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Distribution of microbial fatty acids and fatty alcohols in soils from an altitude transect of Mt. Jianfengling in Hainan, China: Implication for paleoaltimetry and paleotemperature reconstruction

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The temperature gradient along the altitude transect of Mt. Jianfengling provides a good opportunity to establish and evaluate the microbial lipid-based environmental proxies. The soils collected from 14 different altitudes of Mt. Jianfengling contain abundant microbial fatty acids and fatty alcohols, including *iso*/*anteiso* fatty acids (*i*/*a*$C_{12:0}$–*i*/*a*$C_{19:0}$), 10-Me-$C_{16:0}$ fatty acid, *iso*/*anteiso* fatty alcohols (*i*/*a*C_{13}–*i*C_{26}), 10-Me-$C_{16:0}$ fatty alcohol and unsaturated fatty alcohols, which can indicate a strong microbial activity in the Jianfengling soils. The branched and unsaturated fatty alcohols can be only detected when saponification is performed, implying that these lipids are present as the constituents of bacterial wax esters in the soils. The ratio of *a*C_{15}/*i*C_{15} fatty acids is positively correlated with altitude, suggesting that the decrease in temperature can induce the increase in the relative abundance of *anteiso* C_{15} fatty acid. In contrast, the ratio of *a*C_{15}/*i*C_{15} fatty alcohols and of *a*C_{15}/*n*C_{15} fatty alcohols both decrease with increased altitude or decreased temperature. Similarly, the ratio of *n*$C_{18:1}$/*n*$C_{18:0}$ fatty alcohols also decreases with decreased temperature, which is opposite to the previous observation that unsaturation of fatty acids in microorganism increases in response to decreased temperature. Besides, the average chain length (ACL) of long chain fatty alcohols (C_{22}–C_{30}) from leaf waxes and carbon preference index (CPI) of all *n*-fatty alcohols are also significantly correlated with altitude or mean annual temperature, demonstrating their potential for paleoclimate reconstruction. The correlation of microbial fatty acids and alcohols as well as ACL and CPI of plant wax-derived fatty alcohols with altitude may provide novel ways to reconstruct paleotemperature and paleoaltimetry.

***anteiso* fatty alcohols, microorganism, wax ester, paleotemperature, paleoaltimetry**

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The paleotemperature reconstruction has become a focus in current global change research. The paleotemperature variation can help us better understand the climate rhythms, and can provide climate background for evaluating the relative contributions of nature and anthropogenic activities to the modern global warming as well as boundary conditions for paleoclimate modeling (Tierney et al., 2010). The microorganisms are important components of the earth's ecosystem and have been widely used in the paleotemperature reconstruction. Some microorganisms are sensitive to ambient temperature change and the structures and distributions of their lipid biomarkers can indicate the biological taxonomy and also record the temperature signal. For example, UK-37′, TEX_{86} and LDI, which express the unsaturation of long

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chain alkenones of coccolithophores (Müller et al.,1998), the relative number of cyclopentyl moieties in isoprenoid glycerol dialkyl glycerol tetraethers (iGDGTs) of planktonic Thaumarchaeota (Schouten et al., 2002; Kim et al., 2008) and the chain length of long chain diols of algae (Rampen et al., 2012) , respectively, can respond sensitively to changes in the sea surface temperature (SST) and have become important tools in paleo-SST reconstruction (Liu et al., 2009; Li et al., 2013; Pearson et al., 2013). In terrestrial environments, UK-37′ and TEX_{86} proxy can be influenced by numerous factors including salinity, water depth, and biological precursors, etc. and thus their applications are limited to only small numbers of lakes (Sun et al., 2010; Xie et al., 2013). The bacterial branched GDGTs (bGDGTs), which structurally resemble archaeal iGDGTs, are ubiquitous in soils and the MBT proxy, representing the degree of methylation for bGDGTs, is strongly related to the mean annual air temperature (MAAT) and to a lesser extent to the soil pH. Combined with cyclization ratio of bGDGTs (CBT) that is only related to the soil pH, the MBT/CBT index therefore has provided a novel way to reconstruct the paleotemperature in terrestrial environments (Weijers et al., 2007; Peterse et al., 2012), e.g., lakes (Xiong et al., 2009; Sun et al., 2011), peatlands (Zhou et al., 2011), and loess-paleosols (Peterse et al., 2011; Gao et al., 2012). However, due to the great heterogeneity of terrestrial environments and multiple controls on these proxies, the accurate paleotemperature reconstruction for terrestrial environment requires the mutual verification of different proxies. Therefore, it seems necessary to develop more new paleothermometers for terrestrial environments.

The altitude variation can lead to the changes in temperature, precipitation, vegetation, and microbial community. The atmospheric and soil temperature decrease with altitude due to adiabatic cooling of arising air mass, which makes it possible to test and develop new paleotemperature proxies based on the temperature gradient along the altitude transect. The recently developed paleothermometer, clumped isotope (^{13}C-^{18}O isotope) can be used to estimate the soil temperatures and further to reconstruct the paleoaltimetry during different phases of plateau uplift (Ghosh et al., 2006). The TEX_{86} of isoprenoid GDGTs in surface soils of varied altitudes at both humid and arid regions can reveal the temperature change of different altitudes, though the thaumarchaeotal groups producing iGDGTs in soils are different from those in marine environments (Yang et al., 2010; Liu et al., 2013). The MBT/CBT proxy shows great potential in temperature reconstructions at altitude transects of Mt. Jianfengling (Yang et al., 2010), Mt. Gongga (Peterse et al., 2009), Mt. Xiangpi (Liu et al., 2013) in China and Mt. Kilimajaro (Sinninghe Damsté et al., 2008) in Africa, and it has been applied to reconstruct the early Eocene paleoelevation of the northern Sierra Nevada (Hren et al., 2008). Therefore, the paleotemperature reconstruction is an effective way to know the paleo-elevations of different geological ages.

Soils generally have abundant microbial lipids and are easily collected. The surface soils from different altitudes are excellent materials that can be used to study the response of microbial lipids to temperature change. Here we sampled the surface soils from an altitude transect of Mt. Jianfengling in Hainan, China and presented the distribution of microbial fatty acids and fatty alcohols in these soils. Based on this, we established new microbial paleothermometers and paleoaltimetries.

1 Materials and methods

1.1 Soil sampling

Mt. Jianfengling, a rainforest Natural Reserve in the northern flank of tropics, is located in Ledong County in the southwestern Hainan Island of China (Figure 1). Mt. Jianfengling has an altitude of 1405 m at summit and is constituted primarily of felsic granite, which can be converted to laterite and yellow soil after weathering. It is subjected to a tropical island monsoon climate with abundant rainfall in summer and little rain in winter. The perennial mean annual air temperature and mean annual precipitation at the Tianchi meteorological station (Altitude: 820 m) are 19.8°C and 2449 mm, respectively (Zhou et al., 2009).

Soil samples were collected during April, 2008 in the dry season of Hainan. The sampling sites were all under the canopies of trees and were not impacted directly by sunlight. Starting from the summit, one or two surface soils were sampled at ca. 100 m intervals of altitude with a depth of 0–10 cm. The samples were wrapped in the aluminum foil, stored in paper envelopes and then immediately transported to the laboratory. Samples from the summit to piedmont were numbered consecutively from JFL-1 to JFL-14. All samples were laterite or yellow soils, and were not adjacent to the plant root or influenced by anthropogenic activities. One or two *in situ* air and soil temperature(s) were measured using a digital thermometer with a precision of ±0.1°C during each sampling, and finally 22 air temperature values and 22 soil temperatures were obtained. The geographical positions of sampling sites, including longitude, latitude, and altitude were measured by a portable GPS (Table 1). After air dried, the soil samples were ground into powder and homogenized with a pestle and mortar. Then they were passed through a 20 mesh sieve to remove roots and mineral grains.

1.2 Lipid extraction and fractionation

An aliquot of soil samples (ca. 15 g) were weighed and ultrasonically extracted with dichloromethane (DCM) and methanol (MeOH) mixtures for 15 min. The volume ratio of DCM: MeOH was 3∶1, 1∶1 and 1∶3, respectively for each extraction (15 mL×3). The supernatants for each sample

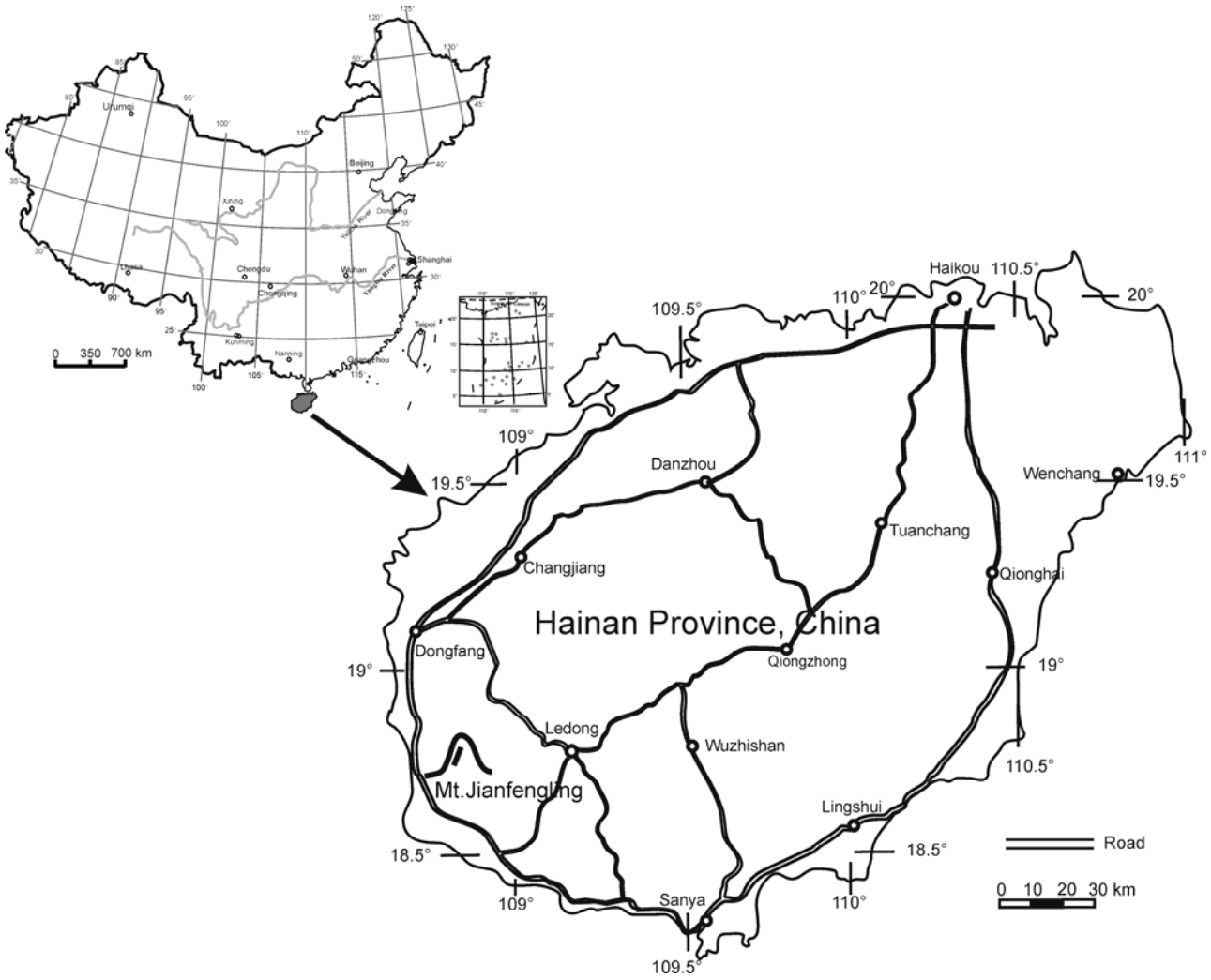

Figure 1 The map of Hainan Province, China, showing the sampling site, Mt. Jianfengling. The shaded part in the map of China is Hainan Island.

Table 1 Soil sample information

Sample No.	GPS position	Altitude (m)	*In situ* air *T* (°C)	*In situ* soil *T* (°C)	Soil type	pH	Estimated MAAT (°C)	Estimated irradiance (kcal cm^{-2} yr^{-1})
JFL-1	18°43.0′N, 108°52.2′E	1405	21.5	20.7	Semi-weathered granite and yellow soils	4.69	16.6	104
JFL-2	18°43.0′N, 108°52.5′E	1296	22.8	20.1	Semi-weathered granite and yellow soils	4.40	17.2	107
JFL-3	18°43.0′N, 108°52.5′E	1262	22.8	19.5	Yellow soil with abundant humus	4.46	17.4	107
JFL-4	18°42.8′N, 108°52.5′E	1137	23.2	21.1	Yellow soil	4.49	18.2	110
JFL-5	18°42.7′N, 108°52.6′E	1002	25.5	23.7	Yellow soil	4.18	19.0	113
JFL-6	18°42.3′N, 108°52.3′E	899	26.6	23	Yellow soil	4.40	19.6	115
JFL-7	18°42.2′N, 108°52.1′E	790	25.5	24.5	Yellow soil	4.24	20.3	117
JFL-8	18°41.8′N, 108°51.3′E	701	27.2	24.5	Yellow soil	4.35	20.8	119
JFL-9	18°42.0′N, 108°51.1′E	597	27.8	27.3	Yellow soil	4.24	21.4	122
JFL-10	18°42.0′N, 108°50.5′E	497	28.1	25.7	Yellow soil	4.03	22.0	124
JFL-11	18°42.1′N, 108°50.2′E	402	28.8	29.3	Yellow soil	4.81	22.6	126
JFL-12	18°41.9′N, 108°49.9′E	290	30.6	27.3	Yellow soil	4.38	23.3	128
JFL-13	18°42.1′N, 108°49.8′E	199	30	28	Yellow soil	5.89	23.8	130
JFL-14	18°42.2′N, 108°47.4′E	86	29.1	28	Yellow soil	6.20	24.5	132

were filtrated to remove soil particles and then combined to form the total lipid extracts (TLE). For some soils with abundant humus, e.g., JFL-3, the filters were washed with a large volume of DCM to remove the absorbed or attached organic residuals. The TLE were condensed to ca. 3 mL at 42°C with a rotary evaporator. They were transferred to 5 mL vials and finally dried on a thermostat water bath.

The activated silica gel (150°C, 5 h) was filled in the chromatography column and condensed. The re-dissolved TLE were separated into apolar and polar fractions by eluting with DCM (5 mL) and MeOH (5 mL), respectively. The apolar fraction contained alkanes and aromatics whereas polar fraction was composed of free fatty acids, fatty alcohols, and esters, etc. After dried under gentle nitrogen gas, the polar fraction was saponified with 2 mL 0.6 mol/L KOH/MeOH (1% H_2O) solution in a 30 °C thermostat oven for 15 min. The cooled solution was mixed with 1 mL ultrapure water and extracted with *n*-hexane (2 mL×5) to recover neutral compounds, e.g., fatty alcohols and ketones, etc. The solution was then acidified with HCl (DCM extracted to remove organisms) and extracted with *n*-hexane (2 mL×5) to obtain fatty acids. The final fractions containing neutral compounds and fatty acids respectively were all dried under nitrogen gas.

The silylation was performed on the neutral compounds as they contain hydroxyl moiety. 30 μL of BSTFA [Bis (trimethylsilyl) trifluoroacetamide] was added to each sample, which was then heated in a 70°C thermostat oven for 1.5 h. The silylated samples were dried immediately under nitrogen gas. The fatty acid fraction was esterified with 14% BF_3/MeOH solution in a 70°C thermostat oven for 1.5 h. After 1 mL ultrapure water added, the solution was extracted with *n*-hexane (2 mL×3). The fatty acid methyl esters were dried under nitrogen gas and then silylated with BSTFA. One sample, JFL-3, was selected to determine whether fatty alcohols, especially branched fatty alcohols, were free or bound in soils. The polar fraction of JFL-3 obtained by column chromatography was directly esterified and silylated without saponification. All the final products above were dried under N_2. All flasks and vials were annealed in a muffle furnace under 500°C for 5 h, and filters and cottons were extracted with DCM in a Soxhlet apparatus for 72 h to avoid possible contamination.

1.3 GC-MS analysis

The derivatized fatty acids and fatty alcohols were analyzed by a Hewlett-Packard (HP) 5890 gas chromatogram and HP 5973 mass spectrometer (GC-MS) equipped with a silica capillary column (DB-5MS; 60 m×0.25 mm×0.25 μm). The GC and MS conditions were as follows. The GC oven temperature ramped from 70°C to 300°C at 3°C/min, held at 300°C for 20 min with helium as the carrier gas. The inlet temperature was 300°C and the injection volume was 1 μL. The ionization energy was 70 eV and the temperature of interface between GC and MS was fixed at 280°C. The oven temperature program was slightly modified for analyzing fatty alcohols of cultured bacteria and unsaponifiable polar fraction of JFL-3. The compounds were identified based on the mass spectra, retention time, and literatures as well as comparison with NIST 02 mass spectra library. The normal fatty acids (or alcohols), *iso* and *anteiso* fatty acids (or alcohols) were denoted, respectively, by $nC_{x:y}$, $iC_{x:y}$ and $aC_{x:y}$, where x represents the carbon number before derivatization and y means the number of double bonds. The carbon preference index (CPI) and average chain length (ACL) of *n*-fatty alcohols were calculated according to following formula:

$$\mathrm{CPI}_{22-31} = 1/2\times\left[\left(\mathrm{C}_{22}+\mathrm{C}_{24}+\mathrm{C}_{26}+\mathrm{C}_{28}+\mathrm{C}_{30}\right)/\left(\mathrm{C}_{23}+\mathrm{C}_{25}+\mathrm{C}_{27}+\mathrm{C}_{29}+\mathrm{C}_{31}\right)+\left(\mathrm{C}_{22}+\mathrm{C}_{24}+\mathrm{C}_{26}+\mathrm{C}_{28}+\mathrm{C}_{30}\right)/\left(\mathrm{C}_{21+}\mathrm{C}_{23}+\mathrm{C}_{25}+\mathrm{C}_{27}+\mathrm{C}_{29}\right)\right];$$

$$\mathrm{ACL}_{22-30} = \left(22\times\mathrm{C}_{22}+24\times\mathrm{C}_{24}+26\times\mathrm{C}_{26}+28\times\mathrm{C}_{28}+30\times\mathrm{C}_{30}\right)/\left(\mathrm{C}_{22}+\mathrm{C}_{24}+\mathrm{C}_{26}+\mathrm{C}_{28}+\mathrm{C}_{30}\right),$$

where C_n represents the peak area of *n*-fatty alcohols with carbon number of *n*.

1.4 Soil pH measurement

The soil sample was mixed with ultrapure water in a ratio of 1∶2.5 (g∶mL) and stirred with a glass rod. After standing for 30 min, the supernatant was measured with a pH meter (IQ Scientific Instruments, USA, precision ±0.01 arbitrary unit). The average of three measurements was taken as the final pH value.

2 Results and discussion

2.1 Soil pH and temperature at sampling sites

The *in situ* air and surface soil temperatures both show significant anticorrelation with altitude at the sampling site of Mt. Jianfengling (R^2=0.90, P<0.001, n=22 and R^2=0.88, P< 0.001, n=22, respectively). The calculated lapse rate during sampling is 0.60°C/100 m, suggesting that soil samples cover excellent temperature gradient along the altitude transect. As soils were collected in spring, the lapse rate calculated by *in situ* temperature may be different from the mean annual lapse rate, which prevents us from estimating MAAT at the sampling sites. However, Zeng et al. (1985) have developed a transfer function between MAAT and altitude based on MAAT monitored perennially at seven sites of different altitudes, i.e., T=25.0–0.006×H, where T is MAAT (°C) and H is altitude (m). According to this equation, MAAT at the sampling sites is estimated to range from 16.6 to 24.5°C (Table 1). In addition, the total radiation amount per year (TRA) at the sampling sites

can be estimated to range from 104–132 kcal cm^{-2} yr^{-1} on the basis of the observation by Zeng et al.(1985) that TRA is negatively correlated with altitude.

The results of soil pH show that all the soil samples are acidic with a range from 4.03 to 6.20. The strong acidity of soils is related to the felsic granite, and also to strong leaching and high accumulation of humic acids in the rainforest. All the soil pH values are quite approximate only with the exception that pH significantly increases at altitude of 199 m and 86 m. The soil pH is an important factor that controls the growth of bacteria (Fierer et al., 2006; Lauber et al., 2009) and it should be considered as an environmental variable that influences the microbial lipid-based proxies.

2.2 The distribution and sources of fatty acids in soils

Abundant fatty acids were detected in all the Jianfengling soil samples, with a carbon number ranging from C_{14} to C_{31}. All the fatty acid profiles are dominated by palmitic acid ($C_{16:0}$), with a strong even over odd predominance (Figure 2). It is generally acknowledged that long chain *n*-fatty acids with a carbon number >20 (C_{21}–C_{32}) come from wax ester, cutin, and suberins of plants (Eglinton et al., 1967) whereas branched fatty acids, including *iso*/*anteiso* fatty acids and mid-methyl fatty acids are contributed by soil bacteria (Kaneda et al., 1991). Obviously, the majority of fatty acids (C_{14}–C_{32}) in Jianfengling soils are derived from plant wax. As samples were saponified, some bound long chain *n*-fatty acids (>C_{20}) can be released from cutin and suberin of plants and may also contribute to the fatty acid profile. The microbial branched fatty acids in Jianfengling soils include *i*/*a* $C_{12:0}$–*i*/*a* $C_{19:0}$ and 10-Me-$C_{16:0}$ with the dominance of *i*/*a* $C_{15:0}$, suggesting a strong microbial activity in the rainforest ecosystem. Among the three isomers of each fatty acid (*iso*-, *anteiso*-, and *n*-fatty acids), the *n*-fatty acids are generally the most abundant components, followed by *iso*-fatty acids. More than three types of isomers of heptadecanoic acid ($C_{17:0}$) with different methyl positions can be observed in all soils and most of them show higher abundances than $C_{17:1}$ (Figure 2). The statistic results of published membrane lipids from cultured bacteria have revealed that the Gram positive bacteria can produce *i*$C_{15:0}$, *a*$C_{15:0}$, *i*$C_{16:0}$, *i*$C_{17:0}$, and *a*$C_{17:0}$ whereas Gram negative bacteria can biosynthesize many kinds of unsaturated fatty acids, e.g., 16∶1ω7c and 18∶1ω7c (Frostegård et al., 1996). The fungi and actinomycetes are characterized by 18∶2ω6,9c (Frostegård et al., 1996) and 10-methyl-hexadecanoic acid (Zelles et al., 1999), respectively. Therefore, the bacterial communities in Jianfengling soils are dominated by Gram positive bacteria with some contribution from the Gram negative bacteria and actinomycetes.

2.3 Distribution and sources of fatty alcohols in soils

The distribution of fatty alcohols in all soils of varied altitudes is quite similar, maximizing at C_{24} or C_{26}. The fatty alcohol profiles have the carbon number ranging from C_7 to C_{32} and show strong even over odd predominance (Figure 3). The long chain *n*-fatty alcohols (>C_{21}) clearly exceed short chain *n*-fatty alcohols in abundance (<C_{21}), indicating a significant contribution of leaf wax to fatty alcohol profile. Similar to the fatty acids, the fatty alcohol profiles contain abundant *iso*/*anteiso* fatty alcohols with carbon number ranging from C_{13} to C_{26}. As *n*-fatty alcohols share quite similar mass spectra with their *iso*, *anteiso* and *mid-methyl*

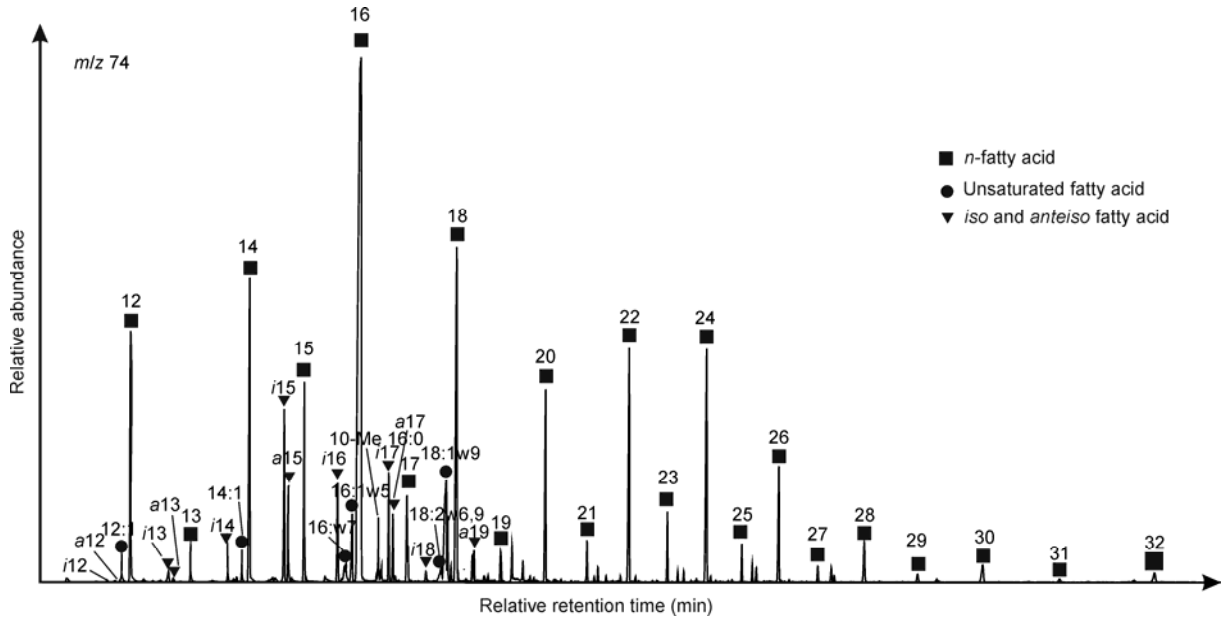

Figure 2 The mass chromatograph of fatty acids (methyl ester, *m/z* 74) in a selected Jianfengling soil (JFL-1).

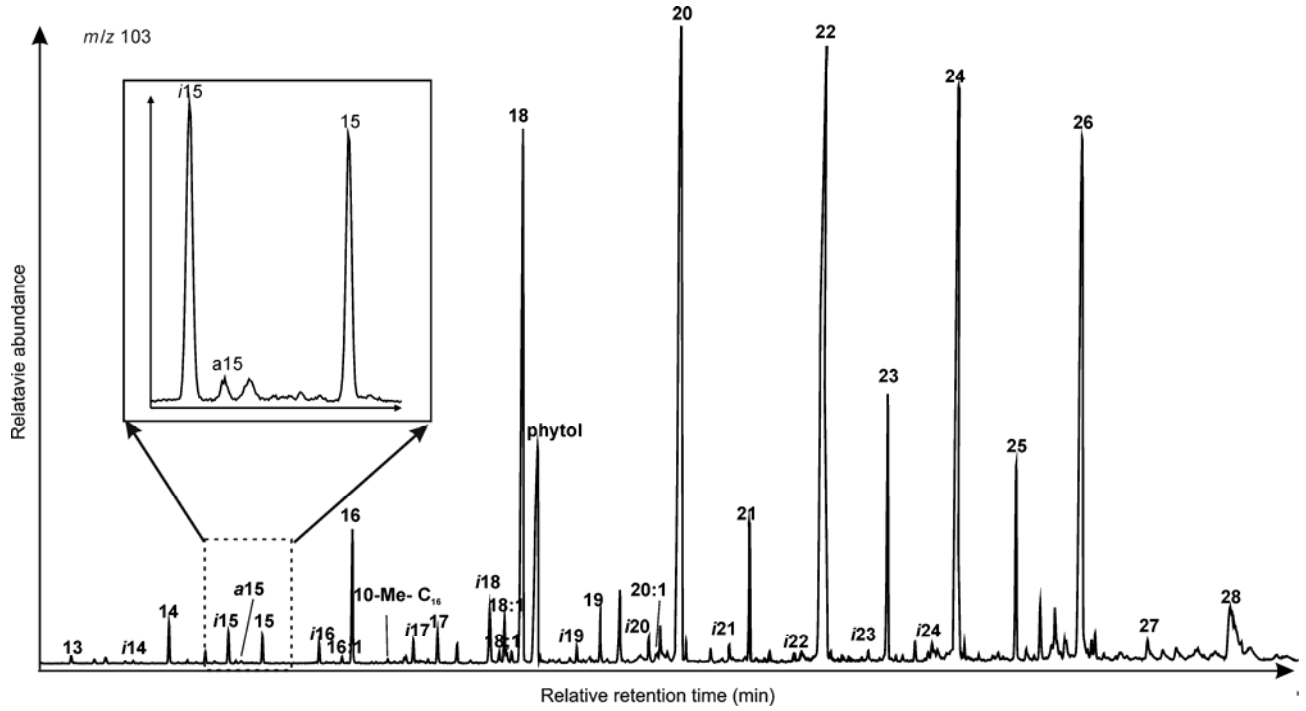

Figure 3 The mass chromatogram of fatty alcohols (trimethylsilyl ether, *m*/*z* 103) in a selected soil sample (JFL-3) from Mt. Jiangfengling.

isomers, they can be identified only by the elution order or relative retention times. For example, the mass spectra of $i\mathrm{C}_{15:0}$ and $a\mathrm{C}_{15:0}$ resemble that of $n\mathrm{C}_{15:0}$ (Figure 4). The boiling points for these isomers are $i\mathrm{C}_{15:0}$<$a\mathrm{C}_{15:0}$<$n\mathrm{C}_{15:0}$ and in turn they will elute in the gas chromatogram in an order of $i\mathrm{C}_{15:0}$, $a\mathrm{C}_{15:0}$, and $n\mathrm{C}_{15:0}$. The identification of other fatty alcohol isomers follows this elution order. It is notable that *n*-heptadecanol has three to five kinds of branched isomers including 10-Me-$\mathrm{C}_{16:0}$ (10-Me-C_{16} in Figure 3), $i\mathrm{C}_{17:0}$, $a\mathrm{C}_{17:0}$, and two $br\mathrm{C}_{17}$ (methyl positions undetermined) in most of the soil samples, e.g., JFL-8 and JFL-10. In some samples, e.g., JFL-12, *n*-heptadecanol has up to seven branched isomers (see supplementary material). The *n*-nonadecanol also possesses four isomers with different methyl positions. In addition, we also detected a series of monounsaturated *n*-fatty alcohols, including $n\mathrm{C}_{16:1}$, $n\mathrm{C}_{17:1}$, $n\mathrm{C}_{18:1}$, $n\mathrm{C}_{19:1}$, $n\mathrm{C}_{20:1}$, $n\mathrm{C}_{22:1}$, $n\mathrm{C}_{23:1}$, $n\mathrm{C}_{24:1}$, $n\mathrm{C}_{25:1}$, $n\mathrm{C}_{26:1}$, and $n\mathrm{C}_{28:1}$, where $n\mathrm{C}_{18:1}$ has 2–3 isomers with different double bond positions. Polyunsaturated fatty alcohol, $n\mathrm{C}_{18:2}$ can be found in some soils including JFL-10, 11, 12, 13 and 14. $i/a\mathrm{C}_{15:0}$ and $i/a\mathrm{C}_{17:0}$ fatty alcohols have ever been identified in marine sediments (Treignier et al., 2006). However, the branched fatty alcohols including $i/a\mathrm{C}_{14}$–$i\mathrm{C}_{26}$ have not been reported yet in any previous soil lipid research. Interestingly, most branched isomers of *n*-fatty alcohol have the corresponding branched fatty acid in the same soil. For example, almost all the soil samples have both 10-Me-$\mathrm{C}_{16:0}$ fatty acid and 10-Me-$\mathrm{C}_{16:0}$ fatty alcohol, and the distribution of C_{14}, C_{15}, C_{16}, and C_{18} fatty alcohols mimics that of their corresponding fatty acids. The $i\mathrm{C}_{15}$ fatty alcohol is generally more abundant than $a\mathrm{C}_{15}$ fatty alcohol in all soils, and similarly, the abundance of $i\mathrm{C}_{15}$ fatty acid is also higher than its *anteiso* isomer. The co-occurrence of short chain *iso*/*anteiso* fatty acid and *iso*/*anteiso* fatty alcohol with the same carbon number in Jianfengling soils suggests that these fatty acids and their corresponding fatty alcohols may share the same biological source.

The *n*-fatty alcohols in natural environments are generally dominated by those with even carbon number, showing a strong even over odd predominance. For example, the *n*-fatty alcohols of planktonic algae generally maximize at $n\mathrm{C}_{22}$ (Volkman et al., 1999). The aquatic plants can produce fatty alcohols with the dominance of $n\mathrm{C}_{28}$ and $n\mathrm{C}_{30}$ and carbon number ranging from C_{20} to C_{32} (Ficken et al., 2000). Similarly, *n*-fatty alcohols of higher plants are generally dominated by $n\mathrm{C}_{24}$, $n\mathrm{C}_{26}$, and $n\mathrm{C}_{28}$ with carbon number ranging from C_{22} to C_{32} (Eglinton et al., 1967; Zhang et al., 2006). However, *iso* and *anteiso* fatty alcohols are considered to be of bacterial origin (Mudge et al.,1997,2008). The $i/a\mathrm{C}_{15:0}$ and $i/a\mathrm{C}_{17:0}$ have been found previously in Shennongjia soils (Huang et al., 2013), Dajiuhu peatlands (Huang et al., 2013), and marine sediments (Treignier et al., 2006), suggesting that branched fatty alcohols may be ubiquitous in both terrestrial and aquatic environments. The distribution of branched fatty alcohols in Jianfengling soils is quite different from that in the marine sediments from cold seep (Thiel et al., 1999). The $i\mathrm{C}_{15:0}$ fatty alcohol dominates over $a\mathrm{C}_{15:0}$ fatty alcohol in Jianfengling soils whereas the cold seep sediments show the opposite and the carbon isotopes of these fatty alcohols are strongly negative, indicating

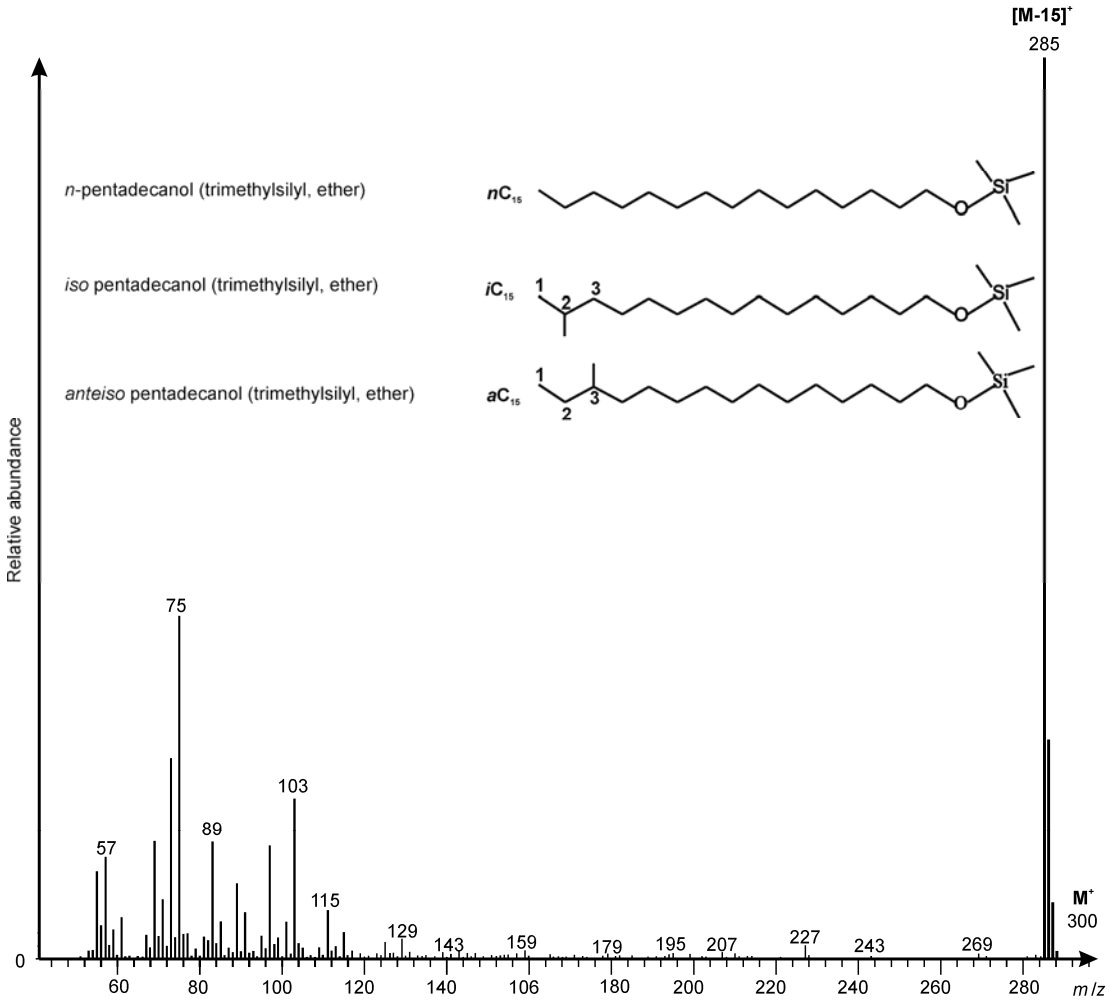

Figure 4 The structures and similar mass spectra of *n*-heptadecanol (trimethylsilyl ether) and its *iso*/*anteiso* isomers.

that these fatty alcohols may be derived from the sulfate reducing bacteria mediating the methane cycle (Thiel et al., 1999; De Boever et al., 2009). In addition, halophilic bacteria isolated from cold seeps of deep sea can biosynthesize large amounts of $C_{16:1}\omega7c$ (Hua et al., 2007), demonstrating that unsaturated fatty alcohols can also come from bacteria.

The production of *iso*/*anteiso* and unsaturated fatty alcohols by bacteria can be proved directly by pure culture of microorganisms in the laboratory. We isolated bacteria from soil with the method of serial dilutions and plate streaking and cultured them in the LB liquid medium (trytone 19 g/L, yeast extract 5 g/L, NaCl 10 g/L, pH 7). Among the nine strains of isolated bacteria , only one strain of Gram positive bacterium (No. 4-1-2) contains a detectable amount of *iso* and unsaturated fatty alcohols in the neutral lipids, including $i\mathrm{C}_{16:0}$, $i\mathrm{C}_{17:0}$, $i\mathrm{C}_{19:0}$, $i\mathrm{C}_{20:0}$, $i\mathrm{C}_{21:0}$, $i\mathrm{C}_{22:0}$, and $n\mathrm{C}_{18:1}$. Apparently, not all bacteria can produce *iso*/*anteiso* and unsaturated fatty alcohols. A fast screening of fatty alcohols in *Erythrobacter* sp. (Gram negative) isolated from the South China Sea (Yang et al., 2009) reveals that Gram negative bacteria can also biosynthesize *iso*/*anteiso* and unsaturated fatty alcohols (Figure 5). Compared with fatty acids, the branched and unsaturated fatty alcohols in *Erythrobacter* sp. are quite low and contain $i\mathrm{C}_{15:0}$, $i\mathrm{C}_{16:0}$, $i\mathrm{C}_{17:0}$, $i\mathrm{C}_{18:0}$, $i\mathrm{C}_{19:0}$, $i\mathrm{C}_{21:0}$, $i\mathrm{C}_{22:0}$, $iC_{23:0}$, and $i\mathrm{C}_{24:0}$, which almost cover the carbon number range (C_{13}–C_{26}) of *iso* fatty alcohols in Jianfengling soils. In addition, the mono-unsaturated fatty alcohols produced by *Erythrobacte*r sp. include $n\mathrm{C}_{17:1}$, $n\mathrm{C}_{18:1}$, and $n\mathrm{C}_{20:1}$, which are also present in the Jianfengling soils.

The fatty acids are the key components of membrane lipids in most bacteria, and they can be bounded to glycerol moiety to form glyceride in cell membrane. The fatty alcohols unlikely occur in the bacterial cell membrane, because fatty alcohols and glycerol both have hydroxyl groups that are not able to be bound together. Instead, the fatty alcohols in natural environments are generally combined with fatty alcohols to form wax ester. The plant wax esters have very important biological function, e.g., inhibiting the vapor evaporation of leaf surface and protecting the leaf from ultraviolet light and pathogens; they are also energy store of plants (Eglinton et al., 1967; Li et al., 2011). The bacterial wax esters may primarily play a role in energy store that

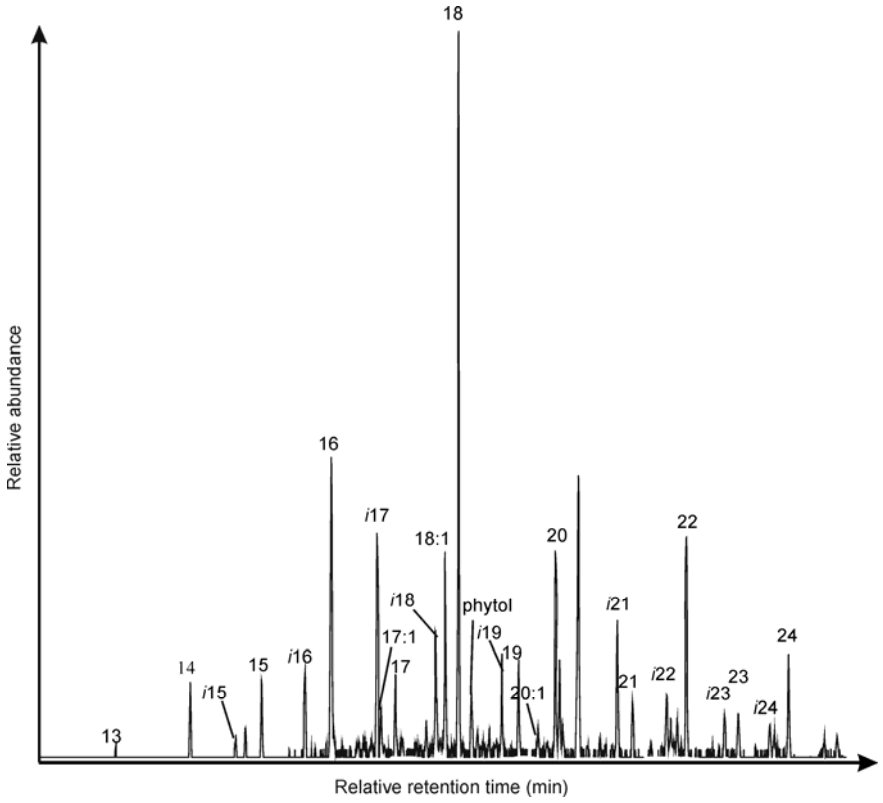

Figure 5 The mass chromatogram of fatty alcohols (trimethylsilyl ether, *m*/z 103) in the aerobic anoxygenic phototrophic bacteria isolated from the South China Sea.

helps bacteria survive in unfavorable environments, though the abundance of them are lower than plants (Ishige et al., 2003; Waltermann et al., 2005). The fatty alcohols can be preserved in free and bound state in sediments. The derivatized free fatty alcohols can be directly detected by GC-MS whereas the bound fatty alcohols may exist primarily in wax esters. As the wax esters in soils are quite low, it is not possible to directly determine whether the branched and unsaturated fatty alcohols are derived from the wax esters. However, the change in the fatty alcohol profile before and after saponification can demonstrate the preservation state of branched and unsaturated fatty alcohols. We extracted the free lipids of JFL-3 and derivatized its polar fraction without saponification. As shown in Figure 6, the long chain *n*-fatty alcohols (>C_{22}) dominate the fatty alcohol profile, maximizing at nC_{22}. The branched and unsaturated fatty alcohols are absent and the short chain fatty alcohols (<C_{22}) only include nC_{15}, nC_{16}, nC_{17}, nC_{18}, nC_{19}, nC_{20}, and nC_{21}. Therefore, most of the short chain and unsaturated fatty alcohols and all branched fatty alcohols may come from the wax esters in the soils. Some protists and bacteria, e.g., Euglena (Dasgupta et al., 2012) and green non-sulfur bacteria (Schouten et al., 2009; van der Meer et al., 2010) can biosynthesize a series of wax esters, in which i/aC_{15}–i/aC_{17} fatty alcohols are important compositions. Chloroflexi, a branch of green non-sulfur bacteria, is widespread in soils (Costello et al., 2006; Janssen et al., 2006), and in turn it may be a potential microbial source that contributes bacterial wax ester and branched fatty alcohols in soils.

2.4 The response of microbial lipids to temperature gradient along the altitude transect

2.4.1 The response of microbial fatty acids to temperature

As the fatty acids and fatty alcohols are rather complex in Jianfengling soils, only microbial lipids, including short chain and branched fatty acids or alcohols, were selected to establish new proxies. Among the microbial fatty acids and alcohols, *iso*/*anteio* pentadecanoic acid (i/aC_{15} fatty acid) and *iso*/*anteio* pentadecanol (i/aC_{15} fatty alcohol) are generally the most abundant compounds in Jianfengling soils and they are ubiquitous in terrestrial environments, e.g., soil, lake, and peat, etc. The ratio of aC_{15}/iC_{15} fatty acid increases with altitude and shows a significant positive correlation with altitude (R^2=0.52, P<0.01) (Figure 7(a)). As the actual MAAT at sampling sites are not available and altitude has a high correlation with air or soil temperature measured in situ, we can use altitude as an independent variable to discuss the relationship between proxies and MAAT. The MAAT decreases with altitude, implying that the ratio of aC_{15}/iC_{15} fatty acid may increase with decreased temperature. In fact, the bacteria cultured under different temperatures also demonstrated that bacteria may produce relatively higher abundance of aC_{15} fatty acid under lower temperatures due

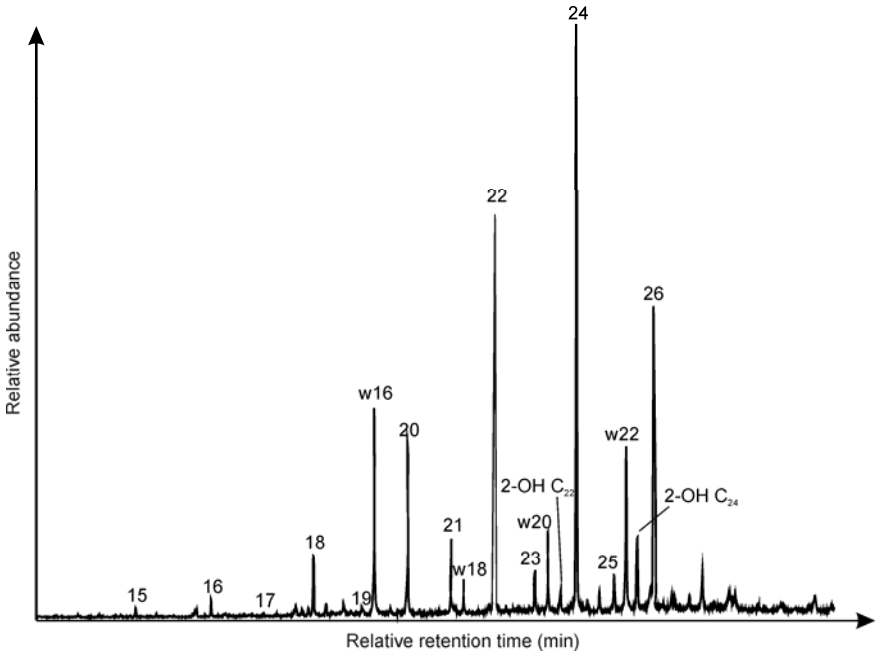

Figure 6 Mass chromatogram of free fatty alcohols, trimethylsilyl ether (*m*/*z* 103) in JFL-3, a selected soil sample from Mt. Jianfengling. ω16 represents hexadecanoic acid with hydroxyl group at ω position and 2-OH C_{22} means dococanoic acid with OH at α position.

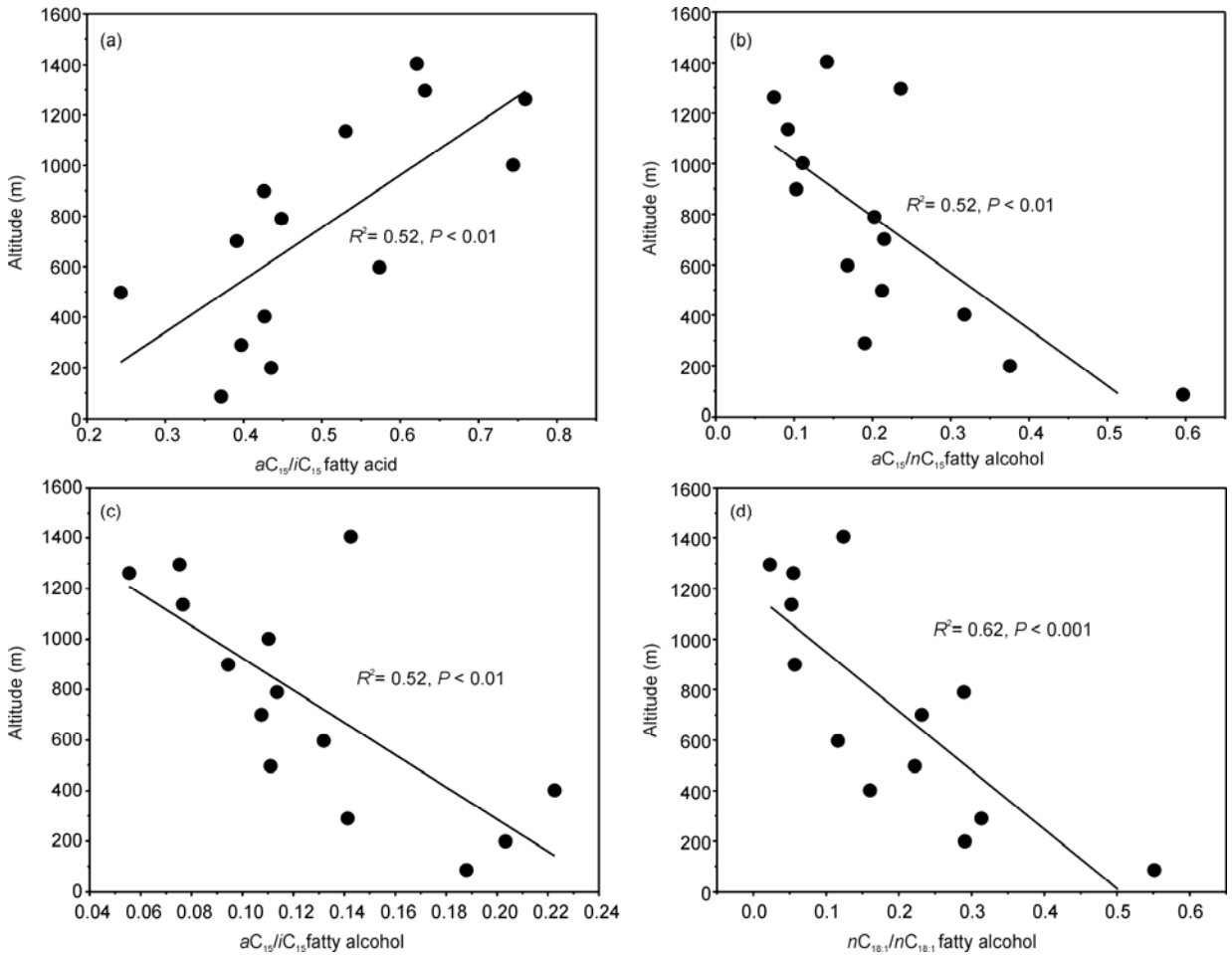

Figure 7 The correlation between altitude vs. aC_{15}/iC_{15} fatty acids (a), aC_{15}/nC_{15} fatty alcohol (b), aC_{15}/iC_{15} fatty alcohol (c) and $nC_{18:1}/nC_{18}$ fatty alcohol (d). P<0.05 means the correlation is significant.

to its special physiochemical characters (Annous et al.,1997; Zhu et al., 2005). The aC_{15} fatty acid has relatively low melting point (25.8°C) and the transition temperature of its phosphatidylcholine between solid and liquid phase (−13.9°C) is much lower than that of iC_{15} fatty acid (−7.0°C) (Suutari et al., 1994), indicating that *anteiso* fatty acid can help regulate the liquid crystalline phase of the microbial cell membrane and keep the fluidity of membrane under low temperature (Edgcomb et al., 2000). Besides, the 3-D structures of *anteiso* fatty acids have a larger cross section than their corresponding *n*- and *iso*-fatty acids, which may help disperse the condensedly packed fatty acyl chains and in turn increase the cell membrane fluidity (Willecke et al., 1971).

2.4.2 *The response of fatty alcohols to temperature*

The fatty alcohols used in paleoenvironmental reconstruction are primarily the long chain *n*-fatty alcohols derived from plant wax esters. The ACL and CPI values of long chain *n*-fatty alcohols can record the change in plant community and microbial degradation induced by the climate fluctuation (Ficken et al., 2000). The ratio of short chain to long chain *n*-fatty alcohol can reflect the intensity of organism degradation in sediments or the input of microorganisms (or marine algae) relative to higher plants in the marine environment, which can be further used to indicate the climate change (Xie et al., 2003; Treignier et al., 2006). The CPI_{21-31} value of long chain *n*-fatty alcohols (C_{21}–C_{31}) ranges from 8.6 to 15.7 in Jiangfengling soils (Table 2), showing no correlation with altitude (or MAAT) (R^2=0.02). However, the CPI_{14-31} value of all *n*-fatty alcohols (C_{14}–C_{31}) has a very significant positive correlation with altitude (R^2=0.68, P<0.001, Figure 8(a)). The CPI_{14-31} value decreases with increased temperature, implying that CPI value of all *n*-fatty alcohols is more sensitive to temperature change than that of long chain *n*-fatty alcohols. A higher temperature may favor the microbial activity, which can facilitate the reworking and degradation of lipid biomarkers and in turn reduce the CPI value of *n*-fatty alcohols from leaf wax in the soils (Xie et al., 2013). The ACL_{22-30} value of long chain *n*-fatty alcohols (C_{22}–C_{30}) varies from 23.1 to 25.2 (Table 2), exhibiting a significant negative correlation with altitude (R^2=0.65, P<0.001, Figure 8(b)). The plants along the altitude transect of Mt. Jianfengling may increase the chain length of long chain *n*-fatty alcohols in response to higher temperatures. The vertical zonality of vegetation in Mt. Jiangfengling has been destroyed by the anthropogenic deforestation and the secondary forest does not show any obvious zonality. Therefore, the ACL variation of long chain *n*-fatty alcohols in Mt. Jiangfengling may not record the change in plant types. Instead, it may reflect the response of plants to temperature change. In fact, the ACL of *n*-alkanes from leaf wax can also increase under higher temperature, and the *n*-alkanes with longer chains appear to be more effective in reducing the water evaporation of leaves under high temperature (Dodd et al., 2003).

Multiple kinds of branched alkanols can be found in Jianfengling soils. We first tested the applicability of BNA_{15} proxy, $(aC_{15}+iC_{15})/nC_{15}$ (Huang et al., 2013) to reconstruct temperature in Mt. Jiangfengling, and found that BNA_{15} shows no correlation with altitude or MAAT, which might be related to large differences in temperature range and microbial communities between Jianfengling soils and Shennongjia soils (16.6–24.5 and 0.9–12.1°C for Jianfengling soils and Shennongjia soils, respectively). We then selected aC_{15}, a widely distributed compound in sediments, to establish the aC_{15}/nC_{15} (alkanols) proxy. The ratio of aC_{15}/nC_{15} (alkanols) shows a significant negative (or positive)

Table 2 Proxies based on fatty acids and alkanols in soils of varied altitudes from Mt. Jianfengling[a)]

Sample No.	Altitude (m)	Estimated MAAT (°C)	CPI_{14-31} (alkanol)	ACL_{22-30} (alkanol)	aC_{15}/nC_{15} (alkanol)	aC_{15}/iC_{15} (alkanol)	$C_{18:1}/nC_{18:0}$ (alkanol)	aC_{15}/iC_{15} (fatty acid)
JFL-1	1405.0	16.6	11.1	23.6	0.14	0.14	0.12	0.62
JFL-2	1296.0	17.2	17.8	23.1	0.24	0.08	0.02	0.63
JFL-3	1262.0	17.4	12.3	23.7	0.07	0.06	0.06	0.76
JFL-4	1137.0	18.2	14.5	24.1	0.09	0.08	0.05	0.53
JFL-5	1002.0	19.0	10.8	23.3	0.11	0.11	–	0.74
JFL-6	899.0	19.6	7.8	24.0	0.10	0.09	0.06	0.43
JFL-7	790.0	20.3	9.3	24.6	0.20	0.11	0.29	0.45
JFL-8	701.0	20.8	6.8	24.3	0.21	0.11	0.23	0.39
JFL-9	597.0	21.4	7.1	24.6	0.17	0.13	0.12	0.57
JFL-10	497.0	22.0	4.8	24.5	0.21	0.11	0.22	0.24
JFL-11	402.0	22.6	6.9	24.0	0.32	0.22	0.16	0.43
JFL-12	290.0	23.3	7.3	24.3	0.19	0.14	0.31	0.40
JFL-13	199.0	23.8	3.6	24.9	0.38	0.20	0.29	0.44
JFL-14	86.0	24.5	6.5	25.2	0.60	0.19	0.55	0.37

a) '–' means data not available.

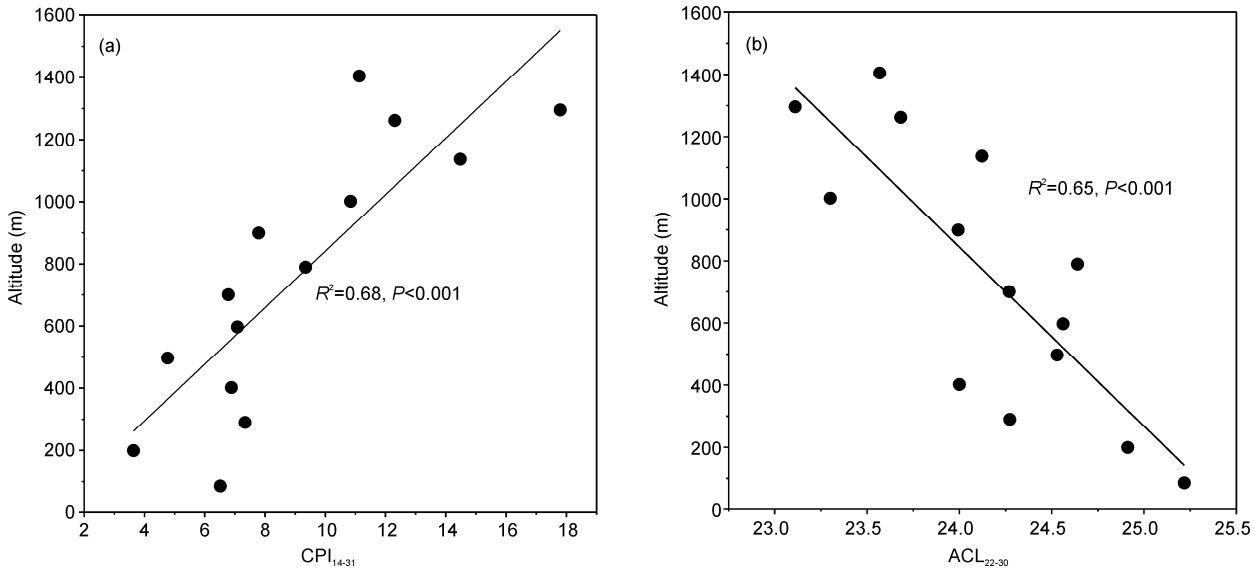

Figure 8 The significant correlation between altitude and CPI of all *n*-alkanols (C_{14}–C_{31}) (a), and ACL of long chain *n*-alkanols (C_{22}–C_{30}) (b). $P<0.05$ means the correlation is significant.

correlation with altitude (or MAAT) (R^2=0.52, P<0.01), implying that lower abundance of aC_{15} relative to nC_{15} occurs in a lower temperature (Figure 7(b)). The minerals in two samples, JFL-1 and JFL-2, were not completely weathered, which may result in a different microbial community from other soil samples. This can partly explain why aC_{15}/nC_{15} ratios of JFL-1 and JFL-2 deviate from the fitting line. The annual rainfall amounts at all altitudes of Jianfengling are abundant (>2000 mm) and thus rainfall seems unlikely to determine the growth of bacteria and to control the microbial membrane lipids. In fact, the well-known environmental factors that can influence the distribution of microbial lipids are temperature and pH (Schouten et al., 2002; Yang et al., 2010). A causal relationship between aC_{15}/nC_{15} ratio and precipitation seems to be unlikely as bacteria can only grow in an aqueous environment and thus more water by means of increased precipitation may not influence the distribution of microbial lipids. The soil pH only shows a slight change along the altitude transect of Mt. Jiangfengling. If soil pH is considered as a factor that affects the distribution of alkanols, the correlation between aC_{15}/nC_{15} ratio, altitude and soil pH obviously has increased determination factor (R^2=0.80, P<0.001) (Figure 9) with a calibration equation as follow:

$$aC_{15}/nC_{15} = 0.137\times \mathrm{pH} - 0.000127\times H - 0.322.$$

Similar to the MBT index of bacterial bGDGTs (cf. Yang et al., 2010), aC_{15}/nC_{15} ratio seems to be also controlled by soil pH. The linear relation between aC_{15}/nC_{15}, altitude (or MAAT) and soil pH may provide a novel way to reconstruct the paleo-altimetry (or paleo-MAAT) in the Quaternary sediments. The aC_{15} and iC_{15} alkanol are widespread in diverse environments, e.g., peat (Huang et al., 2013) and soils, etc, which provides the basis for this new paleothermometer. It should be noted that the estimated MAAT along the altitude transect (16.6–24.5°C) is relatively high as Mt. Jianfengling is located in the tropics. When applied to other regions with lower MAAT, the aC_{15}/nC_{15} proxy may require local calibration. The application of aC_{15}/nC_{15} may be limited to environments with little pH change, e.g., peat. If soil pH variation is estimated to be relatively constant by CBT of

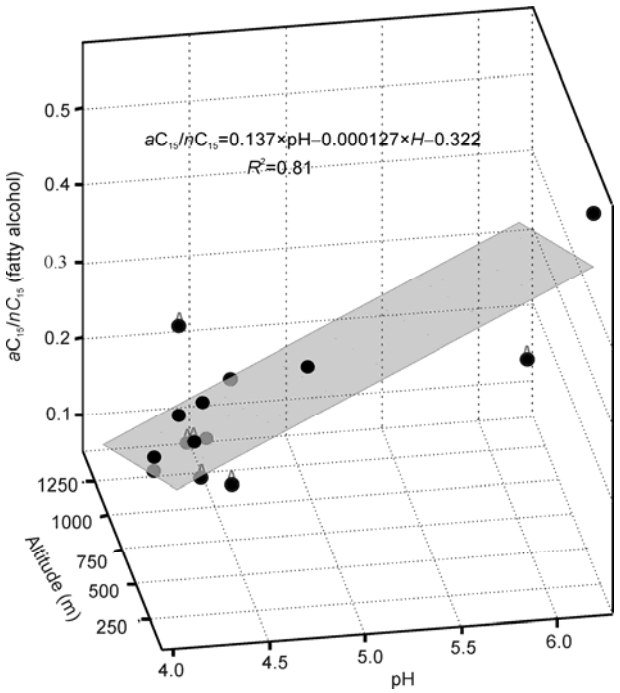

Figure 9 The 3-dimensional correlation between aC_{15}/nC_{15} (alkanol) ratio, altitude and soil pH for Jianfengling soils. H in the equation represents the altitude.

bGDGTs, the aC_{15}/nC_{15} proxy may be applicable to reconstructing paleotemperature in this environment.

The response of aC_{15} alkanol to altitudes (or MAAT) is completely different from that of aC_{15} fatty acid. The relative abundance of aC_{15} alkanol decreases with decreased MAAT. In contrast, aC_{15} fatty acid shows the opposite. Obviously, the relation between aC_{15}/nC_{15} (alkanol) and MAAT cannot be explained by the means of aC_{15} fatty acid as aC_{15} fatty acid may increase under lower temperature. In addition to aC_{15} alkanol, mono-unsaturated octadecanol (all isomers of $nC_{18:1}$) also shows different character from mono-unsaturated octadecanoic acid. The ratio of $nC_{18:1}/nC_{18:0}$ exhibits a negative correlation with altitude or a positive correlation with MAAT, indicating that a higher unsaturation of octadecanol can be observed under higher temperatures (Figure 7(d)). The unsaturation of microbial fatty acids may increase with decreased temperature (Wada et al., 1987; Suutari et al.,1992). Compared with saturated fatty acids, the folded structures of unsaturated fatty acids can reduce the intermolecular forces and thus they generally have lower melting points. The increase in the proportion of unsaturated fatty acids may lower the melting point of cell membrane and keep the fluidity of cell membrane under low temperature. For example, the well-known paleothermometer, UK-37′, is based on increased unsaturation of long chain alkenones of *Haptophyta* with decreased temperature (Prahl et al., 1987). As stated earlier, the *anteiso* and unsaturated fatty alcohols can be only released from wax ester by saponification and may come primarily from bacteria. In contrast, *n*-pentadecanol and *n*-octadecanol may be derived primarily from leaf wax. The decrease in MAAT may result in weaker microbial activity and less biomass of bacteria producing *anteiso* and unsaturated fatty alcohols along the altitude transect, which is subsequently reflected by a decreasing trend of *anteiso* and unsaturated fatty alcohols relative to *n*-pentadecanol and *n*-octadecanol. Therefore, in addition to aC_{15}/nC_{15} alkanols, the ratio of $nC_{18:1}/nC_{18:0}$ may also provide a novel proxy for paleoaltimetry and paleotemperature reconstruction.

The ultraviolet light intensity induced by solar irradiance may affect the composition of plant wax ester (Li et al., 2011). However, *iso*/*anteiso* alkanols may originate primarily from bacteria that flourish in soils. The soils collected in this study were all covered by dense plants and are difficult to be impacted directly by solar irradiance. Therefore, though there is an irradiance gradient along the altitude transect (Table 1), the solar irradiance seems unlikely to influence the functioning of microbial cell in the soils.

3 Conclusions

The soils collected from an altitude transect of Mt. Jianfengling contain abundant microbial fatty acids and fatty alcohols, including *iso*/*anteiso* fatty acids, 10-Me-hexadecanoic acid, *iso*/*anteiso* fatty alcohols, 10-Me-hexadecanol and mono-unsaturated fatty alcohols, etc. These fatty alcohols may be derived primarily from microbial wax esters and can be released only from wax ester by saponification. The distribution of branched fatty alcohols ($<C_{22}$) is consistent with that of branched fatty acids in these soils, indicating they may share a common biological source. The branched fatty acids and branched fatty alcohols can respond to the temperature change along the altitude transect. The ratio of aC_{15}/iC_{15} (fatty acid) increases with altitude, suggesting a higher production of aC_{15} fatty acid under lower temperatures. In contrast, the ratio of aC_{15}/iC_{15} (alkanol) and aC_{15}/nC_{15} (alkanol) both decrease with increased altitude (or decreased MAAT). Besides, the ratio of $nC_{18:1}/nC_{18:0}$ (alkanol), a proxy representing the unsaturation of octadecanol, also decreases with increased altitude (or decreased MAAT), which is in contrast with the consensus that unsaturation of microbial fatty acids increases with decreased temperature. The relation of these microbial proxies with MAAT or altitude may provide new ways for the paleotemperature reconstruction in peats and loess-paleosol.

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