



Vertical variations in plant- and microbial-derived carbon components in grassland soils

Tian Ma · Guohua Dai · Shanshan Zhu · Dima Chen · Litong Chen · Xiaotao Lü · Xiaobo Wang · Juntao Zhu · Yangjian Zhang · Jin-Sheng He · Yongfei Bai · Xingguo Han · Xiaojuan Feng

Received: 9 July 2019 / Accepted: 11 November 2019 / Published online: 23 November 2019
© Springer Nature Switzerland AG 2019

Abstract

Aims Owing to different soil environments and organic carbon sources, soil organic carbon (SOC) composition and preservation mechanisms may vary substantially in deep versus surface soils. This study examines vertical variation of various SOC components (including lignin phenols, cutin, suberin and microbial lipids) across the Chinese-Mongolian grasslands.

Methods Lignin phenols and hydrolysable lipids were isolated by cupric oxide oxidation and alkaline hydrolysis, respectively. A comprehensive list of environmental variables was compiled to disentangle influencing

factors for the variation of various components at four different depths.

Results Lignin phenols, suberin and microbial lipids were most abundant in the surface soil (0–10 cm). Ratios of cutin to suberin and microbial lipids to lignin phenols (in the alpine grasslands) were higher in the bottom-most soil. Calcium (Ca) rather than iron (Fe) plays an important role in the preservation of cutin and microbial lipids in the bottom soil with a high Ca:Fe ratio and high pH. The incorporation efficiency of suberin was higher in deeper than surface soils and showed an increasing trend with depth in the alpine (but not temperate) grasslands.

Responsible Editor: Manuel Delgado-Baqueriz.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11104-019-04371-9>) contains supplementary material, which is available to authorized users.

T. Ma · G. Dai · S. Zhu · D. Chen · Y. Bai · X. Han · X. Feng (✉)
State Key Laboratory of Vegetation and Environmental Change,
Institute of Botany, Chinese Academy of Sciences, Beijing 100093,
China
e-mail: xfeng@ibcas.ac.cn

T. Ma · S. Zhu · Y. Zhang · Y. Bai · X. Han · X. Feng
College of Resources and Environment, University of Chinese
Academy of Sciences, Beijing 100049, China

T. Ma · J.-S. He
State Key Laboratory of Grassland and Agro-ecosystems, College
of Pastoral Agriculture Science and Technology, Lanzhou
University, Lanzhou 730020, China

L. Chen
Key Laboratory of Adaptation and Evolution of Plateau Biota,
Northwest Institute of Plateau Biology, Chinese Academy of
Sciences, Xining 810008, China

X. Lü · X. Wang
Institute of Applied Ecology, Chinese Academy of Sciences,
Shenyang 110016, China

J. Zhu · Y. Zhang
Key Laboratory of Ecosystem Network Observation and Modeling,
Institute of Geographic Sciences and Natural Resources Research,
Chinese Academy of Sciences, Beijing 100101, China

J.-S. He
Department of Ecology, College of Urban and Environmental
Sciences, Peking University, Beijing 100871, China

Conclusions Compared to surface soils, the incorporation of root-derived carbon into SOC is more efficient in deeper soils due to stronger mineral protection and weaker microbial decomposition. Root-derived carbon is more important for SOC accrual in the alpine than temperate grasslands, especially at depth. These findings reveal differential distribution patterns and preservation mechanisms for SOC components in the surface versus deep soils, providing new information to understand SOC stability at different depths.

Keywords Deep soils · Distribution patterns · Preservation mechanisms · Soil organic carbon · Chinese-Mongolian grasslands

Introduction

Soil stores 1500 Gt of organic carbon with varied composition and stability in the first meter (Balesdent et al. 2018; Rumpel and Kögel-Knabner 2011). Small variations in soil organic carbon (SOC) stocks have important impact on atmospheric CO₂ levels. Elucidating SOC compositional characteristics and stabilization mechanisms is pivotal for accurately predicting and mediating soil carbon stock changes in the future. Previous studies have extensively investigated SOC composition in relation to environmental variations and soil carbon accumulation in surface soils (e.g., Amelung et al. 1999; Baumann et al. 2009; Dai et al. 2018; Doetterl et al. 2015; Ma et al. 2019). By comparison, deep soils residing >20 cm below ground contain more than half of global SOC stocks (Rumpel et al. 2012). However, the composition and preservation of SOC components in deeper horizons are under-investigated.

Compared with surface soils, deep soils are characterized by lower organic carbon concentrations (Hobley et al. 2018; Rumpel et al. 2012), reduced fresh plant inputs (Chabbi et al. 2009), lower microbial biomass and activities (Fierer et al. 2003), decreased substrate (and oxygen) availability (Wordell-Dietrich et al. 2017) and longer turnover time of SOC (Balesdent et al. 2018; Rumpel and Kögel-Knabner 2011). Hence, it is expected that SOC exhibits different composition and stability in the deep compared to surface soil. For instance, the abundance of plant-derived lignin components often decreases relative to SOC with soil depth in forests and grasslands due to lower inputs and increased

residence time (and hence decomposition) at depth (Feng and Simpson 2007; Spielvogel et al. 2008). Microbial-derived lipids (e.g., hydrolysable lipids and phospholipid fatty acids) are also reported to decrease with depth in shrub-, cropland and forest soils, likely due to decreased microbial activity and biomass production at depth (Cai et al. 2017; Hsiao et al. 2018; Vancampenhout et al. 2012). However, some other studies reported that deeper layers in forest soils contained more microbially processed or derived components (such as amino sugars and microbial neutral sugars) likely due to prolonged in-situ transformation processes and/or downward accumulation of microbial byproducts (Kögel-Knabner and Amelung 2014; Liang and Balser 2008; Spielvogel et al. 2008). As microbial residues are considered to be relatively stable and contribute to SOC accrual on the long term (Liang and Balser 2008; Ma et al. 2018), quite a few papers have recently reported microbial alteration of organic matter in the forest subsoil at a site level (Angst et al. 2018; Heinze et al. 2018; Heitkotter and Marschner 2018). However, large-scale distribution patterns and preservation mechanisms of microbial-derived SOC components still remains unknown, especially in grasslands. Thus, it is essential to assess their distribution in deeper soils to better understand deep soil carbon stability and its underlying mechanisms. On the other hand, plant root components are considered to be particularly important for deep soil carbon accumulation (Rasse et al. 2005; Hicks Pries et al. 2018). Yet it remains to be investigated whether the vertical distribution and preservation of root-derived component exhibit different patterns from microbial and other plant-derived components such as lignin.

The preservation of SOC may be regulated by differential mechanisms in the sub- versus surface soils (Baumann et al. 2009; Feng and Simpson 2007; Rumpel et al. 2012). In surface soils, SOC contents as well as the abundance of SOC components are strongly affected by contemporary climate and plant variables (e.g., root mass) linked to microbial decomposition and plant inputs (Dai et al. 2018; Hicks Pries et al. 2018; Zhao et al. 2014). By comparison, for deep soils that are often carbon-unsaturated and receive low plant inputs (Rumpel et al. 2012), mineral protection plays a particularly important role in the distribution of SOC components (Fierer et al. 2003; Muneer and Oades 1989; Peinemann et al. 2005; Rumpel et al. 2012). Mineral protection of SOC may be operated via organic matter association with fine-sized particles such as clay (Ye

et al. 2017), iron (Fe) and aluminum (Al) oxides (Eusterhues et al. 2003; Kleber et al. 2005; Moni et al. 2010) and/or via calcium (Ca) bridging (Rowley et al. 2018, and references therein). Given that soil minerals and cation contents vary with depth (Kleber et al. 2005; Rumpel et al. 2012), it is expected that the above mineral-associated mechanisms exert different influences on SOC preservation in the surface versus deep soils. However, such a postulation needs to be tested in natural soil profiles to better understand factors regulating the composition and distribution of SOC at different depths.

Here we utilize two well-characterized grassland transects across China and Mongolia and compare the distribution and preservation of different SOC components in subsurface layers relative to the surface soil. Our previous studies based on the same grassland transects have shown a higher abundance as well as better preservation of plant-derived SOC components (including plant waxes, cutin- and suberin-derived hydrolysable lipids and lignin phenols) in the surface soils of the alpine relative to temperate grasslands (Dai et al. 2018; Ma et al. 2019; Zhu et al. 2019). In addition, different mechanisms are shown to govern the distribution of plant wax lipids, with climate-mediated decomposition processes dominating in the temperate grasslands and plant biomass being more important in the alpine region (Dai et al. 2018). It remains to be investigated whether the vertical distribution patterns of SOC components differ across the Chinese-Mongolian grassland transects. In the present study, we use biomarkers to represent SOC components of varied origins and stabilities, including microbial-derived hydrolysable lipids (referred to as “microbial lipids” hereafter; Cai et al. 2017), cutin and suberin monomers primarily derived from roots along these transects (Ma et al. 2019) and lignin phenols. In particular, following our previous approach (Ma et al. 2019), we calculate root mass normalized incorporation efficiency of suberin to examine the preservation of root-derived components along soil profiles. Coupled with soil mineral analysis and other environmental data, factors controlling the distribution and preservation of SOC components are examined in subsurface layers and compared with the surface soil. Our working hypotheses are: (1) the relative abundance and distribution of SOC components are different in sub- versus surface soils such that microbial lipids and suberin may increase relative to lignin phenols; (2) Ca and clay exert a stronger effect on the preservation of SOC components in the sub- relative to surface soils

due to increasing alkalinity and decreasing particle size at depth; (3) the incorporation efficiency of suberin increases with depth due to better preservation of root-derived components in the carbon-unsaturated and clay-rich deep soils.

Materials and methods

Study area

Two grassland-dominated transects across the alpine and temperate regions of China and Mongolia are included in this study (Supplementary Fig. S1). The alpine transect spans >3200 km with mean annual temperatures (MATs) of -0.5 ± 0.4 °C (mean \pm standard error), mean summer temperatures of 8.4 ± 0.4 °C and a mean annual precipitation (MAP) of 147–446 mm (WorldClim database; <http://www.worldclim.org>). The aridity index, calculated as the ratio of precipitation to potential evapotranspiration, ranges from 0.19 to 0.56 (<http://www.cgiar-csi.org>). The dominant vegetation types include alpine meadow (dominated by *Kobresia pygmaea*, *Kobresia humilis*, and *Kobresia tibetica*), alpine steppe (dominated by *Stipa purpurea*, *Carex ianceolata* and *S. subsessiliflora*) and alpine desert steppe (dominated by *Oxytropis ochrocephala*, etc.). Soil types include Leptosols, Cambisols and Phaeozems according to the IUSS Working Group World Reference Base (WRB) for Soil Resources (IUSS Working Group WRB 2015). The temperate transect spans >4700 km with MATs of 0.6 ± 0.3 °C, mean summer temperatures of 17.9 ± 0.9 °C and MAP of 38–436 mm (aridity index of 0.17–0.57). The dominant vegetation types include temperate meadow steppe (dominated by *S. baicalensis*, *Leymus chinensis* and *C. pediformis*), temperate typical steppe (dominated by *S. grandis*, *L. chinensis* and *Artemisia sacrorum*) and temperate desert steppe (dominated by *S. klemenzi*, *Agropyron desertorum* and *Cleistogenes squarrosa*). Soil types include Arenosols, Kastanozems and Chernozems (IUSS Working Group WRB 2015). Detailed descriptions of the study area can be found in our previous study (Ma et al. 2019).

Field sampling and bulk measurements

Soil samples were collected in the summer (July to August) of 2011–2012 in a collaborative effort to

quantify soil carbon distribution across China and Mongolia. A total of 48 sites with minimal anthropogenic disturbances and grazing activity were selected for soil sampling and biomass survey along the alpine (30 sites) and temperate (18 sites) transects. At each site (100 m × 100 m), five quadrats (1 m × 1 m) were randomly selected with distance of >20 m between each other. Soils were collected by taking three soil cores down to 70 cm (for alpine grasslands) or 80 cm (for temperate grasslands) in each quadrat and divided into five depths (0–10, 10–20, 20–30, 30–50 and 50–70 cm for alpine grasslands; 0–10, 10–20, 20–40, 40–60, and 60–80 cm for temperate grasslands). The three cores were mixed in situ for the same depth as one composite sample for each quadrat. After homogenization and removal of stones and visible roots, soils were passed through a 2-mm sieve and air-dried for subsequent chemical analyses. Given the labor- and time-consuming nature of biomarker analysis, the sub-bottom layer (i.e., 30–40 cm in the alpine and 40–50 cm in the temperate grasslands) with similar soil properties as the bottom layer (Yang et al. 2010) was not chosen in our study. The four selected sampling depths were defined as follows for convenience: surface (0–10 cm), subsurface (10–20 cm), subsoil (20–30 or 20–40 cm), and bottom soil (50–70 or 60–80 cm). Soil properties and biomarker concentrations in the surface soils have been reported previously in Ma et al. (2019) and Zhu et al. (2019). Samples from all other depths were analyzed for the first time in this study. Due to varied depths of soils, not all depths were sampled at all sites.

Belowground biomass (BGB) was separated from dead roots in the temperate grasslands based on root color and resilience. Dead roots were not removed from the alpine samples due to difficulties in distinguishing living roots among the massive volume of dead ones. BGB (for temperate grasslands) and total root mass (for alpine grasslands) were determined after drying plant tissues at 65 °C to a constant weight. To compare root mass normalized incorporation efficiency of suberin into soils, ratios of living-to-total root mass were calculated for different temperate grasslands based on an additional field survey conducted using similar methods along the temperate grassland transect (33 sites in Inner Mongolia; Ma et al. 2019). The average living-to-total root mass ratio was 0.74 for temperate meadow steppe, 0.50 for temperate typical steppe and 0.45 for temperate desert steppe. Soil pH, texture, bulk density, SOC,

nitrogen (N), dithionite-extractable Fe, Al and Ca were measured using methods reported in our previous study (Dai et al. 2018). Dithionite-extractable Fe and Al represent reactive Fe and Al extracted from soils using the citrate-bicarbonate-dithionite method according to Mehra and Jackson (1960). Dithionite-extractable Fe and Al include both poorly crystalline and some crystalline Fe and Al oxides (such as ferrihydrite and varying proportions of hematite and goethite; Mehra and Jackson 1960).

Biomarker analysis

Cutin, suberin and microbial lipids were extracted from dried soil samples using alkaline hydrolysis after first removing solvent-extractable compounds according to Otto and Simpson (2006). Briefly, dried solvent-extracted residues (2–5 g soil containing ~50 mg organic carbon, OC) were heated at 100 °C for 3 h in Teflon-lined bombs with 20 mL of 1 M potassium hydroxide in a methanol:water solution (4:1, v:v). Soil residues were subsequently sonicated twice with 30 mL dichloromethane:methanol (1:1; v:v) while the extracts were combined, spiked with a recovery standard (C₁₉ n-alkanoic acid) and acidified to pH < 2 with 12 M hydrochloric acid (HCl). Compounds were recovered by liquid-liquid extraction with dichloromethane three times. The extracts were dried under nitrogen gas (N₂) and then methylated with methanol:HCl (95:5; v:v) at 70 °C for 14 h. The methylated products were extracted with hexane:dichloromethane (4:1; v:v) three times and concentrated under N₂ for subsequent quantification.

Lignin phenols were subsequently isolated from the dried soil residues using copper oxide (CuO) oxidation method (Feng et al. 2016; Hedges and Mann 1979). About 1–4 g soil residues (containing ~40 mg OC) was mixed with 1 g CuO, 100 mg ammonium iron (II) sulfate hexahydrate [Fe(NH₄)₂(SO₄)₂·6H₂O] and 20 mL of 2 M N₂-purged sodium hydroxide solution in Teflon-lined bombs. All bombs were flushed with N₂ in the headspace for 10 min and heated at 150 °C for 2.5 h. The oxidation products were spiked with a recovery standard (ethyl vanillin), acidified to pH 1 with 6 M HCl, and kept in the dark for 1 h. After centrifugation, oxidation products were liquid-liquid extracted from the clear supernatant with ethyl acetate three times and concentrated under N₂ for further analysis.

Small aliquots (30–50%) of the methylated hydrolysis products and lignin oxidation products were derivatized

with 50 μL N,O-bis-(trimethylsilyl)trifluoroacetamide and 10 μL pyridine for 3 h at 70 °C. Compounds were identified and quantified on a Trace GC 1310 gas chromatograph coupled to an ISQ mass spectrometer (GC-MS, Thermo Fisher Scientific, USA) using a DB-5MS column (30 m \times 0.25 mm i.d., film thickness, 0.25 μm). The oven temperature was held at 65 °C for 2 min, increased to 115 °C at a rate of 6 °C min^{-1} and then to 300 °C at a rate of 4 °C min^{-1} with a final isothermal hold at 300 °C for 15 min. Helium was used as carrier gas (1.2 mL min^{-1}). The mass spectrometer was operated in the electron impact mode at 70 eV ionization energy and scanned from 50 to 650 Da (Cai et al. 2017). Compounds were identified by comparing the mass spectra with authentic standards and MS libraries. Quantification was achieved by comparison with the recovery standards. For hydrolysable lipids, the response ratio was assumed to be 1 for different lipid classes. For lignin phenols, selected ions typical of target compounds were monitored after accounting for response variations using external phenol standards. Based on previous assessment in our laboratory, repeated extraction of the same sample yields the reproducible amounts of the above-mentioned biomarkers with errors <10% of the average concentration.

Biomarker related parameters

As cutin and suberin-specific biomarkers may vary among plant species (Angst et al. 2016; Mueller et al. 2012), we define cutin and suberin biomarkers according to our previous study based on the analysis of root and shoot tissues for the dominant plants in the same transects (Ma et al. 2019). Cutin-specific biomarkers include C_{14} dihydroxyalkanoic acid and C_{16} hydroxyalkanoic acids, C_{15} dihydroxyalkanoic acid and C_{16} hydroxyalkanoic acids for the alpine and temperate grasslands, respectively; suberin-specific biomarkers include C_{20} – C_{30} ω -hydroxyalkanoic acids, C_{20} – C_{30} α , ω -dioic acids and C_{18} 9,10-epoxy-dioic acid for both regions. Biomarkers present in cutin or suberin (cutin/suberin) including C_{15} hydroxyalkanoic acids, C_{16} mono- and dihydroxyalkanoic acids and dioic acid, 9- or 10-hydroxytetradecenoic acid, 7- or 8-hydroxyhexadecane-1,16-dioic acid, α ,16-dihydroxyhexadecanoic acid, and α -chloro- α ,18-hydroxyoctadecanoic acid. In addition, microbial lipids are represented by C_{14} – C_{19} branched alkanolic acids and short-chain (C_{10} – C_{18}) β -hydroxyalkanoic acids (Zelles 1999; Cai et al. 2017). Based on our previous study (Ma et al. 2019),

approximately 79–100% ($97.5 \pm 0.33\%$; $n = 102$) of the suberin-specific markers are supplied by roots to the surface soils due to the high volume of roots in the studied grasslands. We expect even lower contributions of suberin from aboveground litter to the deeper layers. Thus, we assume that all suberin markers are derived from roots. Root mass normalized abundance of suberin-specific biomarkers in soils was used to assess the incorporation efficiency of suberin according to Ma et al. (2019):

Incorporation efficiency of suberin

$$= \frac{M_s \times \rho \times h}{\text{RM}} \times 100\% \quad (1)$$

where M_s is the concentrations of suberin biomarkers in the soil ($\mu\text{g g}^{-1}$ soil); RM is the amount of root mass (g m^{-2}) in the surface soil; ρ is soil bulk density (g cm^{-3}); h is soil thickness. Assuming that suberin is transferred from roots into the soil within the same timeframe as root decomposes, the incorporation efficiency reflects root mass normalized abundance of suberin in the soil.

CuO oxidation releases eight characteristic lignin phenols (Feng et al. 2016; Hedges and Mann 1979), including vanillyl (V; vanillin, acetovanillone, vanillic acid), syringyl (S; syringaldehyde, acetosyringone, syringic acid), and cinnamyl (C; *p*-coumaric acid, ferulic acid) monomers. The SOC-normalized concentration of these monomers is used to indicate the relative abundance of lignin. In addition, CuO oxidation releases *p*-hydroxyl phenols (including *p*-hydroxybenzaldehyde, *p*-hydroxyacetophenone, and *p*-hydroxybenzoic acid) and 3,5-dihydroxybenzoic acid from soils that are commonly considered to be transformation products of proteins, “tannin-like” compounds or lignin (Feng et al. 2016). However, as these non-methoxylated phenols are shown to occur in a high abundance in the roots of typical alpine grass species on the Qinghai-Tibetan Plateau (Zhu et al. 2019), we are refrained from using them to indicate lignin transformation in our study. In addition, contrary to the previous study (e.g., Crow et al. 2009), C and S phenols had higher abundances relative to V phenols in the belowground (rather than aboveground) tissues of some alpine and temperate species, resulting in higher C/V and S/V ratios in the belowground tissues (Zhu et al. 2019). Hence, these ratios cannot be used to differentiate root versus aboveground litter in our study. For the same token, the acid-to-aldehyde (Ad/Al) ratios of V and S phenols show higher values in the roots of some grass species than the others

in our study region (Zhu et al. 2019), complicating the interpretation for lignin oxidation stage (Feng and Simpson 2007; Otto and Simpson 2006). Hence, these parameters are not compared in this study. Instead, ratios of suberin:lignin phenols and microbial lipids:lignin phenols are calculated to investigate the distribution and stability of suberin and microbial lipids relative to lignin in the soil.

Statistics analyses

Differences in the biomarker concentrations and parameters among different soil depths in the corresponding area were examined using two-way ANOVA with soil depth and sample sites as fixed factors, followed by post-hoc analysis (Duncan test; IBM SPSS Statistics 20.0, Chicago, IL). Differences in the biomarker concentrations and parameters between alpine and temperate grasslands were examined using Mann-Whitney U-test. All data (except soil pH) were transformed (square-root transformation for cutin and suberin, ln transformation for the other parameters) to achieve normal distribution (confirmed by Shapiro-Wilk test). Pearson's correlation was used to assess relationships of environmental variables with biomarker concentrations and the incorporation efficiency of suberin. Site-mean values (\pm standard error) were used for sites with spatial replicates. Differences and correlations were considered to be significant at a level of $p < 0.05$. Environmental variables showing significant correlations with the investigated parameter were selected for multiple stepwise regression to further elucidate the most important factors. Among the selected variables, soil N, pH and Al contents were excluded due to their strong correlations with SOC, Ca (except in the bottom layer with $r = 0.11$, $p = 0.65$, $n = 20$) and Fe contents, respectively (i.e., $r > 0.7$).

Results

Soil bulk properties

Over 80% of root mass was located in the surface soil of the alpine grasslands and decreased sharply with soil depth ($p < 0.05$; Fig. 1a). A lower percentage (~60%) of root mass occurred in the surface soil of the temperate grasslands. The alpine grasslands had higher SOC and N contents than the temperate ones for the same soil

layer ($p < 0.05$). Both SOC and N contents decreased with depth ($p < 0.05$; Fig. 1b), resulting in relatively constant SOC:N ratios in both regions ($p > 0.05$; Fig. 1c). The clay content was higher in the alpine than temperate grasslands ($p < 0.05$). Contents of clay were lower in the surface soil compared to other depths in the temperate grasslands ($p > 0.05$; Fig. 1d) but were lower in the bottom compared to other depths in the alpine grasslands ($p < 0.05$). Soil pH and Ca contents exhibited higher values in the bottom soil relative to other depths in both regions ($p < 0.05$; Fig. 1e, f). By comparison, Fe and Al contents showed different vertical changes in the two regions (Fig. 1g, h). The alpine grasslands had higher Fe and Al contents ($p < 0.05$) and similar Ca contents ($p > 0.05$) compared with the temperate grasslands for the same soil layer ($p < 0.05$; raw data listed in Supplementary Table S1).

Depth distribution of biomarkers and related indicators

The absolute abundance of all analyzed biomarkers (per gram soil; Table S2) were closely related to SOC contents. Hence, the SOC-normalized concentrations are used throughout this paper to discuss the relative abundance of different SOC components. Overall, the alpine grasslands had higher abundances of microbial lipids, cutin and suberin in the upper three soil layers compared to the temperate grasslands (except suberin in the subsoil; $p < 0.05$). Both microbial lipids and lignin phenols were relatively more abundant in the surface relative to other soil layer(s) in both regions (Fig. 2a, b). By contrast, cutin had lower abundances in the surface than subsurface and subsoils (for the alpine grasslands) in both regions ($p < 0.05$; Fig. 2c). Suberin had the lowest abundances in the bottom soil of the alpine grasslands ($p < 0.05$) and relatively constant abundances along depths in the temperate grasslands (Fig. 2d).

As a result, the suberin:cutin ratio was higher in the surface than other layers in the temperate grasslands or the bottom soil in the alpine grasslands ($p < 0.05$; Fig. 2e). The ratio of suberin:lignin phenols was relatively constant among depths in both grasslands ($p > 0.05$; Fig. 2f) while the ratio of microbial lipids:lignin phenols tended to increase with depth in the alpine (but not temperate) soils ($p < 0.05$; Fig. 2g). The incorporation efficiency of suberin was the lowest in the surface soil in both regions and displayed an increasing trend with depths in the alpine soils ($p < 0.05$; Fig. 2g, h). Overall, the alpine soils had higher ratio of

suberin:lignin phenols as well as higher incorporation efficiency of suberin than the temperate soils for the same depth ($p < 0.05$). The temperate soils had higher ratios of suberin:cutin in the surface and bottom layers relative to the alpine soils ($p < 0.05$). The ratio of microbial lipids:lignin phenols was comparable between the alpine and temperate soils for the same depth ($p > 0.05$).

Environmental influences

Relationships of biomarker abundances and parameters with environmental variables were first examined by linear correlation analyses for varied soil layers. Cutin and suberin abundances were both positively correlated with SOC and Fe contents except in the bottom soil and

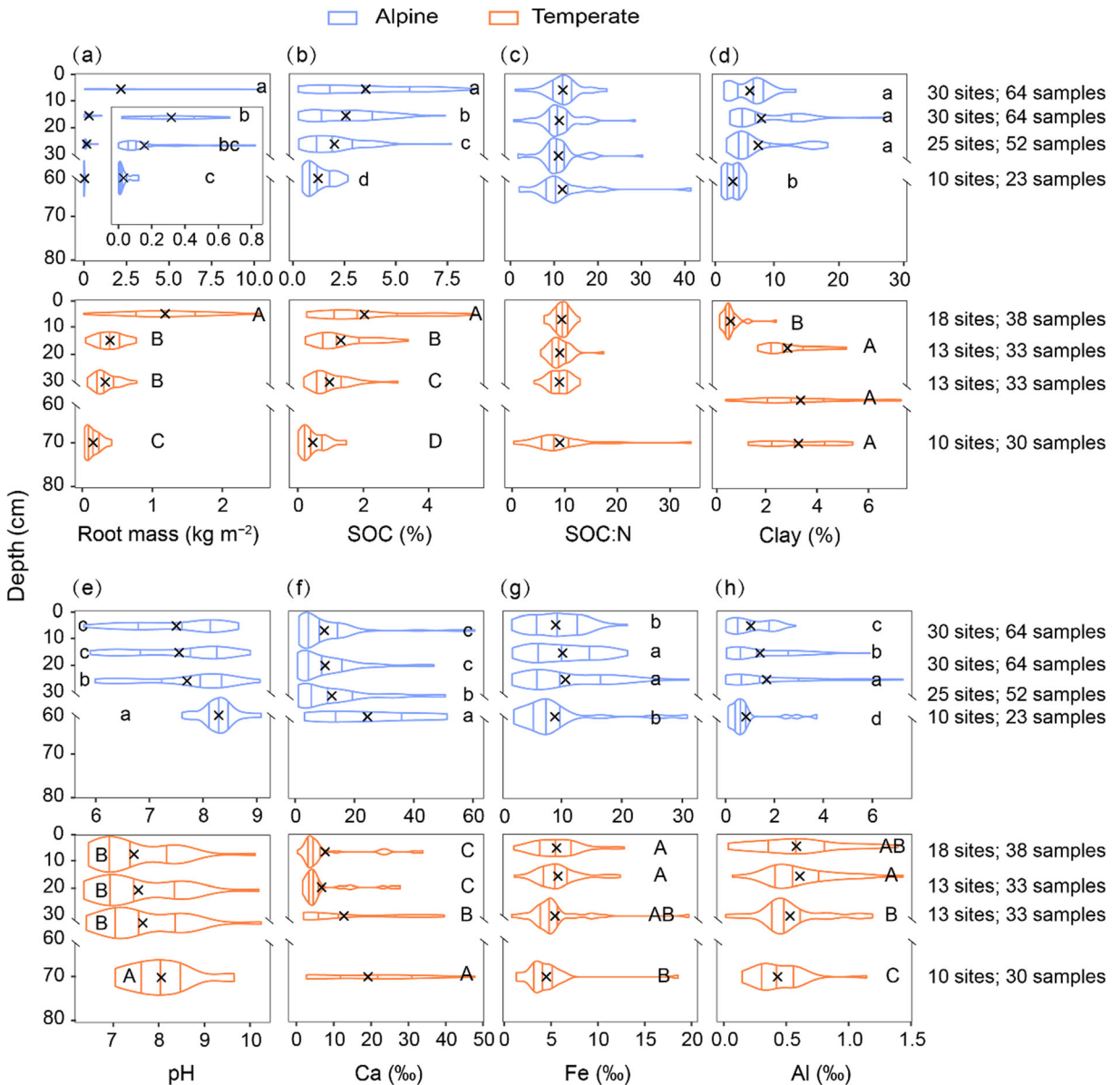


Fig. 1 Root mass and soil properties along soil profiles of the alpine and temperate grasslands. The cross and solid line in the middle of the violin mark the mean and median of each dataset, respectively. The left and right solid lines in the violin denote the 0.25 and 0.75 percentiles, respectively. Shapes of the violin represent the distribution pattern of the corresponding parameters.

Letters indicate different levels among soil depths based on two-way ANOVA analysis for the alpine and temperate grasslands separately (a–d for the alpine grasslands, A–D for the temperate grasslands; $p < 0.05$). SOC: soil organic carbon; N: nitrogen; Fe, dithionite-extractable iron; Al, dithionite-extractable aluminum; Ca, dithionite-extractable calcium

in the subsoil for suberin-SOC relationships ($p < 0.05$; Table 1). Clay contents were positively correlated with cutin and suberin in the surface soil and with cutin in the bottom soil as well ($p < 0.05$). In addition, Ca contents were positively correlated with cutin abundances in the bottom soil ($p < 0.05$). By contrast, lignin phenol

abundances were negatively correlated with SOC contents at all depths ($p < 0.05$). Lignin phenols were also negatively correlated with aridity index in the surface soil and with Fe contents in the bottom soil ($p < 0.05$). Microbial lipids were negatively correlated with Fe in the surface and subsurface soil and positively correlated

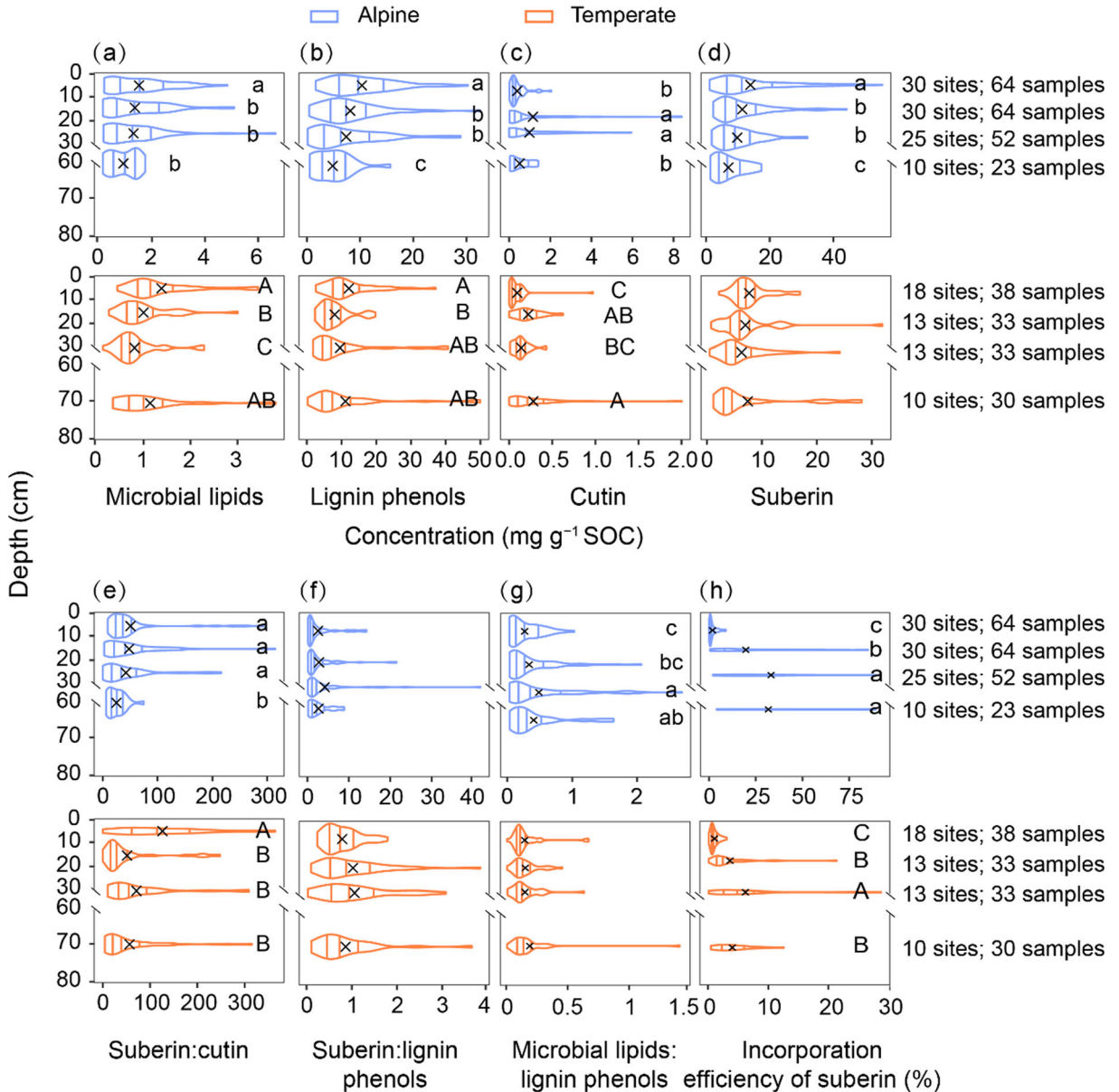


Fig. 2 Soil organic carbon (SOC)-normalized concentrations of biomarkers and parameters along soil profiles of the alpine and temperate grasslands. The cross and solid lines in the middle of the violin mark the mean and median of each dataset, respectively. The left and right solid lines in the violin denote the 0.25 and 0.75 percentiles, respectively. Shapes of the violin represent the

distribution pattern of the corresponding parameters. Letters indicate different levels among soil depths based on two-way ANOVA analysis for the alpine and temperate grasslands separately (a–d for the alpine grasslands, A–D for the temperate grasslands; $p < 0.05$)

with Ca contents in the subsurface and bottom soils ($p < 0.05$).

The suberin:cutin ratio was negatively correlated with Fe and clay contents in surface soils, with SOC contents in the subsurface and subsoil and with Ca contents in the bottom soils ($p < 0.05$; Table 1). By comparison, the ratio of suberin:lignin phenols was

positively correlated with SOC and Fe contents at all depths ($p < 0.05$), with aridity index and root mass in the surface soil, and with clay contents at all depths except the subsoil ($p < 0.05$). The ratio of microbial lipids:lignin phenols was positively correlated with aridity index in the surface soil, with SOC contents in the subsoil and with Ca contents in the bottom soils

Table 1 Correlation coefficient of Pearson's correlations for the soil organic carbon (SOC)-normalized concentrations of biomarkers and the incorporation efficiency of suberin with environmental variables at different depths ($p < 0.05$)

Parameter	Soil layer	n	ln Aridity index	ln Root mass (kg m ⁻²)	ln SOC (%)	ln Fe (%)	ln Ca (%)	ln Clay (%)
ln Microbial lipids (mg g ⁻¹ SOC)	Surface	48	ns	ns	ns	-0.37	ns	ns
	Subsurface	43	ns	ns	ns	-0.38	0.41	ns
	Subsoil	38	na	ns	ns	ns	ns	ns
	Bottom	20	na	ns	ns	ns	0.60	ns
ln Lignin phenols (mg g ⁻¹ SOC)	Surface	48	-0.50	ns	-0.53	ns	ns	ns
	Subsurface	43	ns	ns	-0.44	ns	ns	ns
	Subsoil	38	na	ns	-0.60	ns	ns	ns
	Bottom	20	na	ns	-0.59	-0.46	ns	ns
sqrt Cutin (mg g ⁻¹ SOC)	Surface	48	ns	ns	0.39	0.30	ns	0.54
	Subsurface	43	ns	ns	0.39	0.35	ns	ns
	Subsoil	38	na	ns	0.46	0.44	ns	ns
	Bottom	20	na	ns	ns	ns	0.57	0.63
sqrt Suberin (mg g ⁻¹ SOC)	Surface	48	ns	ns	0.31	0.39	ns	0.43
	Subsurface	43	ns	ns	0.31	0.41	ns	ns
	Subsoil	38	na	ns	ns	0.33	ns	ns
	Bottom	20	na	ns	ns	ns	ns	ns
ln Suberin:cutin	Surface	48	ns	ns	ns	-0.35	ns	-0.45
	Subsurface	43	ns	ns	-0.39	ns	ns	ns
	Subsoil	38	na	na	-0.49	ns	ns	ns
	Bottom	20	na	na	ns	ns	-0.56	ns
ln Suberin:lignin phenols	Surface	48	0.30	0.29	0.50	0.35	ns	0.34
	Subsurface	43	ns	ns	0.53	0.50	ns	0.34
	Subsoil	38	na	ns	0.71	0.50	ns	ns
	Bottom	20	na	ns	0.64	0.50	ns	0.72
ln Microbial lipids: lignin phenols	Surface	48	0.38	ns	ns	ns	ns	ns
	Subsurface	43	ns	ns	ns	ns	ns	ns
	Subsoil	38	ns	ns	0.53	ns	ns	ns
	Bottom	20	ns	ns	ns	ns	0.52	ns
ln Incorporation efficiency of suberin (%)	Surface	48	ns	na	0.43	ns	ns	ns
	Subsurface	43	ns	na	0.56	0.43	ns	0.45
	Subsoil	38	na	na	0.59	0.33	ns	ns
	Bottom	20	na	na	0.59	0.75	ns	ns

All data are transformed (ln or square root); n represents the number of samples in the corresponding soil layer. Bold values denote the most relevant parameters

ns not significant, na not applicable. Abbreviations are defined in Fig. 1

($p < 0.05$). The incorporation efficiency of suberin was positively correlated with SOC contents at all depths, with Fe contents except the surface soil, and with clay contents in the subsurface layer ($p < 0.05$).

Multiple stepwise regression analyses were further conducted to explore the most important environmental factors regulating variations in the above biomarkers and parameters at different depths (Table S3 for the SOC-normalized concentrations; Table S4 for the absolute concentrations which were dominantly influenced by SOC contents). Clay contents best explained the variance of SOC-normalized concentrations of cutin and suberin abundances in the subsurface and subsoils whereas Ca contents best explained cutin distribution in the bottom soil ($p < 0.01$). As a result, clay and Ca contents had a dominant influence on the ratio of suberin:cutin in the surface and bottom soils, respectively ($p < 0.01$). Fe and Ca best explained the variance of microbial lipids in the surface and bottom soils, respectively ($p < 0.01$). SOC contents were the best predictor of lignin phenol abundances at all depths with an additional negative influence by aridity index in the surface soils ($p < 0.01$). SOC contents also best explained the variance in the incorporation efficiency of suberin and the ratio of suberin:lignin phenols at all depths except in the bottom soil ($p < 0.01$), and ratios of suberin:cutin and microbial lipids:lignin phenols in the subsoil ($p < 0.01$). Additionally, aridity index best explained the variation of microbial lipids:lignin phenols in the surface soils ($p < 0.01$), while Ca contents played a more important role in the bottom soil ($p < 0.01$).

Discussion

Vertical distribution of SOC components in grasslands

Consistent with many previous studies (Feng and Simpson 2007; Otto and Simpson 2006; Pisani et al. 2014; Spielvogel et al. 2008), the SOC-normalized abundance of lignin phenols and suberin decreases with soil depth in this study, being highest in the surface soils of both regions (except suberin in the temperate grasslands; Fig. 2). By comparison, the surface soil has the lowest SOC-normalized concentrations of cutin and the highest suberin:cutin ratio compared to other soil layers in both regions, indicating dilution of cutin in the surface soils by other SOC components including

suberin (and lignin). Although roots are a significant contributor of cutin monomers owing to the tremendous root mass in our studied soils (Ma et al. 2019), suberin and lignin phenols are relatively more abundant in root tissues, hence explaining their relative enrichment in the SOC at the soil surface.

The above results also suggest that cutin is better preserved down the soil profile relative to lignin and suberin, possibly owing to the greater persistence of cutin in soils associated with its preferential sorption to minerals (Feng et al. 2005; Kögel-Knabner et al. 2008) as well as its strong interaction with reactive Fe (and/or Al) oxides. Reactive Fe in the soil, including ferrihydrite and, to varying extent, hematite and goethite at pH of ~ 7 (Mehra and Jackson 1960), typically shows strong binding capacities for carboxylic-rich molecules (such as lignin) via ligand exchange in sorption studies (Coward et al. 2018; Lv et al. 2016). However, plant-derived aliphatics are also displayed to be selectively protected by Fe oxides in agricultural soils (Wan et al. 2019) and preferentially retained in the solid phase during reductive release of Fe (Adhikari et al. 2016; Adhikari and Yang 2015). The strong interaction of reactive Fe with cutin (instead of lignin) is evidenced by the strong positive correlation of reactive Fe content with cutin monomers in the upper three layers of our studied soils (Table 1).

Also consistent with some previous studies (Otto and Simpson 2006; Cai et al. 2017; Hsiao et al. 2018), microbial lipids are relatively more abundant in the surface soil relative to other soil layer(s) in the alpine and temperate grasslands (Fig. 2a). This is presumably associated with higher inputs of fresh labile carbon and hence higher microbial activities and production at the surface (Chabbi et al. 2009; Fierer et al. 2003; Wordell-Dietrich et al. 2017). Notably, although abundances of both microbial lipids and lignin phenols decrease with depths in the alpine soils, the ratio of microbial lipids:lignin phenols displays an increasing trend with depths (Fig. 2g). This suggests that microbial lipids are more abundant or better preserved relative to lignin in the deeper alpine soils possibly owing to a stronger association of microbial lipids (relative to lignin) with Ca, reflected by the strong positive correlation of Ca with microbial lipids in the bottom soil (Table 1). In the studied soils with neutral-to-alkaline pHs, Ca^{2+} plays a key role in SOC stabilization via bridging the negatively charged organic matter with clay minerals and reactive Fe with negative surface charges as well (Rowley et al.

2018). This effect may be particularly important for the bottom soil exhibiting the highest pH values and Ca concentrations across the studied transect (Fig. 1e, f). Alternatively, as Ca promotes soil aggregation (Sowers et al. 2018), potentially increasing the occlusion and hence preservation of microbial lipids that tends to be occluded within aggregates (Wiseman and Püttmann 2006).

By comparison, the ratio of microbial lipids:lignin phenols does not vary significantly with depth in the temperate grasslands (Fig. 2g), implying similar degrees of vertical decrease for these SOC components. This result is possibly due to limited protection of microbial lipids in the temperate soils with a lower concentration of reactive minerals as well as opposite down-profile variations in Ca concentrations compared to Fe and Al (Fig. 1f, h). Furthermore, the ratio of suberin:lignin phenols does not show consistent variation along depths in either region (Fig. 2f). Hence, our first hypothesis does not seem to be completely supported by the current data. However, accumulation of non-specific decomposition products may contribute to the concurrent decline of source-specific molecules from both microbes and plants (Cai et al. 2017). Hence, distribution of other microbial component that are less susceptible to degradation (such as amino sugars; Ma et al. 2018) needs to be further assessed to conclude on the relative importance of microbial versus plant carbon in deeper soils.

Environmental influences on the abundance of SOC components along soil profiles

Relationships between the analyzed biomarkers and environmental variables reveal several interesting patterns. First of all, lignin phenol abundances show negative correlations with SOC contents at all depths, in contrast to cutin and suberin displaying positive correlations with SOC in the upper soil layers (Table 1). These patterns confirm our previous observations based only on the surface soil samples (Ma et al. 2019; Zhu et al. 2019) and suggest that lignin phenols may be diluted by other SOC components which are more resistant to degradation (such as cutin; Zhu et al. 2019) or accumulate with SOC accrual (e.g., amino sugars; Ma et al. 2018). It is also notable that cutin is positively correlated with Fe content in the upper three layers, with clay in the surface and bottom layers and with Ca contents in the bottom soil, while lignin phenols show a negative correlation with Fe in the bottom soil

(Table 1). Hence, while the preservation of plant-derived lipids is promoted by reactive Fe oxides and clay in soils (Lin and Simpson 2016; Cai et al. 2017), lignin phenols are diluted by other SOC components that are subject to mineral protective mechanisms. The latter findings fall in line with the depletion of lignin in mineral-associated soil fractions (Kögel-Knabner et al. 2008; Sollins et al. 2009). Alternatively, as both clay and Ca promote soil aggregation (Sowers et al. 2018), potentially increasing the occlusion and hence preservation of organic matter that tends to be occluded within aggregates (Angst et al. 2017; Wiseman and Püttmann 2006). This effect may be stronger or more obvious for cutin than for lignin, contributing to our observed correlations.

In contrast with cutin and suberin, microbial lipids are negatively correlated with Fe contents in the surface and subsurface soils (Table 1). There are two possible explanations for this phenomenon. First, Fe oxides preferentially bind plant-derived lipids with a higher molecular mass compared to microbial-derived, short-chain lipids (Hall et al. 2018; Lv et al. 2016), therefore diluting the latter with increasing contents of reactive Fe in the soil. Second, reactive Fe oxides such as ferrihydrite are known to inhibit microbial activity and growth (Bruun et al. 2010; Saidu et al. 2015) due to toxicity of reactive oxygen species catalyzed by reactive Fe (Kwan and Voelker 2003) and to weaken the stabilization of microbial carbon, especially in neutral to alkaline soils (Ditterich et al. 2016; Pronk et al. 2013). We therefore postulate that reactive Fe may have differential effects on the preservation of plant- versus microbial-derived lipids in the soil, although our current data cannot confirm this postulation.

Moreover, by comparison with the upper soil layers, Ca rather than Fe content is positively correlated with cutin and microbial lipids in the bottom soil (and to a lesser extent in the subsurface soil for microbial lipids; Table 1). Ca content also best explains the abundance variance of these two biomarkers in the bottom soil (Table 1). Extractable Ca in the study area is mainly derived from the weathering of primary and secondary minerals (Likens et al. 1998), exhibiting a relatively high concentration in the neutral-to-alkaline soils, particular in the bottom soil with a higher pH compared to the upper layers (Fig. 1). As Ca is known to play a vital role in the stabilization of SOC via inner- and outer-sphere bridging as well as through promoting soil aggregation (Rowley et al. 2018, and references therein),

its impact on biomarker preservation is stronger in the bottom soil with a higher ratio of Ca:Fe and a higher pH (Czarnes et al. 2000; Rowley et al. 2018), consistent with our second hypothesis. Notably, neither suberin nor lignin phenol abundances are correlated with Ca at any soil depth (Table 1), even though carboxyls and phenols are considered to be subject to Ca bridging (Grünwald et al. 2006; Rowley et al. 2018). Given that the analyzed suberin monomers and lignin phenols are composing units of plant-derived macromolecules, we postulate that the preservation of suberin and lignin macromolecules may be less influenced by Ca bridging relative to the less abundant cutin and microbial lipids in the study soils (Fig. 2), although this postulation needs to be tested by more direct evidence in the future. It is also worth mentioning that clay contents are similar in the bottom three layers of the temperate grasslands and is lowest in the bottom soil of the alpine soil profiles, opposite to the increasing trend of Ca concentrations along depths in both alpine and temperate grasslands (Fig. 1). Hence, the influence of Ca on cutin and microbial lipids is not due to clay effects.

Incorporation efficiency of root carbon components in deep soils

Consistent with our third hypothesis, the incorporation efficiency of suberin is higher in the deeper soils compared with the surface soil (Fig. 2). As the incorporation efficiency of suberin reflects root-mass-normalized abundance of suberin in the soil, it is mainly linked to root carbon preservation rather than root inputs. At least two mechanisms may contribute to this trend. First, microbial activity and decomposition are known to be constrained by labile carbon substrates and/or oxygen availability at depth (Allison et al. 2007; Eilers et al. 2012; Stone et al. 2014), resulting in enhanced preservation of (root) carbon in deep soils. Second, deep soils are unsaturated in carbon compared to the carbon-rich surface soil (Rumpel et al. 2012) and SOC is considered to be better protected by soil minerals at depth (Hobley et al. 2015). This second mechanism is manifested in the stronger correlation (i.e., higher r values) of reactive Fe with the incorporation efficiency of suberin in the bottom than upper soils (Table 1). This observation also implies that, compared to the surface soil where microbial transformation of plant-derived carbon into microbial residues is a key process contributing to SOC accumulation in grasslands (Ma et al. 2018), the

incorporation of plant-derived carbon into SOC is more efficient in deeper soils and may play a more important role in SOC accumulation (Sokol et al. 2019). In addition, given that root tissues of different grasses have varying contents of suberin (Ma et al. 2019) and different species have varying rooting depths (Liu et al. 2018), the incorporation efficiency of suberin may well be affected by root chemistry and hence composition of dominant species at the site. Currently, detailed quantitative data on plant species composition are lacking to prove this idea.

Similar to our previous conclusion based on the surface soils only (Ma et al. 2019), the incorporation efficiency of suberin is positively correlated with and best predicted by SOC contents at all depths (Table 1), suggesting an enhanced preservation of root carbon in SOC-rich soils. It also suggests a coupled accumulation of SOC and suberin throughout soil profiles in the studied grasslands (Hamer et al. 2012; Puget and Drinkwater 2001). The incorporation efficiency of suberin is 2–8 times higher in the alpine than temperate grasslands for the same soil depth (Fig. 2), likely due to cold-constrained decomposition of plant roots in the alpine region. Frequent freeze-thaw processes in the alpine soils may also promote physical fragmentation of plant roots, thereby promoting suberin incorporation into soils. Additionally, the incorporation efficiency shows a strong increasing trend with depth in the alpine (but not temperate) grasslands ($r = 0.59$, $p < 0.05$), suggesting that root-derived carbon is more important for SOC accrual in the alpine than temperate grasslands, especially at depth.

Conclusions

This study compares the vertical distribution and preservation of microbial lipids, lignin phenols, cutin and suberin in soils across the Chinese-Mongolian grasslands. Different from our first hypothesis, the relative abundance of microbial lipids (except in the alpine grasslands) and suberin do not increase compared to lignin phenols along the soil profile. However, the incorporation efficiency of suberin is higher in the deeper soils compared with the surface soil, suggesting better preservation of root carbon mainly due to stronger mineral protection and weaker microbial decomposition processes at depth. Furthermore, due to inhibited decomposition of plant roots at lower temperatures, the

alpine soils have a higher incorporation efficiency of suberin than the temperate counterparts, which shows a strong increasing trend with depth in the alpine soils, suggesting that root-derived carbon is more important for SOC accrual in the alpine than temperate grasslands, especially at depth. Based on regression analysis, the preservation of plant-derived lipids (cutin and suberin) is promoted by reactive Fe and clay-sized particles in soils, while lignin phenols are diluted by other SOC components that are subject to mineral protective mechanisms. Notably, Ca rather than Fe plays an important role in the preservation of cutin and microbial lipids in the bottom soil with a higher Ca:Fe ratio and a higher pH. These findings collectively reveal differential distribution patterns and preservation mechanisms for SOC components in the surface versus deep soils, which is indispensable for better understanding and predicting soil carbon processes at different depths in a changing environment. The sequestration of root-derived carbon in the deep alpine soils deserves more attention, given the wide-spread deepening of grass roots accompanying warming-induced soil moisture decreases. It should also be mentioned that there are other important molecular components of SOC (such as cellulose- and hemicellulose-derived sugars, amino sugars, etc.) that are not analyzed here, whose distribution and behavior along soil profiles also affect the relative abundance of the analyzed biomarkers in SOC and deserve investigation in the future.

Acknowledgements This study was funded by the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDA19050403), the Chinese National Key Development Program for Basic Research (2015CB954201), the National Natural Science Foundation of China (41773067, 31971502) and the CAS Interdisciplinary Innovation Team (JCTD-2018-06). The original data are available from the supplementary information. The authors have no conflict of interest to declare.

References

- Adhikari D, Yang Y (2015) Selective stabilization of aliphatic organic carbon by iron oxide. *Sci Rep* 5:11214
- Adhikari D, Poulson S, Sumaila S, Dynes J, McBeth J, Yang Y (2016) Asynchronous reductive release of iron and organic carbon from hematite–humic acid complexes. *Chem Geol* 430:13–12
- Allison VJ, Yermakov Z, Miller RM, Jastrow JD, Matamala R (2007) Using landscape and depth gradients to decouple the impact of correlated environmental variables on soil microbial community composition. *Soil Biol Biochem* 39:505–516
- Amelung W, Zhang X, Flach KW, Zech W (1999) Amino sugars in native grassland soils along a climosequence in North America. *Soil Sci Soc Am J* 63:86–92
- Angst G, Heinrich L, Kögel-Knabner I, Mueller CW (2016) The fate of cutin and suberin of decaying leaves, needles and roots – inferences from the initial decomposition of bound fatty acids. *Org Geochem* 95:81–92
- Angst G, Mueller K, Kögel-Knabner I, Freeman K, Mueller C (2017) Aggregation controls the stability of lignin and lipids in clay-sized particulate and mineral associated organic matter. *Biogeochemistry* 132:307–324
- Angst G, Messinger J, Greiner M, Häusler W, Hertel D, Kirfel K, Kögel-Knabner I, Leuschner C, Rethemeyer J, Mueller CW (2018) Soil organic carbon stocks in topsoil and subsoil controlled by parent material, carbon input in the rhizosphere, and microbial-derived compounds. *Soil Biol Biochem* 122: 19–30
- Balesdent J, Basile-Doelsch I, Chadoeuf J, Cornu S, Derrien D, Fekiacova Z, Hatté C (2018) Atmosphere–soil carbon transfer as a function of soil depth. *Nature* 559:599–602
- 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 Chang Biol* 15:3001–3017
- Bruun TB, Elberling B, Christensen BT (2010) Lability of soil organic carbon in tropical soils with different clay minerals. *Soil Biol Biochem* 42:888–895
- Cai Y, Tang Z, Xiong G, Xie Z, Liu Z, Feng X (2017) Different composition and distribution patterns of mineral-protected versus hydrolyzable lipids in shrubland soils. *J Geophys Res-Biogeophys* 122:2206–2218
- Chabbi A, Kögel-Knabner I, Rumpel C (2009) Stabilised carbon in subsoil horizons is located in spatially distinct parts of the soil profile. *Soil Biol Biochem* 41:256–261
- Coward E, Ohno T, Plante A (2018) Adsorption and molecular fractionation of dissolved organic matter on iron-bearing mineral matrices of varying crystallinity. *Environ Sci Technol* 52:1036–1044
- Crow S, Lajtha K, Filley T, Swanston C, Bowden R, Caldwell B (2009) Sources of plant-derived carbon and stability of organic matter in soil: implications for global change. *Glob Chang Biol* 15:2003–2019
- Czarnes S, Hallett PD, Bengough AG, Young IM (2000) Root- and microbial-derived mucilages affect soil structure and water transport. *Eur J Soil Sci* 51:435–443
- Dai G, Ma T, Zhu S, Liu Z, Chen D, Bai Y, Chen LT, He J-S, Zhu J, Zhang Y, Lü X, Wang X, Han X, Feng X (2018) Large-scale distribution of molecular components in Chinese grassland soils: the influence of input and decomposition processes. *J Geophys Res-Biogeophys* 123:239–255
- Ditterich F, Poll C, Pronk GJ, Heister K, Chandran A, Rennert T, Kögel-Knabner I, Kandeler E (2016) Succession of soil microbial communities and enzyme activities in artificial soils. *Pedobiologia* 59:93–104
- Doetterl S, Stevens A, Six J, Merckx R, Van Oost K, Casanova Pinto M, Muñoz C, Boudin M, Zagal Venegas E, Boeckx P (2015) Soil carbon storage controlled by interactions between geochemistry and climate. *Nat Geosci* 8:780–783

- Eilers KG, Debenport S, Anderson S, Fierer N (2012) Digging deeper to find unique microbial communities: the strong effect of depth on the structure of bacterial and archaeal communities in soil. *Soil Biol Biochem* 50:58–65
- Eusterhues K, Rumpel C, Kleber M, Kögel-Knabner I (2003) Stabilisation of soil organic matter by interactions with minerals as revealed by mineral dissolution and oxidative degradation. *Org Geochem* 34:1591–1600
- Feng X, Simpson MJ (2007) The distribution and degradation of biomarkers in Alberta grassland soil profiles. *Org Geochem* 38:1558–1570
- Feng X, Simpson AJ, Simpson MJ (2005) Chemical and mineral-organic controls on humic acid sorption to clay mineral surfaces. *Org Geochem* 36:1553–1566
- Feng X, Feakins SJ, Liu Z, Ponton C, Wang RZ, Karkabi E, Galy V, Berelson WM, Nottingham AT, Meir P, West AJ (2016) Source to sink: evolution of lignin composition in the Madre de Dios River system with connection to the Amazon basin and offshore. *J Geophys Res-Biogeosci* 121:1316–1338
- Fierer N, Allen AS, Schimel JP, Holden PA (2003) Controls on microbial CO₂ production: a comparison of surface and subsurface soil horizons. *Glob Chang Biol* 9:1322–1332
- Grünwald G, Kaiser K, Jahn R, Guggenberger G (2006) Organic matter stabilization in young calcareous soils as revealed by density fractionation and analysis of lignin-derived constituents. *Org Geochem* 37:1573–1589
- Hall SJ, Berhe AA, Thompson A (2018) Order from disorder: do soil organic matter composition and turnover co-vary with iron phase crystallinity? *Biogeochemistry* 140:93–110
- Hamer U, Rumpel C, Dignac MF (2012) Cutin and suberin biomarkers as tracers for the turnover of shoot and root derived organic matter along a chronosequence of Ecuadorian pasture soils. *Eur J Soil Sci* 63:808–819
- Hedges JJ, Mann D (1979) The characterization of plant tissues by their lignin oxidation products. *Geochim Cosmochim Acta* 43:1803–1807
- Heinze S, Ludwig B, Piepho HP, Mikutta R, Don A, Wordell-Dietrich P et al (2018) Factors controlling the variability of organic matter in the top- and subsoil of a sandy Dystric Cambisol under beech forest. *Geoderma* 311:37–44
- Heitkötter J, Marschner B (2018) Soil zymography as a powerful tool for exploring hotspots and substrate limitation in undisturbed subsoil. *Soil Biology and Biochemistry* 124:210–217
- Hicks Pries C, Sulman B, West C, O'Neill C, Poppleton E, Porras R, Castanha C, Zhu B, Wiedemeier D, Torn M (2018) Root litter decomposition slows with soil depth. *Soil Biol Biochem* 125:103–114
- Hobley E, Wilson B, Wilkie A, Gray J, Koen T (2015) Drivers of soil organic carbon storage and vertical distribution in eastern Australia. *Plant Soil* 390:111–127
- Hobley E, Steffens M, Bauke SL, Kögel-Knabner I (2018) Hotspots of soil organic carbon storage revealed by laboratory hyperspectral imaging. *Sci Rep* 8:13900
- Hsiao C, Sassenrath G, Zeglin L, Hettiarachchi G, Rice C (2018) Vertical changes of soil microbial properties in claypan soils. *Soil Biol Biochem* 121:154–164
- IUSS Working Group WRB (2015) World reference base for soil resources 2014, update 2015. World soil resources reports, no. 106. FAO, Rome
- Kleber M, Mikutta R, Torn MS, Jahn R (2005) Poorly crystalline mineral phases protect organic matter in acid subsoil horizons. *Eur J Soil Sci* 56:717–725
- Kögel-Knabner I, Amelung W (2014) Dynamics, chemistry, and preservation of organic matter in soils. In: Holland HD, Turekian KK (eds) *Treatise on geochemistry*. Elsevier, Oxford
- Kögel-Knabner I, Guggenberger G, Kleber M, Kandeler E, Kalbitz K, Scheu S, Eusterhues K, Leinweber P (2008) Organo-mineral associations in temperate soils: integrating biology, mineralogy, and organic matter chemistry. *J Plant Nutr Soil Sci* 171:61–82
- Kwan WP, Voelker BM (2003) Rates of hydroxyl radical generation and organic compound oxidation in mineral-catalyzed Fenton-like systems. *Environ Sci Technol* 37:1150–1158
- Liang C, Balsler TC (2008) Preferential sequestration of microbial carbon in subsoils of a glacial-landscape toposequence, Dane County, WI, USA. *Geoderma* 148:113–119
- Likens GE, Driscoll CT, Buso DC, Siccama TG, Johnson CE, Lovett GM, Fahey TJ, Reiners WA, Ryan DF, Martin CW, Bailey SW (1998) The biogeochemistry of calcium at Hubbard brook. *Biogeochemistry* 41:89–173
- Lin LH, Simpson MJ (2016) Enhanced extractability of cutin- and suberin-derived organic matter with demineralization implies physical protection over chemical recalcitrance in soil. *Org Geochem* 97:111–121
- Liu H, Mi Z, Lin L, Wang Y, Zhang Z, Zhang F, Wang H, Liu L, Zhu B, Cao G, Zhao X, Sanders N, Classen A, Reich P, He J-S (2018) Shifting plant species composition in response to climate change stabilizes grassland primary production. *Proc Natl Acad Sci U S A* 115:4051–4056
- Lv J, Zhang S, Wang S, Luo L, Cao D, Christie P (2016) Molecular-scale investigation with ESI-FT-ICR-MS on fractionation of dissolved organic matter induced by adsorption on iron oxyhydroxides. *Environ Sci Technol* 50:2328–2336
- Ma T, Zhu S, Wang Z, Chen D, Dai G, Feng B, Su X, Hu H, Li K, Han W, Liang C, Bai Y, Feng X (2018) Divergent accumulation of microbial necromass and plant lignin components in grassland soils. *Nat Commun* 9:3480
- Ma T, Dai G, Zhu S, Chen D, Chen L, Lü X, Wang X, Zhu J, Zhang Y, Ma W, He J-S, Bai Y, Han X, Feng X (2019) Distribution and preservation of root- and shoot-derived carbon components in soils across the Chinese-Mongolian grasslands. *J Geophys Res-Biogeosci* 124:420–431
- Mehra O, Jackson M (1960) Iron oxide removal from soils and clays by a dithionite-citrate system buffered with sodium bicarbonate. *Clays Clay Miner* 7:317–327
- Moni C, Chabbi A, Nunan A, Rumpel C, Chenu C (2010) Spatial dependence of organic carbon–metal relationships: a multi-scale statistical analysis, from horizon to field. *Geoderma* 158:120–127
- Mueller KE, Polissar PJ, Oleksyn J, Freeman KH (2012) Differentiating temperate tree species and their organs using lipid biomarkers in leaves, roots and soil. *Org Geochem* 52:130–141
- Muneeb M, Oades JM (1989) The role of Ca-organic interactions in soil aggregate stability. II. Field studies with ¹⁴C labelled straw, CaCO₃, and CaSO₄·H₂O. *Aust J Soil Res* 27:401–409

- Otto A, Simpson MJ (2006) Sources and composition of hydrolysable aliphatic lipids and phenols in soils from western Canada. *Org Geochem* 37:385–407
- Peinemann N, Guggenberger G, Zech W (2005) Soil organic matter and its lignin component in surface horizons of salt-affected soils of the Argentinian Pampa. *Catena* 60:113–128
- Pisani O, Hills KM, Courtier-Murias D, Haddix ML, Paul EA, Conant RT, Simpson AJ, Arhonditsis GB, Simpson MJ (2014) Accumulation of aliphatic compounds in soil with increasing mean annual temperature. *Org Geochem* 76:118–127
- Pronk GJ, Heister K, Kögel-Knabner I (2013) Is turnover and development of organic matter controlled by mineral composition? *Soil Biol Biochem* 67:235–244
- Puget P, Drinkwater LE (2001) Short-term dynamics of root- and shoot-derived carbon from a leguminous green manure. *Soil Sci Soc Am J* 65:771–779
- Rasse DP, Rumpel C, Dignac M-F (2005) Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant Soil* 269:341–356
- Rowley MC, Grand S, Verrecchia ÉP (2018) Calcium-mediated stabilisation of soil organic carbon. *Biogeochemistry* 137:27–49
- Rumpel C, Kögel-Knabner I (2011) Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. *Plant Soil* 338:143–158
- Rumpel C, Chabbi A, Marschner B (2012) Carbon storage and sequestration in subsoil horizons: knowledge, gaps and potentials. In: Lal R (ed) *Recarbonization of the biosphere*. Springer, Heidelberg
- Saidy AR, Smernik RJ, Baldock JA, Kaiser K, Sanderman J (2015) Microbial degradation of organic carbon sorbed to phyllosilicate clays with and without hydrous iron oxide coating. *Eur J Soil Sci* 66:83–94
- Sokol NW, Sanderman J, Bradford MA (2019) Pathways of mineral-associated soil organic matter formation: integrating the role of plant carbon source, chemistry, and point of entry. *Glob Chang Biol* 25:12–24
- Sollins P, Kramer MG, Swanston C, Lajtha K, Filley T, Aufdenkampe AK, Wagai R, Bowden RD (2009) Sequential density fractionation across soils of contrasting mineralogy: evidence for both microbial- and mineral-controlled soil organic matter stabilization. *Biogeochemistry* 96:209–231
- Sowers T, Stuckey J, Sparks D (2018) The synergistic effect of calcium on organic carbon sequestration to ferrihydrite. *Geochem Trans* 19:4
- Spielvogel S, Prietzel J, Kögel-Knabner I (2008) Soil organic matter stabilization in acidic forest soils is preferential and soil type-specific. *Eur J Soil Sci* 59:674–692
- Stone MM, DeForest JL, Plante AF (2014) Changes in extracellular enzyme activity and microbial community structure with soil depth at the Luquillo critical zone observatory. *Soil Biol Biochem* 75:237–247
- Vancampenhout K, De Vos B, Wouters K, Swennen R, Buurman P, Deckers J (2012) Organic matter of subsoil horizons under broadleaved forest: highly processed or labile and plant-derived? *Soil Biol Biochem* 50:40–46
- Wan D, Ye T, Lu Y, Chen W, Cai P, Huang Q (2019) Iron oxides selectively stabilize plant-derived polysaccharides and aliphatic compounds in agricultural soils. *Eur J Soil Sci*. <https://doi.org/10.1111/ejss.12827>
- Wiseman C, Püttmann W (2006) Interactions between mineral phases in the preservation of soil organic matter. *Geoderma* 134:109–118
- Wordell-Dietrich P, Don A, Helfrich M (2017) Controlling factors for the stability of subsoil carbon in a Dystric Cambisol. *Geoderma* 304:40–48
- Yang Y, Fang J, Ma W, Smith P, Mohammad A, Wang S, Wang W (2010) Soil carbon stock and its changes in northern China's grasslands from 1980s to 2000s. *Global Change Biology* 16(11):3036–3047
- Ye C, Bai T, Yang Y, Zhang H, Guo H, Li Z, Li H, Hu S (2017) Physical access for residue-mineral interactions controls organic carbon retention in an Oxisol soil. *Sci Rep* 7:6317
- Zelles L (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biol Fertil Soils* 29:111–129
- 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
- Zhu S, Dai G, Ma T, Chen L, Chen D, Lü X, Wang X, Zhu J, Zhang Y, Bai Y, Han X, He J-S, Feng X (2019) Distribution of lignin phenols in comparison with plant-derived lipids in the alpine versus temperate grassland soils. *Plant Soil* 439:325–338

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.