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Accumulation of aliphatic compounds in soil with increasing mean annual temperature

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ABSTRACT

Chemical recalcitrance of biomolecules, physical protection by soil minerals and spatial inaccessibility to decomposer organisms are hypothesized to be primary controls on soil organic matter (SOM) turnover. Previous studies have observed increased sequestration of plant derived aliphatic compounds in experimentally warmed soils but did not identify the mechanisms for this enhanced preservation. To further test the role of environmental conditions in the preservation of aliphatic carbon, we analyzed native soils along a bicontinental, longitudinal, mean annual temperature (MAT) gradient to examine relationships between SOM composition, soil physical properties and temperature. Using biomarker analysis by gas chromatography–mass spectrometry and solid state ^{13}C nuclear magnetic resonance to characterize SOM, we observed that the concentration of aliphatic compounds derived from the waxes and cuticles of plant leaves increased with MAT. We did not observe any significant correlations between aliphatic SOM and clay mineral content which suggests that their inherent chemical recalcitrance is responsible for their persistence in soils. Other SOM components were not correlated with MAT, indicating that temperature alone does not control the overall preservation and biodegradation of soil carbon. As such, other environmental factors (e.g., microbial community structure and activity, litter quality and quantity, mineral surfaces, soil moisture content and antioxidant capacity) also play a role in the selective preservation or accumulation of various SOM components. However, our study shows that the accumulation of aliphatic SOM components is highly correlated to MAT.

1. Introduction

Climate change is predicted to increase the world's average temperature by 0.3–4.8 °C over the next 60–80 years (Collins et al., 2013). Warmer temperatures will likely alter plant species distribution (Walther et al., 2002) and increase vegetation productivity and litterfall (Liu et al., 2004). The response of different SOM components to rising temperature has been examined (Feng and Simpson, 2008; Feng et al., 2008; Haddix et al., 2011), however the mechanisms for stabilization and preservation in soils with a changing climate are still uncertain and further examination is an important step towards elucidating controls on SOM turnover in a changing world (Kirschbaum, 2006; Trumbore and Czimczik, 2008; von Lützow and Kögel-Knabner, 2009). The processes leading to SOM stabilization have been hypothesized to include: (1) selective preservation of chemically recalcitrant compounds, (2) spatial inaccessibility to decomposer organisms, (3) organic matter interactions with minerals, (4) the antioxidant activity of certain compounds and (5) water level fluctuations (Baldock and Skjemstad, 2000; Kögel-Knabner et al., 2008a; Marschner et al., 2008; Abbott et al., 2013; Schlichting et al., 2013). The temperature sensitivity of SOM decomposition can be described by kinetic theory and environmental constraints such as physical and chemical protection of organic matter, drought, flooding and freezing (Davidson and Janssens, 2006; Conant et al., 2011). Recently, it has been suggested that molecular structure alone does not control SOM stability and that this process should be viewed as an ecosystem property 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). Nonetheless, specific SOM components are believed to persist in soils due to their chemical recalcitrance which may be an inherent property of their molecular structure

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(e.g., aliphatic chains in lipids; Lorenz et al., 2007). Aliphatic molecules are abundant in the cuticles of plant leaves (9–25% of leaf carbon) and in the suberin of roots (8–12% of root carbon; Kolattukudy, 1980; Lorenz et al., 2007) and may comprise as much as 40% of the organic carbon in forest soils (Kögel-Knabner et al., 1992). These aliphatic compounds are believed to be relatively stable (Baldock et al., 1992; Riederer et al., 1993) and have been observed to accumulate in various types of soils (Nierop, 1998; von Lützow et al., 2006; Lorenz et al., 2007; Feng et al., 2008). Cuticle derived aliphatic compounds have also been shown to interact with mineral surfaces through selective sorption of polymethylene carbon (Feng et al., 2005), which may provide physical protection against microbial attack (Baldock and Skjemstad, 2000). In addition, cuticle derived compounds have been observed to accumulate with soil warming. For example, Feng et al. (2008) observed that after 14 months of soil warming, the concentration of cutin derived compounds originating from the waxy coatings of leaves, increased significantly, and this was attributed to both increased litter inputs and selective biodegradation of other SOM constituents at elevated temperatures. The mechanism for the stabilization of these aliphatic structures is still unknown. Thus, it is important to study the fate of plant derived aliphatic compounds in soils to better understand SOM turnover patterns at elevated temperatures.

To test the role of temperature as a control of SOM molecular composition, we analyzed soils collected along a mean annual temperature (MAT) gradient (Table 1) using several molecular marker (biomarker) analyses and solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. The bicontinental, longitudinal MAT gradient spans from Indian Head, Saskatchewan to Rondônia, Brazil and represents a MAT range from 2–25.6 °C. We used a set of biomarkers to help elucidate the relationship between temperature and the persistence of SOM compounds of differing stability. The targeted biomarkers include: free lipids (of plant and microbial origin), ester bound lipids (from cutin and suberin biopolymers) and ether bound, lignin derived phenols. These SOM components have different chemical structures and have shown different stabilities and decomposition patterns in response to temperature (Feng and Simpson, 2008; Feng et al., 2008). For example, free lipids including long chain aliphatic compounds and plant steroids, were found to decompose faster at higher temperatures in a soil incubation study (Feng and Simpson, 2008). In addition, suberin is believed to be more resistant to biodegradation than cutin because it has a high content of phenolic units and is embedded in bark and root tissues (Riederer et al., 1993). However, the aliphatic components of suberin have been reported to degrade faster than those derived from cutin in a soil incubation study although no clear degradation trend was observed with increasing incubation temperature (Feng and Simpson, 2008). In fact, cutin derived aliphatic structures have been shown to accumulate in experimentally warmed soils (Feng et al., 2008). Finally, the decomposition of lignin in soils has been reported to increase with increasing temperature (Feng and Simpson, 2008; Feng et al., 2008). The combination of biomarker and NMR techniques has been applied successfully to directly examine the responses of SOM to soil warming (Feng et al., 2008). These techniques are highly complementary and provide detailed information on the source and degradation state of specific compounds (biomarkers) and the overall SOM structure (Simpson et al., 2008).

2. Material and methods

2.1. Soil collection and site characteristics

Soil samples were collected from six locations along a MAT gradient: Indian Head, Saskatchewan; Mandan, North Dakota; Akron, Colorado; Vernon, Texas; Alajuela, Costa Rica; Rondônia, Brazil (Table 1). The MAT ranges from 2 °C in Saskatchewan to 25.6 °C in Brazil. The mean annual precipitation (MAP) ranges from 402 mm in North Dakota to 2700 mm in Costa Rica. The temperate samples were collected from native grassland sites while the samples from Costa Rica and Brazil were collected from native forest sites. Surface litter and aboveground vegetation were cleared away prior to sampling and all samples were collected from 0–20 cm. Three field replicates were collected at each site and combined into one composite sample for laboratory analysis. Because of the low error obtained on the organic carbon analysis of the field replicates (Table S1), these were combined into one composite sample. After sampling, the soils were air dried, passed through a 2 mm sieve, ground with a mortar and pestle and stored at room temperature. Soil pH was measured using a 1:2 ratio of soil to deionized water (Thomas, 1993) with an Accumet® Basic AB15 pH meter (Fisher Scientific).

2.2. Soil organic matter analyses

Sequential chemical extractions (solvent extraction, base hydrolysis and cupric(II) oxide oxidation) were conducted in triplicate on the soil samples to analyze the free lipids, bound lipids and lignin derived phenols, respectively (Otto and Simpson, 2005, 2006a, 2006b). The soils (~15 g) were extracted in triplicate by sonication for 15 min with 30 ml CH_2Cl_2, CH_2Cl_2:CH_3OH (1:1 v:v) and CH_3OH. The combined solvent extracts were filtered through glass fiber filters (Whatman GF/A and GF/F), concentrated by rotary evaporation and dried under a N_2 stream in 2 ml glass vials. The soil residues were subjected to base hydrolysis to yield ester linked lipids (Goñi and Hedges, 1990; Otto and Simpson, 2006a). The air dried soil residues (0.1–2 g) were heated at 100 °C for 3 h in Teflon lined

Table 1
Sample characteristics including geographical location, soil type classification, sand, silt and clay content (%), mean annual temperature (MAT, °C), mean annual precipitation (MAP, mm), dominant vegetation, soil pH, total organic carbon (%) and total nitrogen (%).

Location	Latitude	Longitude	Soil type	Sand (%)	Silt (%)	Clay (%)	MAT (°C)	MAP (mm)	Vegetation	pH	C (%)	N (%)
Indian Head, Saskatchewan	50°53′N	103°52′W	Udic Borroll	28.6	21.1	50.2	2	427	Grassland, mostly cool season grasses	7.40	3.7	0.4
Mandan, North Dakota	46°77′N	100°92′W	Typic Argiboroll	25.5	46.3	28.3	5	402	Warm mixed grass prairie	6.58	3.2	0.3
Akron, Colorado	40°15′N	103°15′W	Aridic Paleustoll	36.1	40.9	23.0	9.2	420	Grassland, mostly C_4 grasses	7.00	1.2	0.1
Vernon, Texas	33°94′N	99°40′W	Typic Paleustoll	17.5	51.6	30.9	17	665	Grassland, mix of C_3 and C_4 grasses	7.47	1.1	0.1
Alajuela, Costa Rica	N/A	N/A	Hydric Melanudand	68.2	23.1	8.7	20	2700	Tropical forest, mostly C_3 species	5.22	20.0	1.7
Rondônia, Brazil	10°17′N	62°82′W	Paleudult & Kandiuldult	59.8	10.0	30.1	25.6	2200	Tropical forest, mostly C_3 species	4.33	1.1	0.1

Table information taken from Haddix et al. (2011) and references therein.

bombs with 20 ml of 1 M methanolic KOH. The extracts were sonicated twice with 15 ml CH_2Cl_2:CH_3OH (1:1 v:v), centrifuged and acidified to pH 1 with 6 M HCl. Solvents were then removed by rotary evaporation and the hydrolysable lipids were recovered from the water phase by liquid–liquid extraction (3×) with 30 ml diethyl ether, dried with Na_2SO_4 to remove any remaining water, concentrated by rotary evaporation and dried under a N_2 stream in 2 ml glass vials. The base hydrolysis residues were air dried and further oxidized with CuO to release lignin derived phenols (Hedges and Mann, 1979; Otto and Simpson, 2006b). Soil residues (0.1–1 g) were extracted with 1 g CuO, 100 mg $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ and 15 ml 2 M NaOH in Teflon lined bombs at 170 °C for 2.5 h. The extracts were acidified to pH 1 with 6 M HCl and kept in the dark for 1 h to prevent polymerization of cinnamic acids. After centrifugation, the supernatants were liquid–liquid extracted (3×) with 30 ml diethyl ether, dried with anhydrous Na_2SO_4, concentrated by rotary evaporation and dried under a N_2 stream in 2 ml glass vials.

2.3. Derivatization and gas chromatography–mass spectrometry

The extracts were re-dissolved in CH_2Cl_2:CH_3OH (1:1 v:v) and aliquots (containing ~1 mg extracts) were derivatized for analysis with gas chromatography–mass spectrometry (GC–MS). Solvent extracts and CuO oxidation products were converted to trimethylsilyl (TMS) derivatives by reaction with 90 μl N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and 10 μl anhydrous pyridine for 1 h at 70 °C. After cooling, hexane was added to dilute the extracts. For fatty acid esterification, base hydrolysis products were first methylated by reacting with 500 μl N,N-dimethylformamide dimethyl acetal (2 milliequivalents/ml in pyridine) at 60 °C for 15 min. After being evaporated to dryness under a N_2 stream, the base hydrolysis extracts were silylated with BSTFA and anhydrous pyridine as previously described. Dodecanoic acid–TMS and tetracosane were used as external standards for the solvent extracts. Tricosanoic acid methyl ester was used as an external standard for base hydrolysis products, while vanillic acid–TMS was used for CuO oxidation products. External standards were used to avoid the co-elution of internal standards with other compounds which are extracted using these techniques. GC–MS analysis was performed on an Agilent 6890N GC coupled to an Agilent 5973 quadrupole mass selective detector. Separation was achieved on a HP-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with helium as the carrier gas (1.2 ml/min). The GC was heated at 65 °C (2 min), increased from 65 °C to 300 °C at a rate of 6 °C/min and held at 300 °C for 20 min. The sample (1 μl) was injected with a 2:1 split ratio using an Agilent 7683 autosampler with the injector temperature set at 280 °C. The mass spectrometer was operated in the electron ionization (EI) mode at 70 eV and scanned from m/z 50–650. Examples of a GC–MS total ion chromatogram (TIC) for a solvent extract, base hydrolysis extract and CuO oxidation extract show the major compounds identified (Supplementary information, Figs. S1, S2 and S3, respectively). Data were acquired and processed with the Chemstation G1701DA software. Individual compounds were identified by comparison of mass spectra with published data, NIST98 and Wiley275 MS library data and authentic standards. The observed differences in the organic carbon content of the soils collected along the MAT gradient (Table 1) is likely due to a combination of environmental factors such as climate, vegetation and soil mineralogy. Because of this variability, the biomarker data was normalized to the soil organic carbon content.

2.4. Solid state ^{13}C nuclear magnetic resonance spectroscopy

Soil samples were repeatedly treated with hydrofluoric acid (HF; 10%) to concentrate the organic matter content and to remove paramagnetic minerals (Schmidt et al., 1997) prior to analysis by solid state ^{13}C cross polarization with magic angle spinning (CP-MAS) NMR spectroscopy. SOM structure is not altered markedly by treatment with HF (Rumpel et al., 2006). The samples were then rinsed with deionized water to remove excess salts and freeze dried. Approximately 100 mg of treated sample was packed into a 4 mm zirconium rotor with a Kel-F cap and the spectra were acquired on a 500 MHz Bruker BioSpin Avance III spectrometer (Bruker BioSpin, Rheinstetten, Germany) equipped with a 4 mm H-X MAS probe and using a ramp-CP pulse program. The spinning rate was set to 13 kHz with a ramp-CP contact time of 1 ms and 1 s recycle delay. The NMR spectra were processed using a zero filling factor of 2 and line broadening of 100 Hz. The spectra were integrated into four chemical shift regions corresponding to alkyl carbon (0–50 ppm) that arises from cutin, suberin, aliphatic side chains and lipids; *O*-alkyl carbon (50–110 ppm) including oxygen and nitrogen substituted aliphatic constituents from carbohydrates, peptides and methoxyl-C found in lignin; aromatic and phenolic carbon (110–165 ppm) from components such as lignin, aromatic amino acids found in peptides and black carbon; and carboxyl and carbonyl carbon (165–215 ppm) from fatty acids and amino acids found in peptides (Baldock et al., 1992; Simpson et al., 2008). Alkyl/*O*-alkyl ratios were calculated by dividing the areas of the alkyl and the *O*-alkyl regions of the spectra (Baldock et al., 1992; Simpson et al., 2008).

2.5. Statistical analyses

A series of simple linear regression models (Netter et al., 1996) were used to examine the relationships between the chemical compounds and each of the potential covariates (MAT, MAP and soil texture data), using Microsoft Excel (Microsoft Office Professional Plus 2010, version 14.0.7113.5005) with the Analysis Toolpak add-in. Regressions were considered significant at the level of $p \leqslant 0.05$. We subsequently compared the concentrations of free and bound aliphatic lipids, lignin derived phenols and solid state ^{13}C NMR spectroscopy integrations related to the mean annual temperature (MAT), mean annual precipitation (MAP) and soil texture of the studied sites using the Bonferroni correction method (Table S2). Multiple regression analysis was also used to examine the relative importance of the different predictor variables. Cluster analysis was also used to delineate the similarities among the six study sites, based on their biomarker values, soil attributes, temperature and precipitation variability. Multiple regression (Table S3) and cluster analyses were performed using Statistica (v. 12; StatSoft) using a HP Pavilion HPE h8–1212c Desktop PC operating 64 bit-Windows version7 (SP3). Our tree clustering exercise used the Euclidean distance, to compute the similarity among the study sites, and the single linkage (or "nearest neighbor") method as the amalgamation rule. The latter method determines the distance between any two clusters based on the location of the closest objects in those two clusters. The final tree plot was created by scaling the individual linkage measure values to a standardized scale (i.e., the maximum distance among the six sites).

3. Results and discussion

Results from biomarker analyses highlight the preferential accumulation of aliphatic structures in SOM (Fig. 1a–d) in soil samples collected along the MAT gradient. The relative abundance of the solvent extractable aliphatic compounds (including *n*-alkanols, *n*-alkanoic acids and *n*-alkanes) in the soils ranged from 42% (Texas) to 73% (Costa Rica) of the total free lipids. The large relative amount of free aliphatic compounds and organic carbon content in the Costa Rica soil (Table 1) may be due to the large amounts of

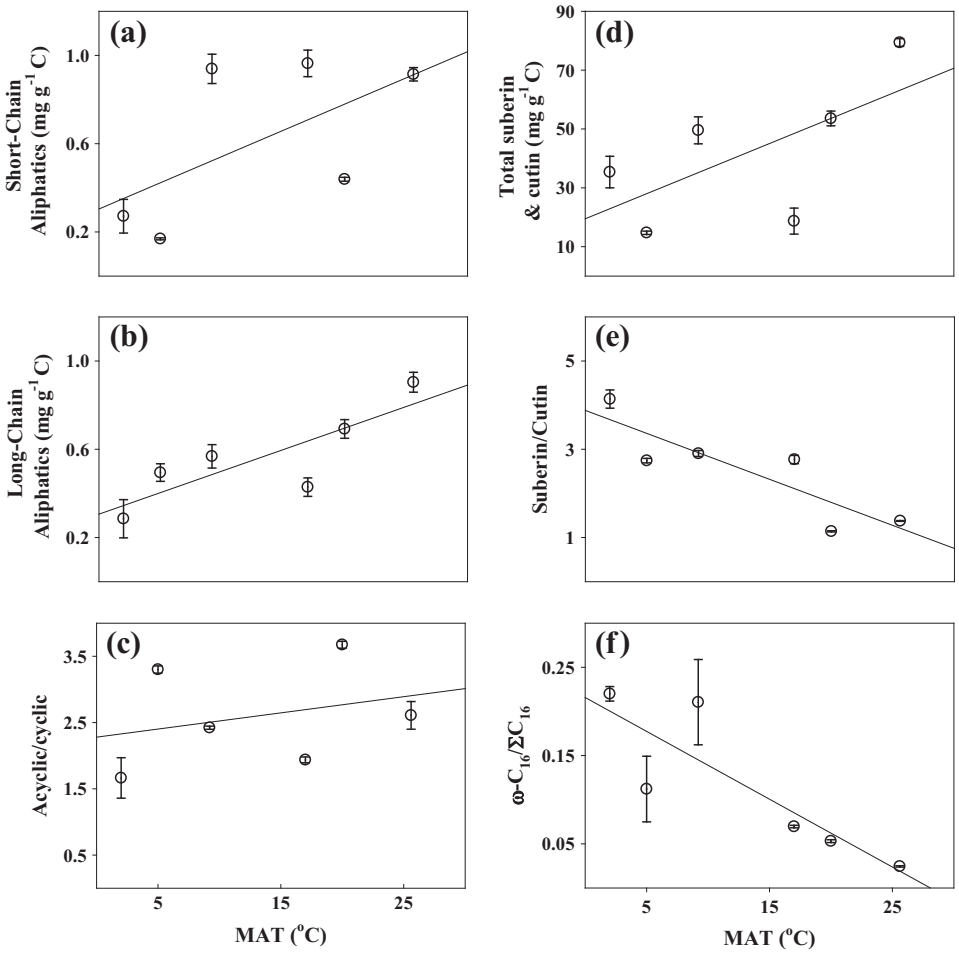

Fig. 1. Relationship between mean annual temperature (MAT) and soil aliphatic components. Correlations between MAT (°C) with (a) short chain free aliphatic lipids (< C_{20}); (b) long chain free aliphatic lipids (⩾C_{20}); (c) acyclic/cyclic aliphatic lipid ratio; (d) total suberin and cutin biomarkers; (e) suberin/cutin ratio; (f) ratio of ω-C_{16} hydroxyalkanoic acid and total C_{16} acids (ω-C_{16}/ΣC_{16}). The total suberin and cutin biomarkers and the suberin/cutin and ω-C_{16}/ΣC_{16} ratios were calculated according to Otto and Simpson (2006a). Error bars depict the analytical error originating from triplicate analyses on composite soil samples (n = 3).

allophane minerals which have been shown to contribute to high SOM concentrations in these types of soils (Boudot et al., 1986; Haddix et al., 2011). The organic carbon normalized concentration of short chain free aliphatic lipids (< C_{20}), which originate from soil microbes (Lichtfouse et al., 1995), increased with temperature (Fig. 1a), but this relationship was not statistically significant. We believe that the lack of a significant correlation between the short chain aliphatic lipids and MAT (Table 2) is an important finding and may be due to the preferential degradation of these compounds compared to their long chain counterparts which are hypothesized to be more recalcitrant (Schulten and Schnitzer, 1990). The concentration of long chain free aliphatic lipids (⩾C_{20}) derived from the epicuticular waxes of plants (Bianchi, 1995), increased significantly with temperature and precipitation (Table 2; Fig. 1b). This trend is likely due to increased plant productivity and litter production in warmer climates and subsequent accumulation of cuticle derived aliphatic compounds because of increased biodegradation of other more labile SOM components (e.g., proteins and carbohydrates). Despite differences in the native vegetation (grassland vs. forest; Table 1), evidence was found for the preferential accumulation of aliphatic structures in the soils collected along the longitudinal MAT gradient.

It has been suggested that warmer temperatures may enable microbes to quickly decompose a larger portion of SOM (Boudot et al., 1986; Haddix et al., 2011) resulting from shifts in the microbial community (Rousk et al., 2012), changes in substrate use (Ågren and Wetterstedt, 2007) or soil priming (Crow et al., 2009a). The accumulation of aliphatic SOM (which likely has a long residence time) in soils at warmer temperatures suggests that they are resistant to biodegradation and are preferentially preserved. Long chain free aliphatic lipids also increased with the soil sand content (Table 2; Fig. 2d). The mineral associated organic matter in acid sandy soils has been reported to be dominated by aliphatic structures (Kögel-Knabner et al., 2008b), and this observed trend in our data set may be related to a lack of substrate constraints for microbial activity resulting in the decomposition of more labile material and the preservation of aliphatic structures (Buurman et al., 2007). Nonetheless, the multivariate regression analysis shows that the squared semi-partial correlation coefficients for MAT and sand are significantly lower than what the simple regressions with one variable at a time suggest (Supporting information, Table S3). Thus, further research is necessary to understand the mechanisms responsible for the preservation of aliphatic structures in soil sand fractions. It has recently been suggested that the persistence of SOM is largely due to complex interactions between organic matter and its environment (e.g., climate, water availability and soil pH; Schmidt et al., 2011). The preferential preservation of aliphatic components has been reported for acidic soils (Bull et al., 2000) and is consistent with our results. The two forest soils have lower pH values compared to the grassland soils (Table 1), which may be enhancing the preservation and accumulation of aliphatic components at these sites. However, despite the

Table 2
Summary of the simple regression analysis performed on the free and bound aliphatic lipids, lignin-derived phenols and solid state ^{13}C NMR spectroscopy integrations with mean annual temperature (MAT), mean annual precipitation (MAP) and soil texture. Values in bold indicate a statistically significant regression model at 5% level of significance.

	R^2 values (slope; intercept)				
	MAT (°C)	MAP (mm)	Sand (%)	Silt (%)	Clay (%)
Free aliphatic lipids					
Short chain (< C_{20})	0.36 (0.30; 0.02)	0.01 (0.57; 0.00004)	0.004 (0.57; 0.001)	0.005 (0.57; 0.001)	0.03 (0.75; −0.005)
Long chain (⩾C_{20})	**0.69** (0.31; 0.02)	**0.61** (0.38; 0.0002)	**0.65** (0.22; 0.009)	0.27 (0.78; −0.007)	0.33 (0.83; −0.009)
Acyclic/cyclic aliphatic lipids	0.08 (2.28; 0.02)	0.32 (2.12; 0.0004)	0.35 (1.71; 0.02)	0.004 (2.70; −0.003)	**0.67** (3.94; −0.05)
Bound aliphatic lipids					
Total suberin + cutin	0.42 (19.50; 1.71)	0.50 (23.16; 0.02)	**0.69** (2.83; 0.99)	**0.68** (80.87; −1.21)	0.06 (54.69; −0.45)
Suberin/cutin	**0.76** (3.88; −0.10)	**0.79** (3.59; −0.0009)	**0.65** (4.24; −0.04)	0.12 (1.76; 0.02)	0.63 (0.65; 0.06)
ω-C_{16}/ΣC_{16}	**0.73** (0.22; −0.008)	0.53 (0.18; −0.00006)	0.23 (0.19; −0.002)	0.04 (0.08; 0.001)	0.24 (0.03; 0.003)
Lignin phenols					
Total lignin	0.10 (10.22; −0.08)	**0.61** (11.19; −0.002)	0.57 (12.63; −0.09)	0.43 (6.12; 0.09)	0.12 (7.45; 0.06)
(Ad/Al)v	0.43 (1.60; −0.02)	0.09 (1.44; −0.00008)	0.08 (1.50; −0.004)	0.04 (1.46; −0.003)	0.47 (0.96; 0.01)
(Ad/Al)s	0.25 (1.02; −0.01)	0.18 (0.97; −0.0001)	0.27 (1.10; −0.006)	0.35 (0.58; 0.009)	0.005 (0.82; 0.001)
Solid state ^{13}C NMR					
Alkyl C	0.13 (28.64; 0.21)	0.003 (31.09; 0.0003)	0.003 (30.84; 0.01)	0.20 (36.06; −0.14)	0.21 (26.16; ; 0.18)
O-alkyl C	0.0002 (41.16; 0.01)	0.25 (37.84; 0.003)	0.37 (33.97; 0.19)	0.15 (46.13; −0.15)	0.18 (47.00; −0.20)
Aromatic C	0.04 (19.10; −0.08)	0.05 (18.98; −0.0008)	0.13 (20.61; −0.06)	0.45 (13.39; 0.15)	0.07 (20.12; −0.07)
Alkyl/*O*-alkyl	0.10 (0.69; 0.007)	0.01 (0.81; −0.00002)	0.03 (0.86; −0.002)	0.02 (0.83; −0.002)	0.19 (0.59; 0.007)

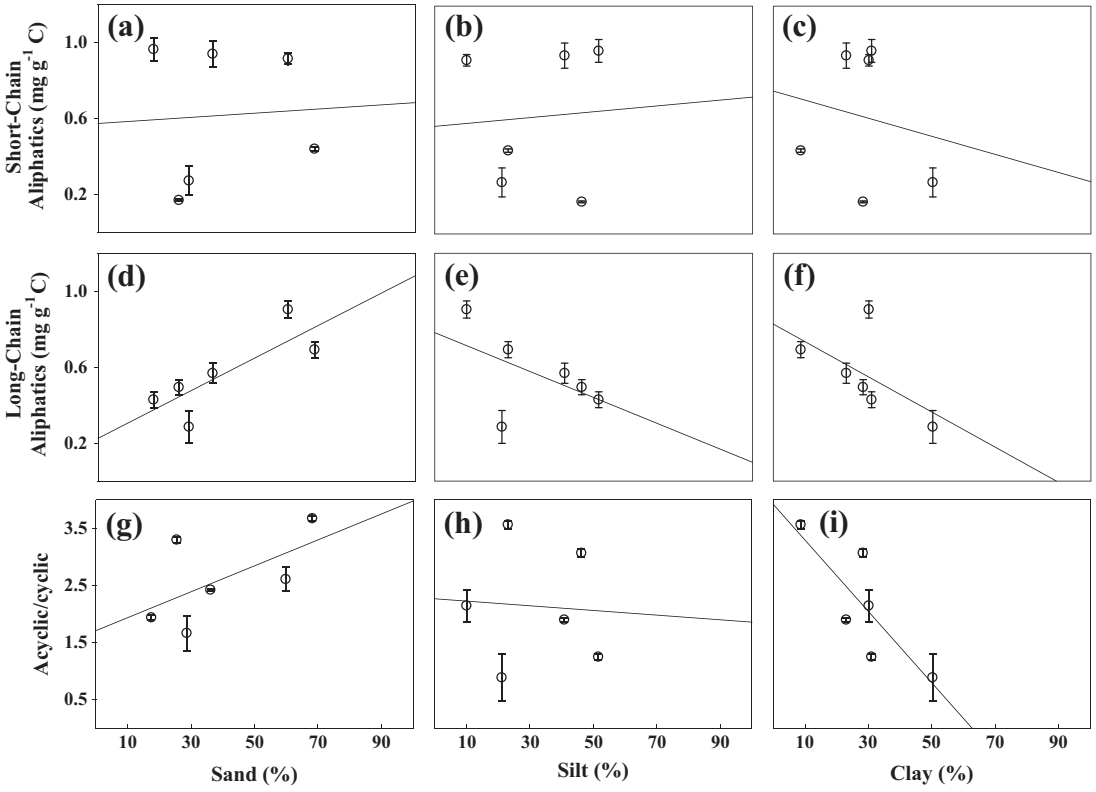

Fig. 2. Relationship between free aliphatic compounds and soil texture. Correlations between short chain free aliphatic lipids (< C_{20}) and soil (a) sand; (b) silt; (c) clay content; long chain free aliphatic lipids (⩾C_{20}) and soil (d) sand; (e) silt; (f) clay content; acyclic/cyclic aliphatic lipid ratio and soil (g) sand; (h) silt; (i) clay content. Error bars depict the analytical error originating from triplicate analyses on composite soil samples (n = 3).

presence of different environmental conditions along the studied transect (e.g., vegetation type, soil moisture, microbial community structure and activity) our results also show an accumulation of aliphatic structures in SOM. The cluster analysis (Fig. 3) shows site similarities based on their biomarker values, soil attributes, temperature and precipitation variability. Based on these characteristics the coldest site (Saskatchewan) was the most similar to another grassland site (Colorado) and less similar to the other sites along the MAT gradient. The greatest difference was observed between the coldest (Saskatchewan) and warmest site (Brazil).

To further test the role of climate in the preservation of aliphatic structures, we examined the degradation state of major lipid classes in soils using the ratio of acyclic aliphatic to cyclic aliphatic lipids (Otto and Simpson, 2005). Acyclic aliphatic lipids, which include *n*-alkanes, *n*-alkanols, and *n*-alkanoic acids, are typically degraded before cyclic aliphatic lipids, such as steroids and terpenoids, which may be better protected by the soil mineral matrix, resulting in lower acyclic/cyclic aliphatic lipid ratios with progressive SOM degradation (Otto and Simpson, 2005). The acyclic/cyclic aliphatic lipid ratio increased with MAT (Fig. 1c) and although this

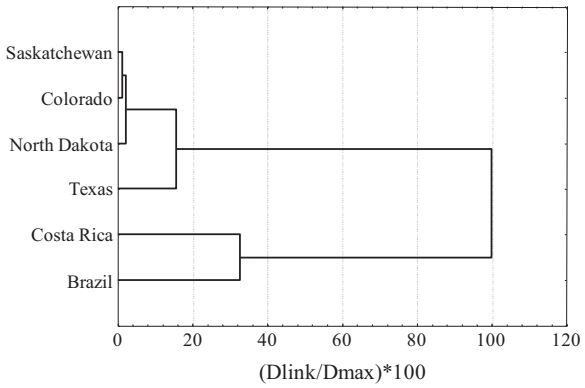

Fig. 3. Cluster analysis showing the grouping of the six study sites based on their biomarker values, soil attributes, temperature and precipitation variability.

relationship was not significant (Table 2) it suggests the accumulation and preservation of acyclic aliphatic components and the relative depletion of cyclic aliphatic compounds which may be more sensitive to higher temperatures. The acyclic/cyclic aliphatic lipid ratio was inversely proportional to the soil clay content (Table 2; Fig. 2i) indicating that cyclic aliphatic lipids may be better protected by the mineral matrix. Once again, the semi-partial correlation coefficients from the multivariate regression analysis for MAT and clay are lower than the simple regressions with one variable (Supporting information, Table S3).

Chemically bound SOM components are more stable than those in the "free" form because they are predominantly linked to soil macromolecules by ester or ether bonds (Riederer et al., 1993; Baldock and Skjemstad, 2000; Feng and Simpson, 2008; Feng et al., 2008). The biomacromolecules suberin and cutin have been recognized as the major sources of bound aliphatic lipids in SOM (Kolattukudy, 1980; Baldock et al., 1992; Kögel-Knabner, 2002; Otto and Simpson, 2006a). The total bound lipids representative of cutin and suberin inputs to SOM (including *n*-alkanols, *n*-alkanoic acids, ω-hydroxyalkanoic acids, α-hydroxyalkanoic acids, *n*-alkane-α,ω-dioic acids, mid-chain hydroxy and epoxy acids) were more abundant compared to the free aliphatic lipids (Fig. 1d). The relative abundance of the bound aliphatic compounds ranged from 46% (Saskatchewan) to 90% (Costa Rica) of the total bound lipids. Again, the high relative abundance of bound aliphatic compounds in the Costa Rican soil may be due to protection by allophane minerals present in this soil (Naafs and van Bergen, 2002; Buurman et al., 2007). The concentration of bound aliphatic compounds increased with MAT (Fig. 1d), although this relationship was not statistically significant (Table 2). An accumulation of cutin derived compounds in experimentally warmed soils has been reported (Feng et al., 2008) and was attributed to an increase in vegetation growth and leaf litter production at higher temperatures. In another study (Crow et al., 2009b), an increase in needle production in an old-growth coniferous forest was suggested to increase the stable soil carbon pool size, comprised mostly of plant derived aliphatic compounds. The total suberin and cutin compounds increased with the soil sand content (Table 2; Fig. 4a), in agreement with the long chain free aliphatic lipid results. This may result from a strong association of aliphatic compounds to sand or from the preferential degradation of more labile SOM components. Although cutin and suberin derived compounds have been reported to accumulate in fine soil fractions (Clemente et al., 2011), these compounds were inversely proportional to the soil silt content (Table 2; Fig. 4b) suggesting that they are not associated with this particular size fraction. Interestingly, the relationship between bound aliphatic compounds and the soil silt content was weaker when MAT was also considered (Supporting information, Table S3). The suberin/cutin ratio which has been used to estimate the cutin vs. suberin inputs to soils (Kögel-Knabner et al., 1989; Otto and Simpson, 2006a) showed an inverse relationship to MAT (Table 2; Fig. 1e) indicating that an increase in plant productivity or a shift from belowground to aboveground inputs at elevated temperatures may cause an increase in cutin derived compound production and accumulation in warmer soils. The preservation of cutin derived aliphatic structures may also be due to higher mean annual precipitation and soil moisture at the two warmer sites (Table 1). Furthermore, suberin derived compounds have been shown to degrade faster than cutin derived compounds (Feng and Simpson, 2008), resulting in low suberin/cutin ratios. The suberin/cutin ratio was also inversely related to the soil sand content (Table 2; Fig. 4d). As in the case of the free aliphatic lipids, the porous nature of sand may enhance the biodegradation of more labile SOM components, resulting in the accumulation of cutin derived compounds because of their inherent chemical recalcitrance. The porous nature of sand may be reducing soil wettability which is considered a key factor for organic matter decomposition because it controls the microbial accessibility of water, nutrients and oxygen and is important for enzyme diffusion in partly saturated soils (Bachmann et al., 2008; Abbott et al., 2013). For example, Abbott et al. (2013) observed that the extent of lignin degradation varied with water table fluctuations in surficial peat soils. Although the soils examined in this study are well-drained, the variation in moisture conditions may also play a role in the observed trends. Changes in the relative abundance of ω-hydroxyalkanoic acids (ω-$C_{16}/\Sigma C_{16}$) have been shown to increase with progressive cutin degradation (Goñi and Hedges, 1990; Otto and Simpson, 2006a) because cutin derived acids containing double bonds and more than one hydroxyl group are preferentially degraded compared to ω-hydroxyalkanoic acids. This ratio was inversely related to MAT (Table 2; Fig. 1f), suggesting that cutin is preserved in soils of warmer climates. It is also possible that the more recalcitrant C_{16} ω-hydroxyalkanoic acid may be more sensitive to elevated temperatures.

To examine potential relationships of other SOM molecular components with temperature, lignin derived phenols were also analyzed. Lignin is a polyphenolic biochemical that occurs as a major component of the conductive tissues of vascular plants, ferns and club mosses (Hedges and Mann, 1979; Kögel-Knabner, 2002). It is composed of the derivatives of three basic structural classes: vanillyls (V), syringyls (S) and cinnamyls (C) and the composition of these lignin phenols is characteristic of major plant groups such as angiosperms and gymnosperms (Hedges and Mann, 1979). The CuO oxidation method does not completely depolymerise lignin and is therefore not quantitative. However, the method releases phenolic monomers from the outer part of the lignin polymer that are indicative of lignin content and composition (Otto and Simpson, 2006b) and the sum of all lignin phenols (VSC) is generally considered as a quantitative measure of soil lignin (Thevenot et al., 2010). Our results show that there is no significant correlation between the total amount of lignin derived phenols and MAT (Fig. 5a). The relationship between VSC and MAT is stronger when MAP is also considered (Supporting information, Table S3), suggesting that the combination of temperature and precipitation may influence the accumulation of lignin in soils. Some studies have proposed that lignin may be recalcitrant in soils (Hedges and Mann, 1979; Kögel-Knabner, 2002; Melillo et al., 2002), but recent studies have shown that lignin may indeed be susceptible to microbial degradation (Feng et al., 2008; Thevenot et al., 2010). Only a small group of fungi (white-rot and brown-rot fungi) are able to biodegrade lignin in terrigenous environments and progressive lignin degradation is reflected by elevated ratios of lignin derived phenolic acids to their corresponding aldehydes (Ad/Al) for both syringyl and vanillyl units (Hedges et al., 1988). The oxidation

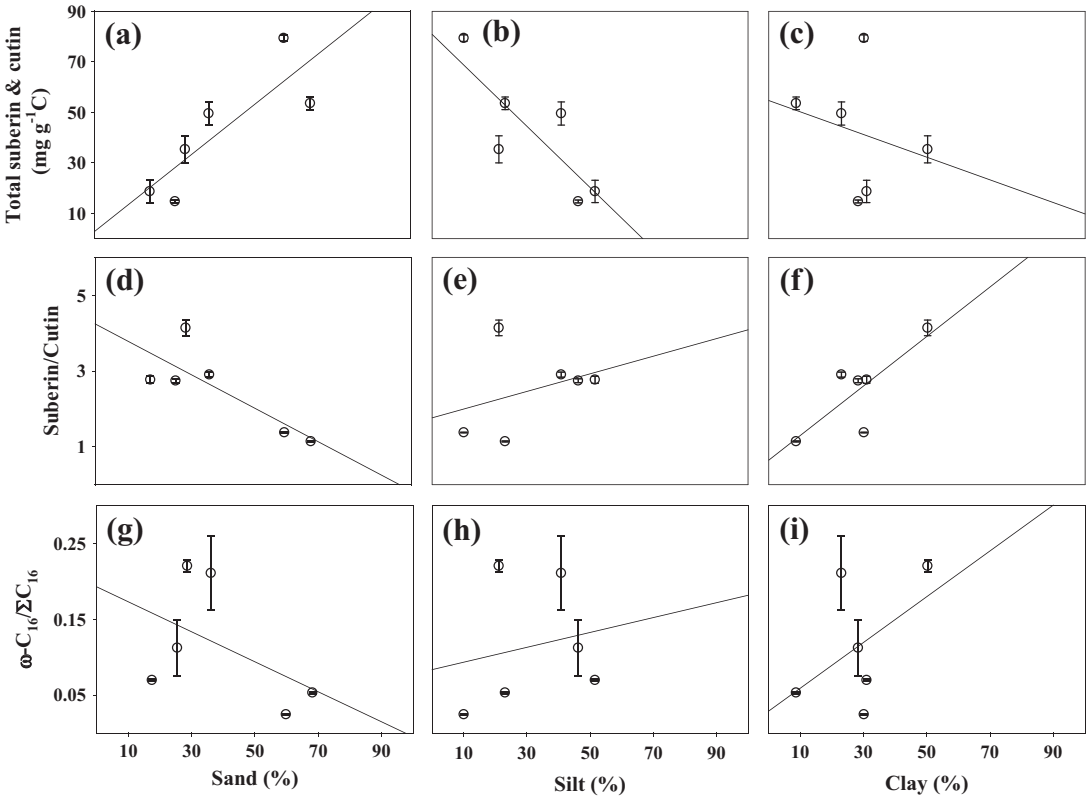

Fig. 4. Relationship between bound aliphatic compounds and soil texture. Correlations between the total suberin and cutin biomarkers and soil (a) sand; (b) silt; (c) clay content; suberin/cutin ratio and soil (d) sand; (e) silt; (f) clay content; ratio of ω-C_{16} hydroxyalkanoic acid and total C_{16} acids (ω-C_{16}/ΣC_{16}) and soil (g) sand; (h) silt; (i) clay content. The total suberin and cutin biomarkers and the suberin/cutin and ω-C_{16}/ΣC_{16} ratios were calculated according to Otto and Simpson (2006a). Error bars depict the analytical error originating from triplicate analyses on composite soil samples (n = 3).

state of lignin, estimated by the Ad/Al ratios, also did not vary significantly with MAT (Fig. 5b). Likewise, the combination of MAT with soil clay and silt content did not provide models that account for a significantly higher amount of the observed variability (Supporting information, Table S3). These results highlight that temperature alone does not control the preservation and degradation of lignin in soils and that there may be other environmental factors (i.e., climate, soil microbial activity and soil antioxidant capacity) involved in the stabilization mechanisms of these compounds (Thevenot et al., 2010).

We also investigated the overall SOM composition in relation to MAT using solid state ^{13}C NMR spectroscopy (Fig. 6). This technique can provide information about the total carbon characteristics of SOM (Cardoza et al., 2004; Simpson et al., 2008, 2012) and has been used in tandem with biomarker analyses for the elucidation of SOM molecular composition and degradation trends (Kögel-Knabner et al., 1992; Feng et al., 2008; Simpson et al., 2008; Huang et al., 2011). The integration results obtained from ^{13}C NMR spectroscopy can be used to compare the relative intensity of different types of carbon present in soil samples. These results showed that the soils collected along the MAT gradient are dominated by *O*-alkyl (36–52%) and alkyl carbon (25–41%). However, no significant correlations between organic carbon moieties (alkyl, *O*-alkyl and aromatic) and MAT were identified (Fig. 7a–c). The alkyl/*O*-alkyl ratio which is used to compare the relative degradation state of SOM (Baldock et al., 1992) also did not significantly correlate with MAT (Fig. 7d). When the Costa Rican soil was removed from the simple regression analysis, the correlation between the alkyl/*O*-alkyl ratio and MAT became statistically significant (r^2 = 0.88; $p \leqslant 0.05$), suggesting that the allophane minerals present in this soil may be protecting *O*-alkyl carbon moieties

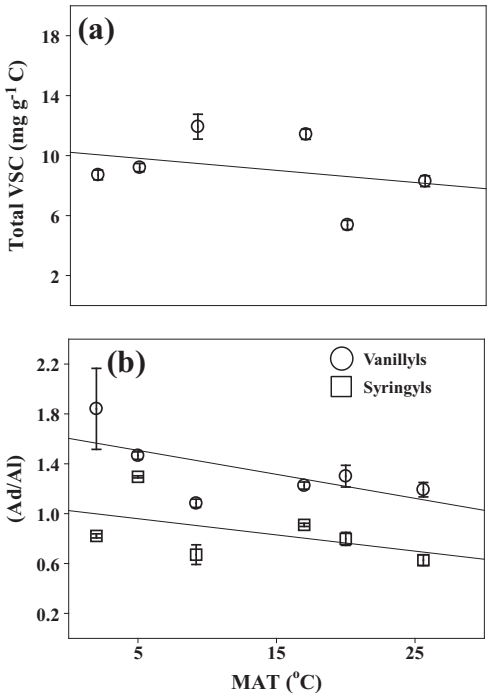

Fig. 5. Relationship between lignin derived phenols and mean annual temperature (MAT). Correlations between MAT (°C) and the (a) total lignin-derived phenols (VSC) and (b) lignin acid/aldehyde (Ad/Al) ratios. The VSC was calculated by taking the sum of all vanillyl, syringyl and cinnamyl units. The Ad/Al ratio was calculated by taking the ratio of the aldehyde and the acid monomer for vanillyl (vanillin/vanillic acid) and syringyl (syringealdehyde/syringic acid) phenols. Error bars depict the analytical error originating from triplicate analyses on composite soil samples (n = 3).

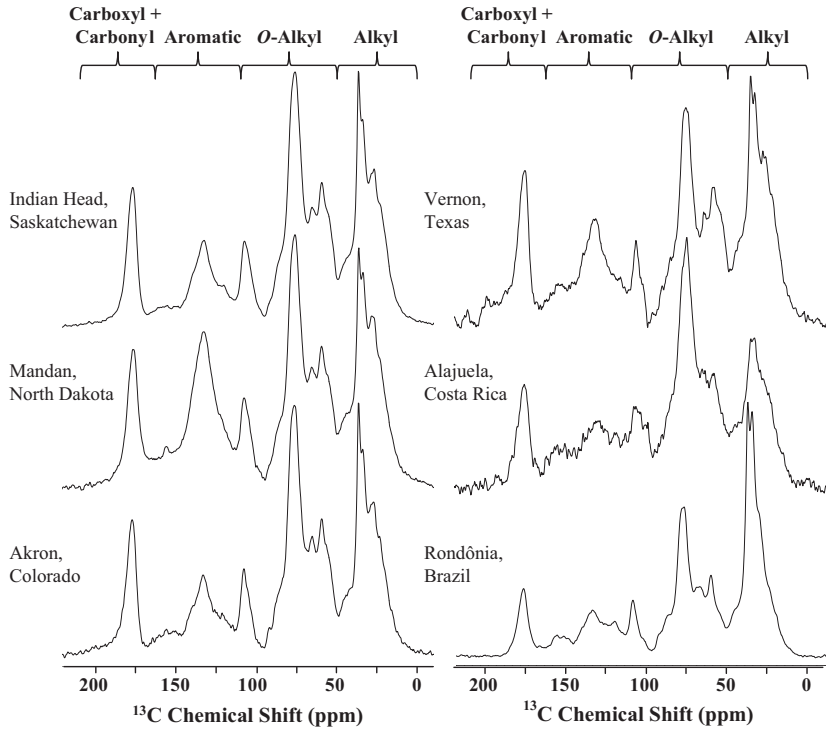

Fig. 6. Solid state ^{13}C nuclear magnetic resonance (NMR) spectra of the grassland and forest soil samples.

from biological attack. The lack of significant correlations may stem from the insensitivity of solid state ^{13}C NMR to subtle changes in SOM composition (Simpson et al., 2012) or the inability to resolve specific resonances for individual SOM components due to spectral overlap. For example, the alkyl carbon region contains signals from terminal methyl groups in lipids, lignin and amino acids as well as signals from mid-chain methylene groups in cutin, suberin, lipids and amino acid side chains (Baldock et al., 1992; Simpson et al., 2008). These results further support our observation that SOM preservation is compound specific and techniques, such as solid state ^{13}C NMR, which do not provide a high level of resolution, may not adequately identify environmental controls on SOM decomposition.

On a final note, the multiple comparisons with the Bonferroni correction method reinforced nearly all of our original assertions (Table S2). For example, the long chain ($\geqslant C_{20}$) compounds display a nearly monotonic increase with the air temperature, as the values measured at 2 °C, 17 °C, and 5 °C formed a homogeneous group

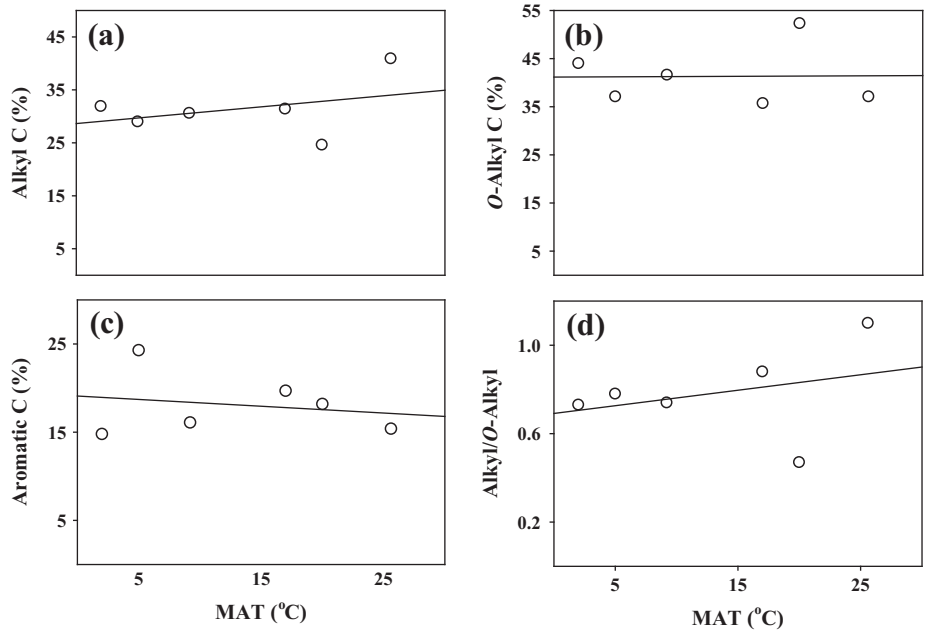

Fig. 7. Relationship between bulk soil organic matter components and mean annual temperature (MAT). Integration results of solid state ^{13}C NMR spectroscopy and correlations with MAT (°C). The integrations are shown as percent (a) alkyl carbon (0–50 ppm); (b) *O*-alkyl carbon (50–110 ppm); (c) aromatic carbon (110–165 ppm); (d) alkyl/*O*-alkyl ratio.

with average concentration lower than the one characterizing the group of temperatures 17 °C, 5 °C, 9.2 °C, 20 °C, which in turn is distinctly lower than the average compound level at 20 °C and 25.6 °C. The opposite holds true for the (monotonically negative) relationship between suberin/cutin and mean annual temperature. Similar inference can be drawn for the influence of sand and clay content on long chain aliphatics as well as the relationship between the percentage of clay and the acyclic/cyclic aliphatic compounds. Generally, the results of these multiple comparisons were on par with the patterns derived from our regression analysis. Namely, strong linear models with high r^2 values were also characterized by clear cut groups that were indicative of monotonically increasing/decreasing patterns. By contrast, we were not able to delineate clear groups in cases with weaker models; neither to provide evidence of non-linear monotonic trends nor of unimodal patterns.

4. Conclusions

The accumulation of aliphatic SOM in soils collected along a bicontinental, longitudinal MAT gradient is likely due to their chemical recalcitrance and the preferential degradation of other SOM components. Our study provides evidence that temperature is highly correlated to aliphatic SOM accumulation in soil. It is important to note that other environmental factors, such as SOM–clay interactions, moisture content and microbial community structure and activity, are also important for the turnover of SOM but did not appear to relate to the accumulation of aliphatic components with varying MAT. With the estimated annual litterfall predicted to increase with rising global temperatures (Liu et al., 2004), our results suggest that future increases in the stabilization and preservation of cuticular carbon in soils may occur despite variation in soil characteristics and environmental controls (e.g., vegetation, soil moisture, microbial community). This shift in SOM composition may result in overall changes in SOM quality and turnover with time. Lastly, our results emphasize the need to consider SOM components individually to better understand soil biogeochemical processes because different mechanisms attributed to the turnover of these various compounds and stabilization processes in soil appear to be component specific. However, we also acknowledge the small sample size used in this study and future research should test the trends reported here using a larger data set.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.orggeochem.2014.07.009.

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