SCIENCE CHINA
Earth Sciences

SPECIAL TOPIC: **Advances in organic proxies for research in climate and environmental changes**
• RESEARCH PAPER •

July 2016 Vol.59 No.7: 1329–1338
doi: 10.1007/s11430-016-5271-2

Distribution of fatty acids in the alpine grassland soils of the Qinghai-Tibetan Plateau

DAI GuoHua[1], ZHU ShanShan[1], LIU ZongGuang[1], CHEN LiTong[2], HE JinSheng[2,3] & FENG XiaoJuan[1*]

[1] *State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China;*
[2] *Key Laboratory of Adaptation and Evolution of Plateau Biota, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810008, China;*
[3] *Department of Ecology, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China*

Received September 29, 2015; accepted January 8, 2016; published online March 25, 2016

Abstract As an important biomarker, fatty acids (FAs) have been extensively used to trace the origin of organic matter in sediments and soils. However, studies of the distribution and abundance of FAs in alpine grassland soils are still rare, especially on the Qinghai-Tibetan Plateau (QTP), the highest plateau in the world, which contributes sediments to many large rivers in Asia. This study investigates the composition, distribution and source of FAs with increasing soil depths from 17 typical alpine grassland sites in the QTP. The most abundant FAs included the ubiquitous C_{16} FA and even-numbered long-chain FAs (C_{20}–C_{30}), indicating mixed inputs from microbial and higher plant sources. Source apportionment showed that higher plants were the dominant contributor of FAs (approximately 40%) in QTP soils. The abundance of FAs decreased with soil depth, with the highest value (1.08±0.09 mg/g C) at a 0–10 cm depth and the lowest value (0.46±0.12 mg/g C) at a 50–70 cm depth, due to much lower plant inputs into the deeper horizons. The total concentration of FAs was negatively correlated to the mean annual temperature (MAT; $P<0.05$) and soil pH ($P<0.01$), suggesting that the preservation of FAs was favored in low-MAT and low-pH soils on the QTP. The abundance of fresh C source FAs increased significantly with the mean annual precipitation (MAP; $P<0.05$), indicating that high MAP facilitates the accumulation of fresh FAs in QTP soils. Other environmental parameters, such as the soil mineral content (aluminum and iron oxide), microbial community composition as well as litter quality and quantity, may also exert a strong control on the preservation of FAs in QTP soils and warrant further research to better understand the mechanisms responsible for the preservation of FAs in QTP soils.

Keywords Qinghai-Tibetan Plateau, Soil organic matter, Biomarker, Fatty acids, Distribution, sources

Citation: Dai G H, Zhu S S, Liu Z G, Chen L T, He J S, Feng X J. 2016. Distribution of fatty acids in the alpine grassland soils of the Qinghai-Tibetan Plateau. Science China Earth Science, 59: 1329−1338, doi: 10.1007/s11430-016-5271-2

1. Introduction

Organic matter biomarkers are compounds with unique structures that are specific to certain organisms (e.g., higher plants and microbes) (Kögel-Knabner, 2002; Otto and Simpson, 2005; Feng and Simpson, 2007). Fatty acids (FAs), derived from plants, microbes and other soil fauna, are the important sources of the aliphatic constituents of soil organic matter (SOM) (Otto and Simpson, 2005, and references therein). They play an important role in the incorporation and transformation of plant and/or microbial residues into soil and in the stabilization of SOM. Over the past few decades, FAs have been successfully used to examine the source and reactivity of organic matter in sediments (Perry et al., 1979; Camacho-Ibar et al., 2003; Hu et al., 2006;

*Corresponding author (email: xfeng@ibcas.ac.cn)

Morgunova et al., 2012; Huang et al., 2015) and have provided valuable information on the composition, source and biogeochemistry of the organic matter in sediments.

A number of studies have assessed the concentrations, distribution patterns and turnover rates of FAs in forest and grassland soils (e.g., Bull et al., 2000; Jandl et al., 2005; Naafs et al., 2004; Otto and Simpson, 2005; Feng and Simpson, 2007). However, as far as we know, studies on the FA distribution in alpine grassland soils are still rare. Furthermore, how environmental factors affect the distribution and abundances of FAs is also poorly understood for this cold region. The Qinghai-Tibetan Plateau (QTP) is the youngest, largest and highest plateau in the world, comprising an area of more than 2.5 million km^2 and an average elevation of over 4000 m a.s.l. Over 60% of the plateau is covered by grasslands, including alpine meadow, alpine steppe and alpine meadow steppe, which composes 40% of the national Chinese grassland area (Yang et al., 2008). Soils on the QTP store a large amount of carbon because of the cold and relatively humid climate (Yang et al., 2008; Shi et al., 2012). Due to the unique geographic and ecological conditions, SOM in the QTP has been reported to play an important role in regional and global carbon cycles and to contribute to the source of sediments in many large rivers in Asia (including the Yangtze, Yellow and Mekong Rivers). Recently, some researchers have noted that the QTP is a highly sensitive area to global climate changes, which may result in the degradation of alpine grasslands and loss of SOM due to microbial decomposition and riverine transport (Baumann et al., 2009; Lin et al., 2011; Chen et al., 2013; Dörfer et al., 2013). Investigations on the molecular compositions of FAs in QTP soils may shed light on soil carbon dynamics under global climate change and also provide source information on FAs in the sediments of rivers that originate in the QTP (Bull et al., 2000; Feng and Simpson, 2008; Feng et al., 2013). In this study, FAs were first investigated in soil profiles at 17 sites in the QTP in August 2012. The main objectives of this study are to (1) determine the distribution and composition of FAs in QTP soils, (2) evaluate the contribution of plant- and microorganism-derived organic matter to the abundance of FAs and (3) assess the influence of temperature, precipitation, and soil properties on the FA distribution in the QTP.

2. Materials and Methods

2.1 Sample collection

A total of 162 soil samples were collected from 17 sites (three profiles per site) in the central part of the QTP (Figure 1) in August 2012. These sites cover a latitudinal gradient of 28.31° to 37.41°N and an elevation gradient of 3130 to 5418 m and are largely dominated by alpine meadow (e.g., *Kobresia pygmaea, Kobresia humilis, Saussurea sp.*) and alpine steppe (e.g., *Stipa purpurea, Stip subsessiliflora, Carex Ianceolata*) (Table 1). The soils are dominated by sand (60%), with a pH of approximately 7.6. Soil samples were taken with a soil corer with an inner diameter of 50 mm from depths of 0–10, 10–20, 20–30 and 50–70 cm where possible. After sampling, soils were air-dried, passed through a 2 mm sieve and ground before chemical analysis. All data on the bulk properties and FAs in QTP soils were

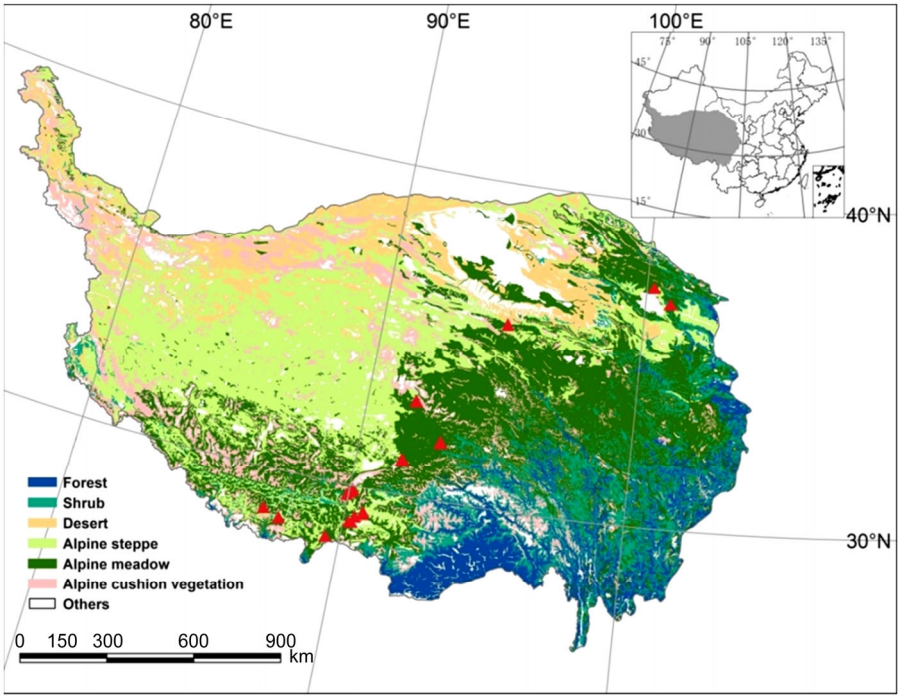

Figure 1 Sampling locations in the central part of the Qinghai-Tibetan Plateau.

Table 1 Sample characteristics, including the geographical location, mean annual temperature (MAT), mean annual precipitation (MAP) and dominant vegetation

Sampling sites	Latitude (N)	Longitude (E)	Altitude (m)	MAT (°C)	MAP (mm)	Primary vegetation
P783	36.98°	100.92°	3130	1.49	437	Alpine meadow steppe
P793	35.71°	94.28°	4374	–4.35	193	Alpine meadow
P804	32.19°	91.68°	4632	–3.01	510	Alpine and subalpine meadow
P806	31.58°	91.86°	4594	–1.22	459	Alpine meadow
P811	30.62°	91.54°	4542	–0.30	529	Alpine meadow
p815	37.41°	100.11°	3370	–1.84	429	Alpine meadow steppe
P816	29.28°	90.64°	3630	3.01	381	Alpine desert steppe
P818	29.20°	90.62°	4721	2.99	369	Alpine and subalpine meadow
P819	29.21°	90.64°	4544	3.01	381	Alpine and subalpine meadow
P821	29.23°	90.63°	4148	3.01	381	Alpine meadow steppe
P826	28.89°	90.30°	4666	–1.80	364	Alpine meadow steppe
P830	28.31°	89.47°	4441	0.92	315	Alpine meadow
P835	28.56°	87.79°	4212	1.96	377	Alpine desert steppe
P839	28.94°	87.44°	5216	–1.40	354	Alpine meadow
P858	29.90°	90.13°	5418	–2.75	452	Alpine and subalpine meadow
P860	29.87°	90.12°	5065	–2.75	452	Alpine and subalpine meadow
P862	29.80°	90.03°	4627	–1.37	419	Alpine and subalpine meadow

expressed as the mean values of three profiles.

2.2 Environmental parameter analyses

In the absence of observations at the exact sampling sites, meteorological data at each site were obtained based on linear models using latitude, longitude and altitude as variables from a 30-year temperature and precipitation record (1981–2010) at 680 well distributed climate stations across China (He et al., 2006). The mean annual precipitation (MAP) ranges from 193 to 529 mm, and more than 80% of the annual precipitation occurs during the summer months from July until September (Table 1). The mean annual temperature (MAT) varies from –4.35 to 3.01°C (Table 1) in the study area.

Soil pH was measured using a pH meter in a soil-water suspension, with a soil∶water ratio of 1∶2.5 (Ding et al., 2015). Soil grain size analysis was conducted following the method described by Sun et al. (2011). Briefly, approximately 1.0 g of dried soils was reacted with an excess of 1 mol/L hydrochloric acid and then with hydrogen peroxide to remove carbonates and oxidize organic matter, respectively. Subsequently, sodium hexametaphosphate was added and the solutions were allowed to settle for 24 h. After ultrasonication for 1 min, the grain size distribution was measured by a Mastersize 2000 Laser Particle Size Analyzer. The scan range was from 0.02 to 2000 μm, and particles were categorized into three fractions: sand (20–2000 μm), silt (2–20 μm) and clay (<2 μm). The total carbon (C,) and total nitrogen (TN) contents of soil samples were measured by the combustion method (Vario EL III, Elementar, Hanau, Germany)

2.3 Extraction and analysis of FAs

FAs were extracted and analyzed as described by Feng and Simpson (2007), with minor modifications. Briefly, soil samples (5–8 g dry soil) were extracted three times with 30 mL of dichloromethane, dichloromethane:methanol (1∶1, *v*∶*v*) and methanol. The solvent extracts (approximately 100 mL), spiked with a surrogate standard (C_{18} alkane), were filtered through glass fiber filters (Whatman GF/F) and evaporated almost to dryness by rotary evaporation. The extracts were further cleaned through a silica gel column (0.5 cm i.d.), with hydrocarbons eluted with 15 mL of hexane and FAs eluted with 10–15 mL of methanol. The FA fraction was dried under nitrogen gas in 2 mL glass vials and redissolved in 1 mL of a dichloromethane:methanol (1∶1, *v*∶*v*) mixture. Aliquots (100 μL) of the extracts were dried in a stream of nitrogen and then converted to trimethylsilyl derivatives by reaction with 90 μL of N, O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and 10 μL of pyridine (9∶1, *v*∶*v*) for 3 h at 70°C. After cooling, 100 μL DCM was added to dilute the extracts prior to instrument analysis.

The molecular composition of FAs was examined on a Trace GC 1310 gas chromatograph coupled to an ISQ mass spectrometer (Thermo Fisher Scientific, USA) using a DB-

5MS column (30 m×0.25 mm i.d., film thickness, 0.25 μm) for separation. The temperature increased from 65°C (initial hold time 2 min) to 300°C at a rate of 6°C/min, and helium was used as the carrier gas (1.2 mL/min). The mass spectrometer was operated in the EI mode at a 70 eV ionization energy and scanned from 50 to 650 daltons. Quantification was achieved by comparison with internal standard C_{19} FA. Errors associated with the lipid concentration data were typically <10% based on replicate analysis of the same soil sample.

2.4 Source apportionment approach

Source apportionment analysis was conducted using principal component analysis followed by multiple linear regression (PCA-MLR) based on the profiles of all FAs to compare the contribution from different sources to total FAs. In the factor analysis model, PCA was used as the extraction method with Kaiser Normalization and varimax rotation. Only factors with eigenvalues >1 were used for the identification of the possible sources. The relationship between the principal component and FAs is indicated by the factor loadings. Stepwise MLR was then performed on the significant factors to determine the mass apportionment of each source to the total concentration. After normalization, the multiple regression model is represented by the simple formula:

$$\hat{Z}_{\text{SumFAs}} = \sum B_k t_k, \tag{1}$$

where SumFAs is the total concentration of FAs in this study, $\hat{Z}$ is the standard normalized deviation of the SumFAs values, B_k is the modeled regression coefficient and t_k is the factor score calculated by PCA.

The mean percentage contributions of each factor are calculated by eq. (2):

$$\text{Mean contribution of source } k(\%) = 100 \times \left(B_k / \sum B_k \right). \tag{2}$$

The contribution of each source k to the SumFAs is calculated by eq. (3):

$$\begin{aligned} &\text{Contribution of source } k\left(\text{mg/gOC}\right) \\ &= \text{mean}_{\text{SumFAs}} \times \left(\frac{B_k}{\sum B_k} \right) + B_k \sigma_{\text{SumFAs}} t_k, \end{aligned} \tag{3}$$

where $\text{mean}_{\text{SumFAs}}$ is the mean concentration of SumFAs and σ_{SumFAs} is the standard deviation of SumFAs for all samples.

2.5 Statistical analysis

Mann-Whitney U analysis was conducted to examine the differences in bulk properties and FAs in soils from different soil depths using SPSS 18.0. Linear regression models were used to examine the relationship between the abundance of FAs and potential environmental covariates (MAT, MAP and soil properties). In all cases, $P<0.05$ were considered to be significant.

3. Results and discussion

3.1 Bulk soil properties

The bulk properties of the QTP grassland soils are listed in Table 2. The grains in the investigated soil samples were dominated by sand (58.7±2.7%, mean±s.e.), followed by silt (35.3±2.4%) and clay (6.0±0.4%). The clay content generally increased with soil depth, with Site P830 exhibiting the highest accumulation of clay (17.9±0.3%) at a 50–70 cm depth. The soil pH ranged from 5.83 to 8.47 in QTP soils with the highest value at Site P804 and the lowest value at Site P862 (Table 2). The soil pH increased with soil depth, with a pH of approximately 7.2 in the first 30 cm of the soils and 8.2 at a 50–70 cm depth. The C contents ranged from 0.6% to 13.0%, with the highest value at Site P862 and the lowest value at Site P835 (Table 2). In general, the C and TN contents seemed to decrease with soil depth, with mean values ranging from 5.0±0.7% (C) and 0.4±0.05% (TN) at a depth of 0–10 cm to 2.5±0.6% (C) and 0.1±0.02% (TN) at a depth of 50–70 cm, respectively. However, the decrease was not significant ($P>0.05$), which may be due to the limited number of samples in the deeper horizons (50–70 cm). A strong correlation was observed between C and TN ($r=0.909$, $n=71$, $P<0.01$), suggesting that nitrogen is primarily associated with soil carbon. It is notable that the atomic C/N ratio (C/N_a) showed quite high values, approximately 16 in the first 30 cm of the soils. Moreover, the C/N_a ratio increased to ~25 at a 50–70 cm depth. Usually the organic C/N_a ratio shows values of approximately 10 in well-developed grasslands (Otto and Simpson, 2005; Feng and Simpson, 2007; Zhao et al., 2014), our higher C/N_a values likely due to the presence of inorganic carbon. Another possible explanation for the increase of the C/N ratio at a 50–70 cm depth is the presence of an oxic-anoxic boundary near the depth of 50 cm in QTP soils, which prohibits SOM degradation (Baumann et al., 2009; Dörfer et al., 2013).

3.2 Composition and distribution of FAs in QTP soils

The distribution of FAs in the QTP soil samples is summarized in Figure 2. The distribution of FAs (C_9 to C_{32}) was similar among different soil depths, dominated by C_{16}, C_{18} and even-numbered homologues in the range of C_{22}–C_{32} (Figure 2). Generally, FAs exhibited a bimodal distribution with the maximum concentration (C_{max}) at C_{16} for short-chain FAs (C_{14}–C_{18}) and C_{max} at C_{24} for long-chain FAs (C_{20}–C_{32}) (Figure 2). This distribution pattern is characteristic of a mixed source of microbes and higher plants and has been previously reported in soils from other regions (Otto

Table 2 Bulk soil properties, abundance of fatty acids (FAs; mg/g C) and FA-based parameters (CPI and ACL) of the organic matter sources and degradation stages used in this paper [a)]

Sites	Depth (cm)	C (%)	TN (%)	C/N_a	pH	Clay (%)	Slit (%)	Sand (%)	SumFAs (mg/g C)	CPI	ACL	t_1	t_2	t_3	t_4
P783	0–10	4.36	0.43	12	8.02	7.3	39.1	53.6	0.38	5.7	22.4	0.73	0.46	0.45	0.62
	10–20	3.52	0.34	12	8.09	5.8	54.9	39.3	0.41	5.9	22.1	0.82	0.32	0.35	0.83
	20–30	3.15	0.28	13	8.07	4.2	54.9	40.9	0.30	5.5	20.8	0.68	0.31	0.32	0.84
	50–70	3.50	0.16	25	8.29	7.6	37.1	55.3	0.06	4.1	20.3	0.60	0.27	0.36	0.59
P793	0–10	5.03	0.35	17	7.95	5.2	31.0	63.8	1.06	9.3	20.3	0.01	0.73	1.82	0.65
	10–20	4.09	0.25	19	8.09	5.5	31.9	62.6	0.53	8.7	21.2	0.60	0.54	0.71	0.59
	20–30	3.63	0.18	23	8.17	6.0	36.6	57.4	0.19	9.6	22.6	0.68	0.31	0.38	0.61
P804	0–10	1.77	0.05	38	8.28	2.0	12.3	85.7	0.39	8.7	19.3	0.50	0.57	0.40	0.79
	10–20	1.59	0.05	37	8.41	0.6	2.4	97.0	1.24	6.5	22.0	1.02	1.09	0.71	0.67
	20–30	1.88	0.06	35	8.35	1.1	5.1	93.8	0.40	6.2	21.7	0.78	0.48	0.38	0.63
	50–70	2.28	0.07	36	8.47	5.7	20.0	74.4	0.57	5.0	21.7	0.88	0.54	0.38	0.74
P806	0–10	3.85	0.32	14	7.13	5.3	19.1	75.6	0.70	6.5	22.3	0.99	0.42	0.47	0.81
	10–20	2.89	0.24	14	7.17	3.6	15.5	81.0	0.40	5.8	23.5	0.95	0.36	0.34	0.62
P811	0–10	6.59	0.55	15	5.85	4.8	19.8	75.3	0.94	13.6	20.6	0.09	0.44	1.78	0.68
	10–20	4.48	0.42	12	5.99	5.7	24.6	69.7	3.32	10.5	21.3	1.30	0.56	0.98	3.65
	20–30	4.84	0.43	13	6.29	5.4	20.8	73.8	1.34	9.7	23.1	1.59	0.49	0.49	0.93
P815	0–10	8.04	0.70	14	7.21	7.4	36.1	56.4	0.68	6.2	21.3	0.46	0.67	0.81	0.70
	10–20	6.03	0.59	12	7.60	6.8	37.5	55.7	0.48	5.1	22.8	0.85	0.45	0.50	0.60
	20–30	4.12	0.42	11	7.84	7.4	34.9	57.8	0.22	4.8	22.7	0.78	0.35	0.35	0.59
	50–70	3.25	0.17	23	8.22	6.0	33.7	60.3	0.14	3.8	22.3	0.69	0.27	0.34	0.64
P816	0–10	1.22	0.15	10	7.67	4.6	24.0	71.4	0.18	6.0	18.8	0.53	0.47	0.37	0.61
	10–20	0.98	0.12	9	7.81	5.1	32.2	62.7	0.17	7.3	20.2	0.59	0.44	0.32	0.60
	20–30	0.82	0.09	11	7.88	6.2	34.0	59.8	0.25	6.7	20.0	0.63	0.54	0.28	0.61
P818	0–10	5.44	0.45	14	6.39	5.9	35.7	58.4	1.09	5.5	22.6	1.41	0.63	0.47	0.65
	10–20	4.36	0.37	14	6.48	7.5	40.3	52.1	1.55	5.0	23.4	2.01	0.67	0.46	0.67
P819	0–10	6.00	0.50	14	6.60	5.6	30.8	63.5	1.00	5.3	22.4	1.12	0.49	0.92	0.59
	10–20	4.17	0.38	13	6.50	7.0	38.5	54.5	0.75	5.1	23.7	1.32	0.39	0.44	0.60
	20–30	3.29	0.26	15	6.55	6.1	38.3	55.6	0.92	4.6	23.8	1.56	0.47	0.39	0.56
P821	0–10	3.98	0.31	15	7.06	7.6	38.1	54.3	0.55	6.0	22.9	1.07	0.42	0.35	0.65
	10–20	2.16	0.17	15	7.11	6.9	44.9	48.2	0.44	5.9	22.8	0.98	0.39	0.34	0.63
	20–30	1.21	0.13	11	7.11	6.8	43.4	49.8	0.34	5.5	22.8	0.91	0.37	0.33	0.63
P826	0–10	3.15	0.29	12	7.97	4.6	44.7	50.7	0.93	5.0	22.6	1.34	0.64	0.51	0.54
	10–20	2.40	0.22	13	8.10	4.0	48.0	48.0	0.58	5.4	22.5	1.01	0.59	0.37	0.55
	20–30	1.89	0.16	14	8.12	4.3	48.5	47.2	1.56	1.3	23.5	2.04	0.98	0.19	0.62
	50–70	0.77	0.06	15	8.09	6.0	50.3	43.7	1.62	5.1	20.0	0.57	2.59	0.38	0.45
P830	0–10	4.41	0.14	40	7.93	11.6	81.5	6.9	0.15	7.1	22.2	0.69	0.30	0.32	0.62
	10–20	4.59	0.10	54	7.84	11.1	80.1	8.8	0.08	6.1	23.0	0.65	0.26	0.34	0.60
	20–30	4.84	0.19	30	7.73	13.4	80.4	6.2	0.08	5.8	24.2	0.68	0.24	0.34	0.59
	50–70	4.67	0.12	45	7.74	17.9	73.8	8.3	0.09	5.4	23.7	0.69	0.24	0.35	0.59
P835	0–10	0.61	0.14	5	8.38	0.6	5.3	94.1	1.27	9.5	20.0	0.35	2.10	0.47	0.59
	10–20	0.76	0.20	4	8.36	2.1	13.5	84.4	0.53	10.1	20.5	0.55	0.80	0.45	0.64
	20–30	0.64	0.15	5	8.39	1.9	16.6	81.4	0.59	11.5	19.8	0.47	1.02	0.40	0.61
	50–70	0.87	0.17	6	8.33	2.5	27.9	69.7	0.28	7.1	21.0	0.67	0.51	0.32	0.62

(*To be continued on the next page*)

(*Continued*)

Sites	Depth (cm)	C (%)	TN (%)	C/N_a	pH	Clay (%)	Slit (%)	Sand (%)	SumFAs (mg/g C)	CPI	ACL	t_1	t_2	t_3	t_4
P839	0–10	4.00	0.30	16	6.57	3.5	18.3	78.2	2.33	6.5	23.7	3.28	0.80	0.35	0.71
	10–20	3.41	0.28	14	6.33	6.2	30.5	63.3	1.70	6.2	23.9	2.77	0.52	0.19	0.67
	20–30	2.01	0.17	14	6.49	6.9	34.2	58.9	1.92	6.0	21.3	1.78	0.77	0.06	1.79
P858	0–10	6.82	0.46	17	6.76	5.0	30.1	64.9	1.41	5.6	22.8	1.80	0.68	0.58	0.66
	10–20	3.66	0.27	16	6.49	4.2	23.1	72.7	2.04	4.3	23.2	2.89	1.11	0.38	0.47
P860	0–10	6.90	0.38	21	6.25	5.5	32.0	62.5	2.93	8.0	23.6	3.49	0.40	0.85	0.89
	10–20	5.03	0.28	21	6.21	6.6	38.0	55.3	1.14	5.6	24.0	1.83	0.39	0.44	0.63
	20–30	4.02	0.22	21	6.04	7.2	42.0	50.8	1.91	5.4	20.1	0.90	0.92	0.35	2.16
P862	0–10	13.04	0.77	20	6.04	7.7	33.6	58.8	2.38	5.5	23.6	2.93	0.63	1.46	0.07
	10–20	10.91	0.64	20	5.87	9.4	45.1	45.5	1.27	6.6	23.5	1.77	0.47	0.61	0.57
	20–30	10.48	0.60	20	5.83	8.8	39.2	52.0	1.48	4.9	23.4	2.01	0.46	1.21	0.27

a) C, total carbon; TN, total nitrogen; C/N_a Atomic C/N ratio; SumFAs, the total concentrations of fatty acids (FAs); CPI, carbon preference index: $\mathrm{CPI}=\left[\left(\sum C_{20+22+24+26+28+30}/\sum C_{19+21+23+25+27+29}\right)+\left(\sum C_{20+22+24+26+28+30}/\sum C_{21+23+25+27+29+31}\right)\right]/2$; ACL, average chain length: $\mathrm{ACL}=\sum(z_n\times n)/\sum z_n$, where z_n is the abundance of FAs with a n number of carbon (in the range of 16 to 32); t_1, t_2, t_3, and t_4 were calculated by eqs. (2) and (4) in the main text. t_1, plant-derived FAs, including saturated long-chain FAs (C_{20}-C_{32}); t_2, FAs derived from a mixed source of both plant and microorganisms, including C_9 to C_{18}; t_3, microbial-derived FAs, including branched FAs (*iso*-C_{15} and *iso*-C_{17}); t_4, fresh FAs, including unsaturated C_{18} FAs ($C_{18:1}$ and $C_{18:2}$).

and Simpson, 2005; Feng and Simpson, 2007; Zhao et al., 2014). FAs from higher plant waxes show a distribution from C_{20} to C_{32}, with a C_{max} at C_{24} or C_{26}, whereas those from microorganisms range from C_{14} to C_{18}, with a C_{max} at C_{16} (Otto and Simpson, 2005, and reference therein). Branched FAs (*iso*-C_{15} and *iso*-C_{17}) were detected at trace amounts in all soil samples, which are mainly biosynthesized by bacteria and fungi, confirming microbial inputs in the study area. In addition to saturated FAs, unsaturated C_{18} FAs ($C_{18:1}$ and $C_{18:2}$) were also detected at considerable concentrations in all soil samples. Their presence reflects the contribution of relatively fresh materials and indicates low to moderate levels of SOM degradation, as unsaturated FAs are easy to degrade in soils (Moucawi et al., 1981; Wiesenberg et al., 2010).

The concentrations of FAs in QTP soils are given in Table 2. The total concentrations of FAs ranged between 0.40 mg/g C (P830) and 5.99 mg/g C (P860), with a mean of 2.89±1.91 mg/g C. The concentrations of FAs decreased with increasing soil depth, with the highest value (1.08± 0.09 mg/g C) at a 0–10 cm depth and the lowest value (0.46±0.12 mg/g C) at a 50–70 cm depth. The abundance of FAs in the surface soils (0–10 cm) in this study is higher than in grassland soils from western Alberta (0.10–0.16 mg/g C) (Otto and Simpson, 2005; Feng and Simpson, 2007) and similar to that of grassland soils from Inner Mongolia in Northern China (0.38–1.20 mg/g C) (Zhao et al., 2014). The abundance of total FAs and higher-plant-derived long-chain FAs at depths of 50–70 cm was significantly lower than that in the first 30 cm of QTP soils (P<0.05; Figure 3). This phenomenon is thought to be mainly caused by a substantially lower input of higher-plant-derived SOM in deeper soil profiles compared with the upper layer because 90% of the total root biomass occurred in the top 30 cm of alpine grassland soils in the QTP (Yang et al., 2009). By comparison, the concentration of short-chain FAs remained constant in the soil profile (Figure 3), which may be attributed to *in situ* microbial production, which remains active in the soil profiles.

3.3 Sources and degradation of FAs in QTP soils

The average chain length (ACL) and the carbon preference index (CPI) of FAs can be used to assess the degradation stages of SOM at the molecular level (Gleixner et al., 2001). The ACL value typically increases with increasing degradation due to a decrease of easily degradable short-chain FAs and a selective enrichment of long-chain FAs with increasing degradation, while the CPI value decreases with ongoing degradation (Kolattukudy et al., 1976; Wiesenberg et al., 2010). The ACL values were 21.8±0.4, 22.6±0.3, 22.1±0.4 and 21.5±0.6 at 0–10, 10–20, 20–30 and 50–70 cm depths, respectively, showing no clear trends with the soil depth. While the CPI values decreased with the soil depth, with the highest value at 0–10 cm (7.1±0.5), intermediate values at 10–20 cm (6.5±0.4) and 20–30 cm (6.3±0.7), and the lowest value at 50–70 cm depths (5.1±0.5) (Table 2). This trend may be attributed to the progressive degradation of plant-derived organic matter with increasing soil depths.

The distributions of FAs in QTP soil extracts (Figure 2) are consistent with the dominant input of higher-plant-derived organic matter to soil FAs, with microbial-derived organic matter as a minor contributor. To further quantify the carbon sources based on FA profiles, a PCA-MLR model was performed for all soil samples. Four principal components (PC1, PC2, PC3 and PC4) were identified after varimax

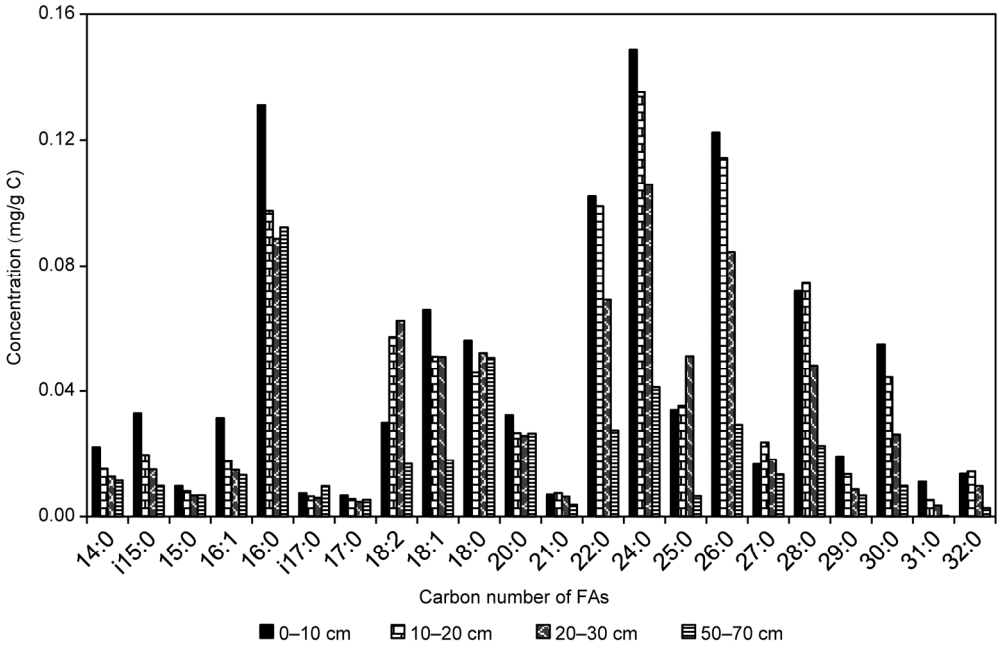

Figure 2 Abundance of fatty acids (FAs) in QTP soils from different depth. Fatty acids are given as carbon numbers followed by the number of double bonds after the colon.

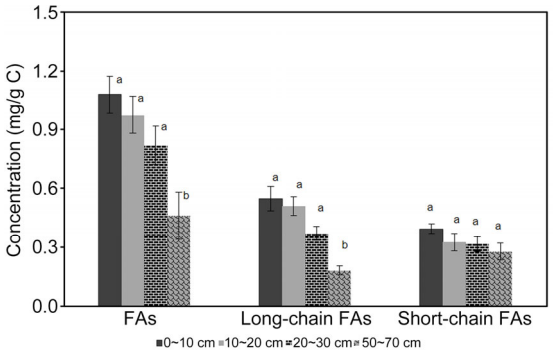

Figure 3 The distribution of total fatty acids (FAs), long-chain FAs and short-chain FAs with soil depth in QTP soils. Different letters (a, b) above each column bar indicate significant difference (P<0.05).

rotation; these components accounted for 32.4%, 23.0%, 15.6% and 13.9% of the total variance, respectively (Table 3). The first rotated component (PC1) was closely associated with long-chain FAs (Table 3) and hence indicative of high plant wax input. The second rotated component (PC2) was characterized by high loadings of saturated short-chain FAs (Table 3). Because short-chain FAs are synthesized by both plants and microorganisms (Otto and Simpson, 2005, and references therein), this indicates a mixed source. The profile in the third rotated component (PC3) had a high loading of branched FAs (*iso*-C_{15} and *iso*-C_{17}) (Table 3), which is mainly biosynthesized by bacteria and fungi, indicating microbial input. Finally, PC4 was only correlated with unsaturated C_{18} FAs ($C_{18:1}$ and $C_{18:2}$). Because fresh plant and microbes are enriched in $C_{18:1}$ and $C_{18:2}$ (Wiesenberg et al., 2010), PC4 represents fresh C sources.

MLR analysis of elements in the factor scores matrix (t_k) against the normal standard deviation of the SumFAs values ($\hat{Z}$) was performed on the PCA scores to determine the mass apportionment of the four components in all soil samples. The resulting equation was as follows:

$$\hat{Z}_{\mathrm{SumFAs}} = 0.393t_1 + 0.204t_2 + 0.176t_3 + 0.249t_4 \ (R^2 = 0.996,\ P < 0.001), \quad (4)$$

where t_1 is defined as higher-plant-derived FAs, t_2 is a mixed source FAs from both plant and microorganisms, t_3 is microorganism-derived FAs and t_4 is fresh C source FAs. Then, the percentage contributions calculated by eq. (2) were 38.4% for t_1, 20.0% for t_2, 17.2% for t_3 and 24.4% for t_4. Overall, approximately 40% of the FAs in QTP soils were from higher plants.

The contribution of each source k to the SumFAs can be calculated by eq. (3). In this study, $\mathrm{mean}_{\mathrm{SumFAs}}$ is 3.0 mg/g C and σ_{SumFAs} is 2.0 mg/g C. For sampling Sites P818, P819, P839, P858, P860 and P862, the contribution from t_1 was particularly high (42%–52%), suggesting a strong input of higher plants at these sites. At different soil depths, contributions from t_1 (higher-plant input) and t_3 (microbial input) decreased with increasing soil depth mainly due to the lower input of plant- and microbial-derived organic matter in the deeper soil layers (Yang et al., 2009). Contributions from t_2 (a mixed source from both higher-plant and microbial inputs) and t_4 (fresh material input) were highest at 50–70 and 20–30 cm depths, respectively. This may be partly explained by the leaching of short-chain FAs in the soil profile, which can be present in solutions, colloids and

Table 3 Varimax-rotated component matrix following principal component analysis (PCA) of all samples[a)]

FA	Rotated component number			
	1	2	3	4
9:0	0.037	**0.863**	–0.119	0.078
10:0	0.498	0.616	0.416	–0.098
12:0	0.140	**0.773**	0.219	0.223
14:0	0.315	0.**752**	0.253	0.024
i15:0	0.243	0.083	**0.859**	0.052
i15:0	0.263	0.356	**0.780**	0.365
15:0	0.310	0.625	0.532	0.318
16:1	0.318	0.277	0.687	0.190
16:1	–0.157	**0.757**	0.361	0.153
16:0	0.435	**0.778**	0.337	0.233
i17:0	0.138	0.491	**0.816**	0.043
17:0	0.464	**0.774**	0.331	0.081
18:2	0.060	0.078	0.130	**0.969**
18:1	0.140	0.165	0.196	**0.913**
18:1	0.246	0.177	0.548	**0.782**
18:0	0.379	**0.786**	0.097	0.378
20:0	**0.722**	0.637	–0.016	0.041
21:0	**0.837**	0.311	0.170	0.212
22:0	**0.796**	0.231	0.307	0.347
24:0	**0.916**	0.136	0.229	0.146
25:0	0.590	0.139	0.010	0.133
26:0	**0.871**	0.051	0.313	0.197
27:0	**0.734**	0.362	–0.002	–0.148
28:0	**0.861**	0.217	0.275	0.258
29:0	**0.785**	0.252	0.217	-0.201
30:0	**0.859**	0.177	0.167	0.219
31:0	**0.846**	0.008	0.273	–0.167
32:0	**0.717**	0.066	0.375	0.461
Variance explained	32.4%	23.0%	15.6%	13.9%

a) Fatty acids (FAs) are given as the carbon number followed by the number of double bonds after the colon. Bold values denote a PCA loading higher than 0.7. i indicates the branched FAs.

micelles in the soil (Piccolo et al., 1996; Nierop and Buurman, 1998; Feng and Simpson, 2007).

3.4 Relationship of environmental parameters with FAs in QTP soils

A number of studies have demonstrated that temperature, precipitation and soil properties are the most important factors that govern SOM composition and distribution (Feng and Simpson, 2008; Baumann et al., 2009; Schmidt et al., 2011; Pisani et al., 2014; Toriyama et al., 2015). In this study, the effects of MAT, MAP, soil pH and soil texture on the preservation of FAs in QTP soils were examined. As shown in Table 4, the total concentration of FAs was negatively correlated with MAT (r=–0.301, P=0.027; Table 4), indicating that the abundance of FAs decreased with increasing MAT due to increased biodegradation of FAs in warmer climates. As for MAP, the abundance of fresh C source FAs (t_4) increased significantly with MAP (r=0.270, P=0.049; Table 4), indicating that high precipitation facilitates the accumulation of fresh FAs in QTP soils; similar results have also been reported by Pisani et al. (2014). This is probably caused by increased plant productivity and/or increased preservation of fresh FAs at higher MAP. This explanation is supported by the recent findings on the QTP, where soil moisture plays an important role in soil C storage (Baumann et al., 2009; Dörfer et al., 2013).

Soil pH has a profound effect on SOM preservation at the bulk (formation of humic horizon) and molecular levels (e.g., FAs; Bull et al., 2000). As shown in Table 4, the abundance of FAs, except for those derived from mixed sources of both higher-plant and microbial inputs (t_2), is negatively correlated with soil pH, suggesting that the preservation of FAs was favored in low-pH soils. Bull et al. (2000) and Nierop et al. (2005) both observed a similar relationship between the FA concentration and soil pH in grassland and oak forest soils, respectively. They believe that apart from direct input from vegetation, the most likely source for FAs is oxidation of other lipids, such as *n*-alkanes and *n*-alkanols in acidic soils (Moucawi et al., 1981; Amblès et al., 1994). Additionally, soils with a low pH appear to preserve biomolecules by retarding the activities of micro-organisms (Bull et al., 2000; Nierop et al., 2005). By comparison, short-chain FAs derived from mixed sources of both plants and microorganisms (t_2) increased with the soil sand content (r=0.300, P=0.027; Table 4). However, because sand has little sorption capacity, this relationship may not be causal. Overall, the significantly negative correlations of the total concentration of FAs with MAT (P<0.05) and soil pH (P<0.01) indicate that the preservation of FAs is favored in low-MAT and low-pH soils on the QTP. This result is consistent with previous studies (Bull et al., 2000; Nierop et al., 2005; Pisani et al., 2014). Recently, it has been suggested that the molecular structure alone does not control SOM stability and that this process should be viewed as an ecosystem property that is controlled by several environmental factors, such as the presence of reactive mineral surfaces, climate, water availability, soil acidity, soil redox state and soil microbial community (Schmidt et al., 2011). Therefore, other environmental parameters, such as the soil mineral content, microbial community composition as well as litter quality and quantity, may also exert a strong control on the preservation and degradation of FAs in QTP soils, but are not included in the analysis here. Thus, further research is necessary to understand the mechanisms

Table 4 Pearson correlation coefficient between the abundance of various fatty acids (FAs) and climatic parameters and soil properties[a)]

Environmental parameters	Pearson parameters	t_1	t_2	t_3	t_4	SumFAs
MAT	*r*	–0.190	–0.130	–0.245	–0.080	**–0.301**
	P	0.168	0.350	0.074	0.566	0.027
MAP	*r*	0.134	–0.019	0.051	**0.270**	0.232
	P	0.335	0.890	0.714	0.049	0.092
Clay	*r*	0.016	**–0.387**	–0.028	–0.026	–0.159
	P	0.910	0.004	0.840	0.850	0.251
Silt	*r*	–0.065	**–0.277**	–0.192	–0.086	–0.254
	P	0.638	0.042	0.165	0.536	0.064
Sand	*r*	0.055	**0.300**	0.172	0.079	0.246
	P	0.694	0.027	0.214	0.569	0.073
pH	*r*	**–0.620**	0.136	**–0.343**	**–0.272**	**–0.644**
	P	0.000	0.329	0.011	0.047	0.000

a) Bold values denote significant correlations ($P \leqslant 0.05$, n=54).

responsible for the distribution of FAs in QTP soils.

4. Conclusions

This study investigated the composition, source and distribution of FAs in the soil profile of 17 sampling sites from the QTP. In general, plant-derived even-numbered long-chain FAs ($C_{22–30}$) dominate in all soil samples. Source apportionment showed that approximately 40% of the FAs in QTP soils were from higher plants. The great abundance of unsaturated C_{18} FAs, mainly derived from fresh C sources, together with high C/N_a ratios, indicate a low to moderate level of SOM degradation in QTP soils. The abundance of FAs decreased with soil depth, and there was a significant decrease at depths of 50–70 cm for total FAs and long-chain FAs. This phenomenon may be mainly caused by a substantially lower input of higher-plant-derived FAs in deeper soils compared with the upper layers. By comparison, short-chain FAs remained constant with soil depth. The total concentration of FAs was negatively correlated to MAT (P<0.05) and soil pH (P<0.01), suggesting that the preservation of FAs was favored in low-MAT and low-pH soils on the QTP. Further research is necessary to understand the mechanisms responsible for the preservation and distribution of FAs in QTP soils.

Acknowledgements *This study was supported by the Chinese National Key Development Program for Basic Research (Grant Nos. 2014CB954003 & 2015CB954201), the National Natural Science Foundation of China (Grant Nos. 31370491 & 41503073), National 1000 Young Talents Program and the "Strategic Priority Research Program- Climate Change: Carbon Budget and Relevant Issues" of the Chinese Academy of Sciences (Grant No. XDA05050404).*

References

Amblès A, Jambu P, Parlanti E, Joffre J, Riffe C. 1994. Incorporation of natural monoacids from plant residues into an hydromorphic forest podzol. Eur J Soil Sci, 45: 175–182

Baumann F, He J S, Schmidt K, Kühn P, Scholten T. 2009. Pedogenesis, permafrost, and soil moisture as controlling factors for soil nitrogen and carbon contents across the Tibetan Plateau. Glob Change Biol, 15: 3001–3017

Bull I D, Nott C J, van Bergen P F, Poulton P R, Evershed R P. 2000. Organic geochemical studies of soils from the Rothamsted classical experiments-VI. The occurrence and source of organic acids in an experimental grassland soil. Soil Biol Biochem, 32: 1367–1376

Camacho-Ibar V F, Aveytua-Alcázar L, Carriquiry J D. 2003. Fatty acid reactivities in sediment cores from the northern Gulf of California. Org Geochem, 34: 425–439

Chen H, Zhu Q, Peng C, Wu N, Wang Y, Fang X, Gao Y, Zhu D, Yang G, Yian J, Kang X, Piao S, Ouyang H, Xiang W, Luo Z, Jiang H, Song X, Zhang Y, Yu G, Zhao X, Gong P, Yao T, Wu J. 2013. The impacts of climate change and human activities on biogeochemical cycles on the Qinghai-Tibetan Plateau. Glob Change Biol, 19: 2940–2955

Ding S, Xu Y, Wang Y, He Y, Hou J, Chen L, He J S. 2015. Distribution of branched glycerol dialkyl glycerol tetraethers in surface soils of the Qinghai-Tibetan Plateau: Implications of brGDGTs-based proxies in cold and dry regions. Biogeosciences, 12: 3141–3151

Dörfer C, Kuehhn P, Baumann F, He J S, Scholten T. 2013. Soil organic carbon pools and stocks in permafrost-affected soils on the Tibetan Plateau. PLoS One, 8: e57024

Feng X J, Simpson M J. 2007. The distribution and degradation of biomarkers in Alberta grassland soil profiles. Org Geochem, 38: 1558– 1570

Feng X J, Simpson M J. 2008. Temperature responses of individual soil organic matter components. J Geophys Res, 113: G03036

Feng X J, Vonk J E, van Dongen B E, Gustafsson Ö, Semiletov I P, Dudarev O V, Wang Z H, Montluçon D B, Wacker L, Eglinton T I. 2013. Differential mobilization of terrestrial carbon pools in Eurasian Arctic river basins. Proc Natl Acad Sci USA, 110: 14168–14173

Gleixner G, Czimczik C I, Kramer C, Lühker B, Schmidt M W I. 2001. Plant compounds and their turnover and stabilization as soil organic matter. In: Schulze E D, Heimann M, Harrison S, Holland E, Lloyd J, Prentice I C, Schimel D, eds. Global Biogeochemical Cycles in the Climate System. San Diego: Academic Press. 201–215

He J S, Wang Z H, Wang X P, Schmid B, Zuo W Y, Zhou M, Zheng C Y, Wang M F, Fang J Y. 2006. A test of generality of leaf trait relationships on the Tibetan Plateau. New Phytologist, 170: 835–848

Hu J F, Zhang H B, Peng P A. 2006. Fatty acid composition of surface sediments in the subtropical Pearl River estuary and adjacent shelf, Southern China. Estuar Coast Shelf Sci, 66: 346–356

Huang X, Zeng Z G, Chen S, Yin X B, Wang X Y, Ma Y, Yang B J, Rong K B, Shu Y C, Jiang T. 2015. Abundance and distribution of fatty acids

in sediments of the South Mid-Atlantic Ridge. J Ocean Univ China, 14: 277–283
Jandl G, Leinweber P, Schulten H R, Ekschmitt K. 2005. Contribution of primary organic matter to the fatty acid pool in agricultural soils. Soil Biol Biochem, 37: 1033–1041
Kögel-Knabner I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol Biochem, 34: 139–162
Kolattukudy P E, Croteau R, Buckner J S. 1976. Biochemistry of plant waxes. In: Kolattukudy P E, ed. Chemistry and Biochemistry of Natural Waxes. Amsterdam: Elsevier. 290–348
Lin X, Zhang Z, Wang S, Hu Y, Xu G, Luo C, Chang X, Duan J, Lin Q, Xu B, Wang Y, Zhao X, Xie Z. 2011 Response of ecosystem respiration to warming and grazing during the growing seasons in the alpine meadow on the Tibetan plateau. Agric For Meteorol, 151: 792–802
Morgunova I P, Ivanov V N, Litvinenko I V, Petrova V I, Stepanova T V, Cherkashev G A. 2012. Geochemistry of organic matter in bottom sediments of the Ashadze hydrothermal field. Oceanology, 52: 345–353
Moucawi J, Fustec E, Jambu P. 1981. Decomposition of lipids in soils: Free and esterified fatty acids, alcohols and ketones. Soil Biol Biochem, 13: 461–468
Naafs D F W, van Bergen P F, Boogert S J, de Leeuw J W. 2004. Solvent-extractable lipids in an acid andic forest soil; variations with depth and season. Soil Biol Biochem, 36: 297–308
Nierop K G J, Buurman P. 1998. Composition of soil organic matter and its water-soluble fraction under young vegetation drift sand, central Netherlands. Eur J Soil Sci, 49: 605–615
Nierop K G J, Naffs D F W, van Bergen P F. 2005. Origin, occurrence and fate of extractable lipids in Dutch coastal dune soils along a pH gradient. Org Geochem, 36: 555–566
Otto A, Simpson M J. 2005. Degradation and preservation of vascular plant-derived biomarkers in grassland and forest soils from western Canada. Biogeochemistry, 74: 377–409
Perry G J, Volkman J K, Johns R B, Bavor H J. 1979. Fatty-acids of bacterial origin in contemporary marine-sediments. Geochim Cosmochim Acta, 43: 1715–1725
Piccolo A, Nardi S, Concheri G. 1996. Micelle-like conformation of humic substances as revealed by size exclusion chromatography. Chemosphere, 33: 595–602
Pisani O, Hills K M, Courtier-Murias D, Haddix M L, Paul E A, Conant R T, Simpson A J, Arhonditsis G B, Simpson M J. 2014. Accumulation of aliphatic compounds in soil with increasing mean annual temperature. Org Geochem, 76: 118–127
Schmidt M W I, Torn M S, Abiven S, Dittmar T, Guggenberger G, Janssens I A, Kleber M, Kögel-Knabner I, Lehmann J, Manning D A C, Nannipieri P, Rasse D P, Weiner S, Trumbore S E. 2011. Persistence of soil organic matter as an ecosystem property. Nature, 478: 49–56
Shi Y, Baumann F, Ma Y L, Song C, Kuehn P, Scholten T, He J S. 2012. Organic and inorganic carbon in the topsoil of the Mongolian and Tibetan grasslands: Pattern, control and implications. Biogeosciences, 9: 2287–2299
Sun D Y, Tan W B, Pei Y D, Zhou L P, Wang H, Yang H, Xu Y P. 2011. Late Quaternary environmental change of Yellow River Basin: An organic geochemical record in Bohai Sea (North China). Org Geochem, 42: 575–585
Toriyama J, Hak M, Imaya A, Hirai K, Kiyono Y. 2015. Effects of forest type and environmental factors on the soil organic carbon pool and its density fractions in a seasonally dry tropical forest. Forest Ecol Manag, 335: 147–155
Wiesenberg G L B, Dorodnikov M, Kuzyakov Y. 2010. Source determination of lipids in bulk soil and soil density fractions after four years of wheat cropping. Geoderma, 156: 267–277
Yang Y H, Fang J Y, Tang Y H, He J S, Ji C J, Zheng C Y, Zhu B. 2008. Storage, patterns and controls of soil organic carbon in the Tibetan grasslands. Glob Change Biol, 14: 1592–1599
Yang Y H, Fang J Y, Ji C J, Han W X. 2009. Above- and belowground biomass allocation in Tibetan grasslands. J Veg Sci, 20: 177–184
Zhao L, Wu W, Xu X, Xu Y. 2014. Soil organic matter dynamics under different land use in grasslands in Inner Mongolia (northern China). Biogeosciences, 11: 5103–5113