results suggested that elevation, soil density, SOC and C/N displayed significant correlations with PLFA concentrations.

^{13}C Values of PLFAs

$\delta^{13}C$ of 16 representative PLFAs varied from –33‰ to –17‰, suggesting a difference in microbial C_3 to C_4 plant resource use across vegetation types (evergreen broadleaved subtropical, mixed temperate, coniferous alpine forest, and alpine meadow) (Figure_10_Supp., Figure 2). In general, $\delta^{13}C$ of PLFAs ranged between –32‰ to –25 ‰, reflecting C3 plant dominance, i.e., $\delta^{13}C \approx$–29‰, consist with $\delta^{13}C$ of plants investigation (–31‰ to –25‰) on the same elevation of Mount Gongga (Wang et al. 2004a). The most abundant bacterial PLFAs (iC15:0, aC15:0, and C16:1w9) were found in all soils of different vegetation types, and their $\delta^{13}C$ ranked as coniferous alpine (west slope) > alpine meadow > coniferous alpine (east slope) > mixed temperate > evergreen broadleaved subtropical (Figure 2). Together with the PLFAs mentioned above, ^{13}C values of anaerobe-representative cyclopropyl PLFAs (cyC17 and cyC19) and bacillus-representative branched chain PLFAs (brC16 and brC17) in forest soils followed the rank: coniferous alpine > mixed temperate > evergreen broadleaved subtropical (Figure 2). As elevation, MAT and MAP were significantly intercorrelated, they all showed considerable significant relationships with PLFA $\delta^{13}C$ variation (Figure 2; Table_1_Supp.). The^{13}C values of PLFAs representing bacteria (C14:0, iC15:0andC16:1w9c) and bacillus (brC16 and brC17) exhibited higher variance than other PLFAs (over 5‰) across all vegetation types (Figure 2).

Compound-Specific ^{14}C Values of Forest Soil PLFAs and ^{14}C Values of SOC

$\Delta^{14}C$ of PLFA and C age of SOC and DOC calculated from the $\Delta^{14}C$ allow us to estimate the C resource use and variation with microbial isotopes along the elevation gradient (Figure 3 and Table 4). Results showed a huge ^{14}C value variation (–315~132‰), revealing an obvious disparity of microbial C sources from modern to pre-nuclear weapons testing age among different forest soils (Figure 3). The $\Delta^{14}C$ of TOC and DOC were similar and always positive, showing no significant difference among three forest types (75~156‰; Tables 3 and 4).

Table 2 Results of redundancy analysis. Environmental variables, soil properties thatcorrelated with the PLFA concentrations. Permutation: free; number of permutations: 999.

	RDA1	RDA2	r2	Pr (>r)
Longitude	–0.11796	–0.99302	0.5050	0.101
Elevation	0.94038	0.34013	0.6220	0.040**
MAP[1]	0.99000	–0.14104	0.3842	0.205
Soil density	–0.80494	–0.59336	0.5207	0.090*
SOC[2]	0.08677	–0.99623	0.5492	0.057*
C/N[3]	0.99607	0.08851	0.0698	0.765
pH	–0.90885	–0.41712	0.5830	0.055*

*Correlation is significant at the 0.1 level.
**Correlation is significant at the 0.05 level.
[1]MAP: mean annual precipitation.
[2]SOC: soil organic carbon inventory.
[3]C/N: ratio of soil carbon to nitrogen

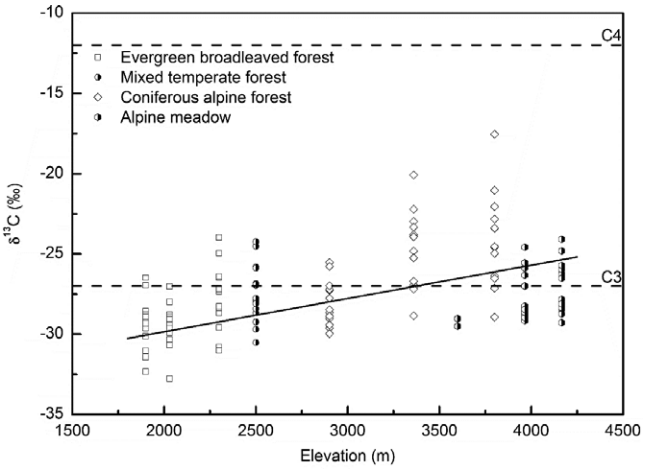

Figure 2 Compound-specific ^{13}C values of soil microbial PLFAs across the elevation gradient (R^2=0.9017 $p < 0.001$).

The $\Delta^{14}C$ values of common microbes C16:0 and anaerobes cyC17 and cyC19 PLFAs showed no significant difference among different forest types. The $\Delta^{14}C$ values of PLFAs representing bacteria (i/a C15, C16:1w9) and bacillus (brC17) ranged from positive to negative across forest types, decreasing with elevation. The $\Delta^{14}C$ difference among the three forest types was largest in bacterial C16:1w9, followed by bacillus (brC17) and common bacteria (C15), common microbes (C16:0), and anaerobes (cyC17 and cyC19) (Figure 3). Microbial pre-bomb C ($\Delta^{14}C < 0‰$) varied more than microbial post-bomb C ($\Delta^{14}C > 0‰$) across three forest types. SOC turnover rate did not vary much between the three forest types, but DOC turnover rate decreased with elevation (Table 4).

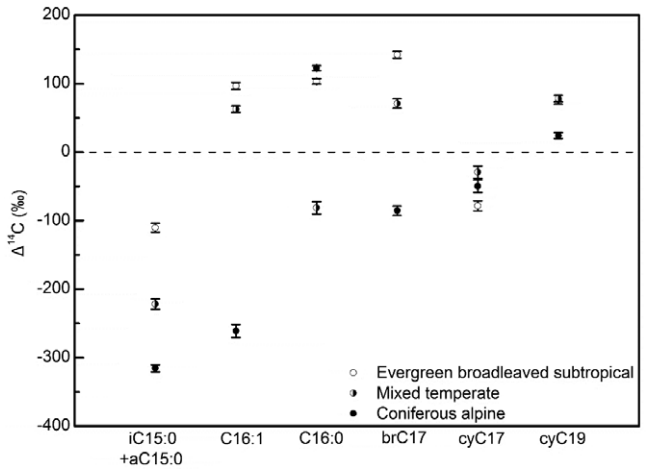

Figure 3 Compound-specific ^{14}C values of soil microbial PLFAs across three forest types on Mount Gongga.

DISCUSSION

Environmental Influences on Microbial C Resource Use

It is known that soil C and N content is influenced by both climate and vegetation (Wang et al. 2004b), and that resultant SOM influences soil microbial community structure (Smith et al. 2014). In the present study, we found a positive correlation between SOC and TN content across all sampling sites (Table_1_Supp.). This is consistent with previous results from Mount Gongga (Wang et al. 2004b). Differences across the climate-vegetation gradient also influenced microbial plant C resource use, as reflected by the $\delta^{13}C$ pattern of PLFAs (Figure 2). C_4 vegetation is known to be more productive in warm habitats with sufficient sunlight and moderate water content (Wang et al. 2005a). Topographical differences resulted in less precipitation, higher temperatures and more C_4 plants on the west slope than at the same elevation on the east slope. Additionally, conditions in the high elevation east slope meadow areas are generally more suitable for C_4 plant growth compared with the cold and moist environment in the low elevation east slope forest areas (Sage and Sage 2002; Su et al. 2011; Wang et al. 2004a). Existing evidence confirms that C_4 plants are rare above 2000 m asl on the east slope of Mount Gongga (Li et al. 2009) whereas there are C_4 plants at 4250 m asl on the Tibetan Plateau (Wang et al. 2004a). In our results, the $\delta^{13}C$ values of most microbial PLFAs (–32‰ to –25‰) reflected the $\delta^{13}C$ signature of dominant C_3 plants on Mount Gongga (–31‰ to –25‰) (Li et al. 2009), while the $\delta^{13}C$ of PLFAs in the coniferous forest on the west slope and meadow on the east slope reflected the presence of C_4 plants (Figure 2). The positive correlation between the $\delta^{13}C$ values of SOC and elevation in our study provided evidence for such a C_3-C_4 plant distribution (Table_1_Supp.). Other work has shown that the $\delta^{13}C$ values of C_3 plants above 2000 m asl on the east slope of Mount Gongga are linearly positively correlated with the elevation (Li et al. 2009). Likewise in the present work, the $\delta^{13}C$ values of forest microbial PLFAs were linearly and positively correlated with the elevation (Figure 2). Additionally, the PLFA structure is most correlated with elevation in this study (Table 2), suggesting that vegetation conversion in the elevation gradient altered the microbial metabolism of plant carbon source through

Table 3 $\Delta^{14}C$, C age, and C turnover rate of SOC and DOC across three forest types in Mount Gongga.

	Evergreen broadleaved subtropical			Mixed temperate			Coniferous alpine		
	$\Delta^{14}C \pm$ STED (‰)	CY[1] (a BP, 2σ)	TR[2] (k yr^{-1})	$\Delta^{14}C \pm$ STED (‰)	CY[1] (a BP, 2σ)	TR[2] (k yr^{-1})	$\Delta^{14}C \pm$ STED (‰)	CY[1] (a BP, 2σ)	TR[2] (k yr^{-1})
SOC[a]	101 ± 3	–51∼47	0.068	133 ± 3	–46∼–45	0.054	89 ± 3	–53∼–50	0.081
DOC[b]	75.3 ± 3	–56∼–55	0.118	89 ± 3	–53∼–50	0.081	156 ± 4	–42∼–40	0.047

[a]SOC: soil organic carbon.
[b]DOC: dissolved organic carbon.
[1]CY: calibrated year (BP: pre-bomb, 2σ: confidence, dataset from Hua et al. 2013).
[2]TR: C turnover rates of SOC and DOC (k = 1/turnover time [yr] method from Wang et al. 2005b).

Table 4 $\Delta^{14}C$ of microbial PLFAs in global different natural ecosystems.

Location	Year	Material	Status	$\Delta^{14}C$ of PLFA (‰)						Reference
				iC15/aC15	C16:1	C16:0	brC17	cyC17	cyC19	
Mount Gongga (China)	2014	Forest soils	Three natural forests	–315~–110	–261~91	–81~123	–85~142	–78~–29	24~79	This study
Rotthalmünster (Germany)	2002	Agricultural soil	Artificial plants	98	79.1	118	—	—	—	Rethemeyer et al. (2004)
Rotthalmünster (Germany)	2002	Agricultural soil	Artificial plants	19~98	49.4~79.1	19~141	—	—	—	Rethemeyer et al. (2005)
Rotthalmünster (Germany)	2002	Agricultural soil	C_3-C_4 plant evolution	98.3~122.1	78.7~87.6	126~128.8	– 33.3~43.8	100.4	112.1~127	Kramer & Gleixner (2006)
Halle (Germany)	2000	Agricultural soil	C_3-C_4 plant evolution	–9.3~–6.3	52.3~56.2	33.75~46.8	–17.5	–85.1~–60.4	–87~48.9	Kramer et al. (2010)
Tennessee (USA)	2002–2004	Temperate forest soil	Artificial litterfall replacement	153~166	206~251	195~206	183~195	—	—	
Northeast Pacific Ocean	1996–2002	Sediment	Three natural cores	–425~–99	–425~–15	–425~54	—	—	—	Zhang et al. (2010)

modifying soil microbial structures. Thus, the $\delta^{13}C$ pattern of microbial PLFAs strongly reflected the C_3-C_4 plant distribution across this natural climate-vegetation gradient.

Soil Microbial C Resource Use across Natural Forest Gradient

In forest ecosystems, there are many potential C resources for microbes, e.g., organic C from plant litter, plant rhizospheric C and native soil C (Kramer et al. 2010; Schwab et al. 2019). Our results suggest that bacteria (PLFAs iC15 and aC15) were assimilating ancient C, with the $\Delta^{14}C$values decreasing with forest elevation. This finding corresponds with previous observations of bacterial $\Delta^{14}C$ (C15) in a natural ocean sediment (Zhang et al. 2010). In this study, bacterial PLFAs of C16:1w9 tended to use modern C in the mixed temperate forest, but ancient soil C in coniferous alpine forest ecosystems. The same phenomenon was observed in bacillus PLFA of brC17, but with a higher $\Delta^{14}C$ value (Figure 3). This reflected the importance of climate as a regulator of productivity, decomposition and microbial access to C resources for both bacteria and actinomycetes. C16:0 PLFA is common to most soil microbes, and thus its $\Delta^{14}C$ values reflect the range of C sources used by the microbial community (Keinänen et al. 2003; Pelz et al. 2001). Our results showed that soil microbes in evergreen broadleaved and coniferous alpine forest used a higher proportion of modern C than microbes in mixed temperate forest. This might be attributable to significant differences in vegetation composition and associated C inputs. In mixed temperate forest, the moderate temperature compared with that in broadleaved and coniferous forest in the elevation gradient might influence the balance of the plant litter decomposition and microbial metabolism into SOC, and finally changed the ^{14}C signature of common microbes. Anaerobes (PLFAs of cyC17and cyC19) tended to use modern C, irrespective of forest types, indicating their preference for more recent C inputs. The changed $\Delta^{14}C$ pattern of PLFAs (i/a C15, C16:1, and brC17) suggested that the source of microbial C resource was older in coniferous alpine forest than in other forest types. Considering the lower MAT (5.7°C) and the higher SOC stock (538.6 g/cm^2; Figure 1) in coniferous alpine forest soils, it might be attributing to higher input of surface biomass and low bacterial activity as soils are frozen over a long period of time (Hagedorn et al. 2019), microbes in coniferous alpine forest tend to use older SOC reserves.

Microbial C Metabolism Flexibility in Different Ecosystem

The differences in the $\Delta^{14}C$ values of PLFAs observed across the forest gradient represented the changes in C resource use and, thus, offered information about the microbial C metabolism flexibility. We compared the $\Delta^{14}C$ values of six PLFAs representing different microbes with previous studies on a range of ecosystems including a C_3-C_4 transition (Kramer and Gleixner 2006), agricultural soils (Rethemeyer et al. 2005; Rethemeyer et al. 2004), an artificial litterfall replacement experiment (Kramer et al. 2010) and sediment (Zhang et al. 2010) (Table 4). These studies suggested that microbial C metabolism varied across different ecosystems due to the change in C resource use environments. In reported soil ecosystems, the $\Delta^{14}C$-variation (–315~251‰) of PLFAs with ^{14}C signatures decreased with elevation (i/a C15, C16:1 and brC17) is also bigger than the $\Delta^{14}C$-variation (–87~206‰)of PLFAs with no difference (C16:0, cyC17, and cyC19) in our study (Table 4).

In our study and a previous study in ocean sediment, the $\Delta^{14}C$ value of i/a C15 is always reported as <0‰, indicating that this bacterial C metabolism remains pre-bomb C (Zhang et al. 2010) (Table 4). The $\Delta^{14}C$ values of C16:1w9 and brC17 in reported soil ecosystems

changed significantly (i.e., 350‰ and 220‰), indicating the considerable microbial C metabolism flexibility of bacteria and bacillus related with two PLFAs across different ecological environments (Table 4).

The lower Δ^{14}C-variation (–81~206‰) of C16:0 among different soils is speculatively caused by its high representative of different microbes. The Δ^{14}C values of anaerobes representative cyC17and cyC19 biomarkers varied by <50‰, suggesting a narrower specificity of C metabolism reflected by larger differences in their abundances across ecosystems (Table 4).

CONCLUSIONS

Bacteria and bacillus represented by C16:1 and brC17 both showed a more flexible microbial C metabolism across the climatic zones and vegetation types (Figures 2 and 3, Table 4). This indicates their potential as regulators of terrestrial carbon cycling as climate change warms these ecosystems. Besides, microbial pre-bomb C ($\Delta^{14}C < 0‰$) varied more than microbial post-bomb C ($\Delta^{14}C > 0‰$) across different forest types (Figure3), revealing that microbes using ancient C were more "flexible" than microbes using younger C, possibly contributing to the greater sensitivity of the old soil organic C pool to temperature (Hilasvuori et al. 2013). Our findings reveal specific C source differences between and within soil fungal and bacterial groups across forest ecosystems that could have implications for their resilience to future climate change.

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SUPPLEMENTARY MATERIAL

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