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Molecular composition of soil organic matter with land-use change along a bi-continental mean annual temperature gradient

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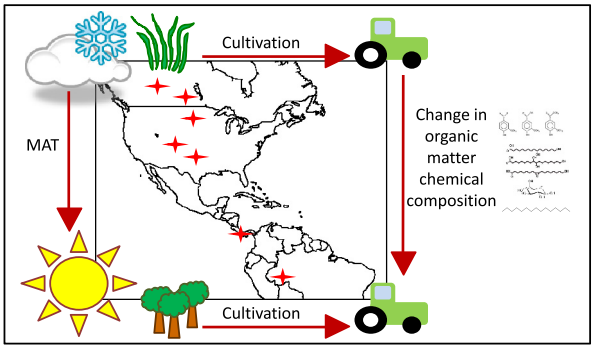
HIGHLIGHTS

- Soil organic matter composition was examined across a bi-continental gradient.
- SOM chemical composition varied with MAT and land-use change.
- Cultivation of native sites resulted in a decrease in cutin-derived organic matter.
- Lignin oxidation also increased with cultivation.
- Cultivation may enhance the degradation of recalcitrant compounds.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil organic matter (SOM) is critical for maintaining soil fertility and long-term agricultural sustainability. The molecular composition of SOM is likely altered due to global climate and land-use change; but rarely are these two aspects studied in tandem. Here we used molecular-level techniques to examine SOM composition along a bi-continental (from North to South America) mean annual temperature (MAT) gradient from seven native grassland/forest and cultivated/pasture sites. Biomarker methods included solvent extraction, base hydrolysis and cupric (II) oxide oxidation for the analysis of free lipids of plant and microbial origin, ester-bound lipids from cutin and suberin, and lignin-derived phenols, respectively. Solid-state ^{13}C nuclear magnetic resonance (NMR) was used to examine the overall composition of SOM. Soil cultivation was found to increase the amount of microbial-derived compounds at warmer temperatures (up to 17% increase). The cultivated soils were characterized by much lower contributions of plant-derived SOM components compared to the native soils (up to 64% lower at the coldest site). In addition, cultivation caused an increase in lignin and cutin degradation (up to 68 and 15% increase, respectively), and an increase in the amount of suberin-derived inputs (up to 54% increase). Clear differences in the molecular composition of SOM due to soil cultivation were observed in soils of varying mineral composition and were attributed to disturbance, different vegetation inputs, soil aggregate destruction and MAT. A high organic allophanic tropical soil was characterized by its protection of carbohydrates and nitrogen-containing compounds. The conversion of native to cultivated land shows significant shifts in the degradation stage of SOM. In particular, cutin-derived compounds which are believed to be part of the stable SOM pool may undergo enhanced degradation with long-term cultivation and disruption of soil aggregates. On a per year basis, the total amount of cutin decreased only at the two forest sites that were converted to pasture, likely due to cutin degradation or to changes in vegetation and litter quality associated with land-use change. Overall, our study highlights

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that the implementation of different agricultural management practices enhances the degradation of recalcitrant SOM compounds that may become a source of atmospheric CO_2 with increasing land-use and climate change.

1. Introduction

Soil organic matter (SOM) contains two-thirds of the global terrestrial carbon storage (Batjes, 1996) and is critical for maintaining soil fertility and long-term agricultural sustainability (Lal, 2004). The accumulation and turnover of SOM are major factors in ecosystem functioning and determine whether soils act as sinks or sources of carbon in the global carbon cycle (Post and Kwon, 2000). The onset of global warming and elevated atmospheric CO_2 levels and the conversion of native to agricultural land may alter the global carbon cycle through potential shifts in soil processes and CO_2 emissions. The molecular-level role of SOM in ecological responses to global climate and land-use change is an emerging aspect of SOM research and understanding the fate of SOM with ecological change may help improve predictions of potential ecosystem shifts (da Silva Oliveira et al., 2016; Simpson and Simpson, 2012). There is current interest in trying to understand how climate and land-use change may impact the molecular composition of SOM (Bahri et al., 2006; da Silva Oliveira et al., 2016; Feng et al., 2008; Nierop et al., 2001; Pisani et al., 2014, 2015; Rumpel and Chabbi, 2010; Schulten et al., 1995). Knowledge of their combined influence on the accumulation and turnover of molecularly distinct SOM components is still lacking.

By the year 2100, the mean global temperature is projected to increase by 0.3–6.4 °C (IPCC, 2007). These rising temperatures are predicted to alter many biogeochemical processes in terrestrial ecosystems (Shaver et al., 2000) and may have a direct impact on the molecular composition of SOM (Feng et al., 2008; Pisani et al., 2014, 2015). Changes in SOM composition are likely to occur through vegetation shifts (Feng et al., 2008) and through changes in the soil microbial community composition and microbial decomposition patterns of SOM (Feng et al., 2008; Frey et al., 2013). The conversion of natural vegetation to cultivated use may result in a rapid decline in SOM (Davidson and Ackerman, 1993; Lal, 2004; Post and Kwon, 2000) and long-term management of agricultural soils may alter the quantity and the chemical structure of SOM (da Silva Oliveira et al., 2016; Conant et al., 2007; Nierop et al., 2001; Paul et al., 2003; Schulten et al., 1995). The influence of mean annual temperature (MAT) and land-use change on the bulk composition of SOM for various soils has been studied (Conant et al., 2007; Davidson and Ackerman, 1993; Haddix et al., 2011; Li et al., 2013; Plante et al., 2011), but a detailed molecular-level analysis of the SOM is needed to better understand how cultivation as well as environmental factors may alter SOM biogeochemistry.

This study uses molecular-level methods to characterize SOM in soils that vary in both MAT and land-use. Soils were collected from native grassland/forest and cultivated/pasture sites along a bi-continental, longitudinal MAT gradient (Table 1). The molecular composition of the SOM was analyzed using biomarkers and gas chromatography–mass spectrometry (GC–MS) in combination with solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. The biomarker methods employed include solvent-extraction for the analysis of free lipids of plant and microbial origin (Otto and Simpson, 2005), base hydrolysis for the determination of ester-bound lipids from leaf cuticles and root suberin (Goñi and Hedges, 1990a; Otto and Simpson, 2006a) and cupric (II) oxide (CuO) oxidation for the analysis of lignin-derived phenols (Hedges and Mann, 1979; Otto and Simpson, 2006b). While biomarker methods provide detailed information on specific compounds, solid-state ^{13}C NMR provides information on the overall structure of SOM (Simpson et al., 2008). These techniques have been successfully used in tandem for the determination of SOM molecular-level responses to environmental change and land management (Feng et al., 2008; Feng and Simpson, 2011; Huang et al., 2011; Pisani et al., 2014, 2015).

2. Materials and methods

2.1. Sample collection

Soil samples were collected in 2005 from seven locations along a MAT gradient: Indian Head, Saskatchewan; Mandan, North Dakota; Akron, Colorado; Vernon, Texas; Alajuela, Costa Rica; Rondônia, Brazil (Table 1). The Melfort, Saskatchewan soil sample was collected in 2000 from a cultivated site and compared with a similar Orthic Black Chernozem (Udic Boroll) grassland soil from Edmonton, Alberta that has previously been molecularly characterized (Mitchell and Simpson, 2013; Otto et al., 2005). The MAT ranges from 0.8 °C in Melfort to 25.6 °C in Rondônia, while the mean annual precipitation ranges from 402 mm in Mandan to 2700 mm in Alajuela. The temperate samples were collected from both a native grassland site and a cultivated site at each location, while the samples obtained from Costa Rica and Brazil were collected from both a native forest and pasture site located on previously forested land. Soil textural characteristics, dominant vegetation and cultivation type, and elemental composition at each location (taken from Haddix et al., 2011 and references therein) are summarized in Table 1. Surface litter and aboveground vegetation were cleared away prior to sampling and all soil samples were collected from 0 to 20 cm. Three field replicates were collected at each site and combined into one composite sample for laboratory analysis. After sampling, the soils were air-dried, passed through a 2 mm sieve, ground with a mortar and pestle and stored at room temperature.

2.2. Soil organic matter biomarker extraction, analysis and quantification

Sequential chemical extractions (solvent extraction, base hydrolysis and CuO oxidation) were conducted on the soil samples to analyze the free lipids, ester-bound lipids and lignin-derived phenols, respectively (Otto and Simpson, 2007). The free lipids obtained through solvent extraction can be indicative of SOM sources and stage of degradation in terrestrial environments (Medeiros and Simoneit, 2007; Simoneit, 2005; Simpson and Simpson, 2012). For example, the carbon chain length of alkyl lipids can be used to elucidate SOM sources because short-chain compounds ($<C_{20}$) are frequently considered to originate from soil microbes (Lichtfouse et al., 1995) whereas long-chain compounds ($\geq C_{20}$) are typically derived from plant epicuticular waxes (Medeiros and Simoneit, 2007). The carbon chain length maximum (C_{max}) is indicative of the relative source input to soils (Bull et al., 2000; Otto and Simpson, 2005; Otto et al., 2005). Free lipids also include sterols and triterpenoids. Sterols such as campesterol, stigmasterol and β-sitosterol are among the most common phytosterols in vascular plants (Hartmann, 1998). The sterol ergosterol has been used as a signature lipid for fungi in soil environments (Ruzicka et al., 2000) while cholesterol has been reported in soil microbes and plant waxes and is not source specific (Hartmann, 1998; Volkman, 2003). Triterpenoids such as α- and β-amyrin, lupeol and ursolic acid, typically occur in angiosperms (Bianchi, 1995). Other free lipids include the C_{16} mono-unsaturated monoacylglyceride which is a major constituent of cell membranes and storage lipids (fats) that are produced by many organisms (Harwood and Russell, 1984). Ferulic acid originates from suberin-associated waxes (Otto and Simpson, 2005). Carbohydrates, including glucose, mannose and sucrose, have been observed in animals, plants

Table 1
Characteristics of the soil samples collected in native grassland (NG), cultivated (C), native forest (NF) and pasture (P) sites including soil type classification and texture (percent sand, silt and clay), mean annual temperature (MAT) and precipitation (MAP), dominant vegetation and type of cultivation, soil pH, percent organic carbon (C) and carbon to nitrogen ratio (C:N).

Sample ID	Location	Latitude	Longitude	Soil type	Sand (%)	Silt (%)	Clay (%)	MAT (°C)	MAP (mm)	Vegetation/cultivation	pH	C (%)	C:N
AL-NG	Edmonton, Alberta	53°55′N	113°50′ W	Udic Borroll	30	55	15	2	452	Grassland, C_3 fescue grasses	6.22	5.3	13.2
AL-C	Melfort, Saskatchewan	52°52′N	104°36′ W	Udic Borroll	10	50	40	1	411	Continuous corn cultivation	5.41	5.1	10.2
SK-NG	Indian Head, Saskatchewan	50°53′N	103°52′ W	Udic Borroll	29	21	50	2	427	Grassland, cool season grasses	7.40	3.7	10.4
SK-C	Indian Head, Saskatchewan	50°53′N	103°52′ W	Udic Borroll	28	11	61	2	427	Spring wheat crop rotations	8.05	2.3	11.2
ND-NG	Mandan, North Dakota	46°77′N	100°92′ W	Typic Argiboroll	26	46	28	5	402	Warm mixed grass prairie	6.58	3.2	11.0
ND-C	Mandan, North Dakota	46°77′N	100°92′ W	Typic Argiboroll	26	41	33	5	402	Continuous no-till spring wheat	5.89	2.8	11.7
CO-NG	Akron, Colorado	40°15′N	103°15′ W	Aridic Paleustoll	36	41	23	9	420	Grassland, C_4 grasses	7.00	1.2	8.6
CO-C	Akron, Colorado	40°15′N	103°15′ W	Aridic Paleustoll	39	33	28	9	420	Continuous till wheat-fallow treatments	6.41	0.7	6.9
TX-NG	Vernon, Texas	33°94′N	99°40′W	Typic Paleustoll	17	52	31	17	665	Grassland, mix of C_3 and C_4 grasses	7.47	1.1	8.9
TX-C	Vernon, Texas	33°94′N	99°40′W	Typic Paleustoll	15	41	44	17	665	Continuous wheat with conventional tillage	7.95	1.0	8.1
CR-NF	Alajuela, Costa Rica	N/A	N/A	Hydric Melanudand	68	23	9	20	2700	Tropical forest, mostly C_3 species	5.22	20.0	12.1
CR-P	Alajuela, Costa Rica	N/A	N/A	Hydric Melanudand	61	27	12	20	2700	Warm season grasses, C_4	5.49	14.2	13.5
BR-NF	Rondônia, Brazil	10°17′N	62°82′W	Paleudult & Kandiuldult	60	10	30	26	2200	Tropical forest, mostly C_3 species	4.33	1.1	8.7
BR-P	Rondônia, Brazil	10°17′N	62°82′W	Paleudult & Kandiuldult	65	10	25	26	2200	Warm season grasses, C_4	5.59	1.4	10.6

Land management at each site began in the following years: Alberta (AL; 1958), Saskatchewan (SK; 1907), North Dakota (ND; 1910), Colorado (CO; 1907), Texas (TX; 1980), Costa Rica (CR; 1979) and Brazil (BR; 1972).
Table information was taken from Haddix et al. (2011) and references therein.
AL-NG soil characteristics were taken from Janzen et al. (1998), soil texture from Salloum et al. (2000), soil pH from Mitchell and Simpson (2013), and elemental composition from Otto et al. (2005).

and soil microbes (Simoneit et al., 2004) and are not source specific. Trehalose is synthesized as a stress protectant and energy reserve carbohydrate by soil fungi and bacteria (Koide et al., 2000) although it can also be biosynthesized by some plants (Wingler, 2002). To obtain these free lipids, the soils (~15 g) were solvent-extracted in triplicate by sonication for 15 min with 30 ml CH_2Cl_2, CH_2Cl_2:MeOH (1:1 v/v) and MeOH. 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 remaining soil residues from solvent extraction were air-dried and then subjected to base hydrolysis to yield ester-linked lipids (Goñi and Hedges, 1990a; Otto and Simpson, 2006a). Ester-linked lipids can be attributed to cutin (short-chain mid-chain hydroxy acids, C_{16} mono- and dihydroxy acids and dioic acids), suberin (long-chain ω-hydroxyalkanoic and dioic acids, and 9,10-epoxy-ω-hydroxy C_{18} acid) or both macromolecules (C_{16} ω-hydroxyalkanoic acid, C_{18} di- and trihydroxy acids, C_{16} and C_{18} dioic acids; Kolattukudy, 1980; Otto and Simpson, 2006a). In addition, the suberin to cutin ratio (S/C) can be used to determine inputs of fresh root litter and leaf cuticles to soils (Kögel-Knabner et al., 1989; Otto and Simpson, 2006a). Changes in the relative abundances of the ω-hydroxy acids can be expressed as the ω-C_{16}/ΣC_{16} ratio which has been reported to increase with progressive cutin degradation in coastal marine sediments (Goñi and Hedges, 1990b) and in soils from western Canada (Otto and Simpson, 2006a). For base hydrolysis, the soil residues from solvent extraction (0.1—3 g) were heated at 100 °C for 3 h in Teflon-lined bombs with 20 ml of 1 M methanolic KOH. The extracts were sonicated twice with 15 ml CH_2Cl_2:MeOH (1:1 *v/v*), centrifuged and acidified to pH 1 with 6 M HCl. The solvents were removed by rotary evaporation and the lipids were recovered from the water phase by liquid-liquid extraction (3×) with 30 ml diethyl ether. The extracts were dried with anhydrous Na_2SO_4, concentrated by rotary evaporation and dried under a N_2 stream in 2 ml glass vials. The base hydrolysis soil residues were air-dried and further oxidized with CuO to release lignin-derived phenols (Hedges and Mann, 1979; Otto and Simpson, 2006b). Alkaline oxidation with CuO cleaves aryl ether bonds and releases phenolic monomers from the outer part of the lignin macromolecule that are indicative of lignin content and composition. The composition of lignin-derived phenols is characteristic of major plant groups because gymnosperm wood contains only vanillyl derivatives while angiosperm wood contains approximately equal quantities of vanillyls and syringyls (Hedges and Mann, 1979). The non-woody vascular plant tissues of gymnosperms and angiosperms (e.g., conifer needles, grass and angiosperm leaves) contain cinnamyl units that are part of the lignin macromolecule or the ligno-cellulose complex (Lam et al., 2001). As such, the ratios of syringyl to vanillyl (S/V) and cinnamyl to vanillyl (C/V) monomers can be used to determine the botanical origin of lignin in soils and sediments (Ertel and Hedges, 1984; Goñi et al., 2000; Otto and Simpson, 2006b; Prahl et al., 1994). In addition, a decrease in S/V and C/V ratios can be indicative of progressive lignin degradation (Hedges et al., 1988) because syringyls and cinnamyls preferentially degrade resulting in the relative enrichment of vanillyls (Otto and Simpson, 2006b). Lignin oxidation is also reflected by elevated ratios of lignin-derived phenolic acids and their corresponding aldehydes (Ad/Al) of both vanillyl and syringyl units (Hedges et al., 1982) and these ratios have been commonly used as indicators of the level of lignin degradation in soils and sediments (Ertel and Hedges, 1984; Goñi et al., 2000; Otto and Simpson, 2006b). For CuO oxidation, the soil residues from base hydrolysis (0.1–3 g) were extracted with 1 g CuO, 100 mg ammonium iron (II) sulfate hexahydrate $[Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O]$ and 15 ml of 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 for 1 h at room temperature in the dark to prevent the polymerization of cinnamic acids. After centrifugation, the supernatants were liquid-liquid extracted (3×) with 30 ml diethyl

ether. The ether extracts were dried with anhydrous Na_2SO_4, concentrated by rotary evaporation, transferred to 2 ml glass vials and dried under a N_2 stream.

The soil extracts were re-dissolved in CH_2Cl_2:MeOH (1:1 v/v) and aliquots (containing ~1 mg extracts) were derivatized for GC–MS analysis. 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, 400 μl of hexane was added to dilute the extracts. For fatty acid esterification, base hydrolysis products were first methylated by reacting with 500 μl of *N,N*-dimethylformamide dimethyl acetal (2 mEq/ml in pyridine) at 60 °C for 15 min (Thenot et al., 1972). After being evaporated to dryness under a N_2 stream, the base hydrolysis products were silylated with BSTFA and anhydrous pyridine. For the solvent extracts lauric acid, trehalose and ergosterol (as TMS esters) were used as external standards along with tetracosane. Tricosanoic acid methyl ester was used as an external standard for the base hydrolysis products, while vanillic acid-TMS was used for the 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^{-1}). The GC operating conditions were as follows: 65 °C (hold for 2 min), increased from 65 to 300 °C (at a rate of 6 °C min^{-1}) 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 inlet temperature set at 280 °C. The mass spectrometer was operated in the electron ionization mode (EI) at 70 eV and scanned from *m/z* 50 to 650. 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.

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

Solid-state ^{13}C NMR has been widely used to characterize SOM because it provides basic structural information on the whole soil sample (Kögel-Knabner, 2000; Simpson et al., 2008; Simpson and Simpson, 2012). The soil samples for solid-state ^{13}C Cross Polarization with Magic Angle Spinning (CP-MAS) NMR were repeatedly treated with 10% hydrofluoric acid to concentrate the organic matter and to remove paramagnetic minerals (Schmidt et al., 1997; Rumpel et al., 2006). The treated samples were rinsed with deionized water to remove excess salts and freeze-dried. Approximately 100 mg of sample was packed into a 4 mm zirconium oxide rotor equipped with a Kel-F cap. 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, using a ramp-CP pulse program (Cardoza et al., 2004). The following acquisition parameters were employed: 13 kHz spinning rate, 1 ms ramp-CP contact time and 1 s recycle delay. The spectra were processed using a zero filling factor of 2 and line broadening of 100 Hz. The resulting NMR spectra were integrated into the following four chemical shift regions: alkyl carbon (0–50 ppm) from methyl groups in alkyl chains and methylene carbons in lipids, waxes, cutin and/or suberin; O-alkyl carbon (50–110 ppm) from carbon bonds and methoxy groups in carbohydrates and lignin; aromatic and phenolic carbon (110–165 ppm) from lignin, proteins and black carbon; carboxyl and carbonyl carbon (165–215 ppm) from fatty acids and peptides (Baldock et al., 1992; Simpson and Hatcher, 2004; Simpson et al., 2008). All chemical shifts were calibrated using an external glycine standard and the total NMR signal (0–215 ppm) was normalized to 100% to compare the relative contents of each type of carbon. The alkyl/O-alkyl ratio is often used as an indicator of the stage of degradation of SOM because O-alkyl compounds are labile and more easily degraded compared to alkyl compounds, resulting in increased ratios with progressive SOM degradation. The alkyl/O-alkyl ratios were calculated by dividing the areas of the alkyl and the O-alkyl regions of the NMR spectra (Baldock et al., 1992; Simpson et al., 2008).

2.4. Data analyses

A one-way analysis of variance (ANOVA) was applied using the Holm-Sidak multiple comparisons test for the determination of statistically significant differences ($P \leq 0.05$) in SOM molecular composition between the native grassland/forest and the cultivated/pasture soil samples. Statistical analyses were performed using SigmaPlot version 11.0.

Changes in SOM compound classes with time since cultivation were calculated using the following equation:

$$\text{Change} = ([BC] - [BN])/(t_2 - t_1) \quad (1)$$

where [BC] is the concentration of a particular compound class in the cultivated/pasture soils, [BN] is the concentration in the native grassland/forest soils, t_2 is the year of sample collection (2000 or 2005) and t_1 is the year that the site was converted to the current land-use (Table 1).

3. Results

3.1. Soil organic matter biomarkers

The native and cultivated soils collected along the MAT gradient were different in terms of texture, pH, organic C content and C:N ratios (Table 1). These parameters have been described and interpreted in previous studies (Janzen et al., 1998; Haddix et al., 2011 and references therein; Mitchell and Simpson, 2013; Otto et al., 2005; Salloum et al., 2000). They are included in the present study to assist in the interpretation of molecular-level data. The solvent-extractable compounds identified in the native and cultivated soil samples collected along the MAT gradient consisted of alkyl compounds (*n*-alkanols, *n*-alkanoic acids and *n*-alkanes), cyclic compounds (steroids and triterpenoids), monoacylglycerides, phenols and carbohydrates (Supplementary Tables S1 and S2). In the native soils, the *n*-alkanols ranged from C_{16} to C_{32} with a C_{max} at C_{26}, except the soils from Colorado and Texas which had a C_{max} at C_{18}. The *n*-alkanoic acids ranged from C_9 to C_{30} and also included some short-chain mono-unsaturated compounds. Most soils had a short-chain homologue as their C_{max} (C_{16}, $C_{16:1}$ or $C_{18:1}$) except for the soils from Alberta, North Dakota and Costa Rica which had a long-chain C_{max} (C_{24} or C_{26}). The *n*-alkanes comprised a small component of the alkyl compounds and only long-chain homologues, ranging from C_{24} to C_{33}, were found. The distribution of alkyl components of the native soils showed no particular trend with MAT. The cyclic compounds identified in the native soils included sterols and triterpenoids (Table S1). The major sterols were campesterol, stigmasterol and β-sitosterol, which was the most abundant sterol in all the soils (Table S1). In addition to these phytosterols, cholesterol and ergosterol were also detected. The triterpenoids identified included α- and β-amyrin, lupeol and ursolic acid and were most abundant in the soils from the native forest sites. Other lipids detected included a C_{16} mono-unsaturated monoacylglyceride and the phenol ferulic acid. The carbohydrates identified include glucose, mannose, sucrose and the disaccharide trehalose. The highest amount of carbohydrates was found in the Texas and Brazil soils. However, the distribution of these compounds showed no clear trend with MAT.

In the cultivated soils (Table S2), the *n*-alkanols ranged from C_{16} to C_{30} and generally had a short-chain C_{max} (C_{18}). The soils from the two colder sites (Melfort and Indian Head, Saskatchewan) and the soil from Costa Rica had a long-chain C_{max} (C_{28} or C_{26}). The *n*-alkanoic acids ranged from C_{14} to C_{28} with a C_{max} at C_{16} for all the soils except

the North Dakota (C_{24}) and the Texas soil ($C_{16:1}$). The *n*-alkanes were a small component of the solvent extracts of the cultivated soils and only long-chain compounds were found (C_{24}–C_{31}). The identified cyclic compounds include sterols and triterpenoids. As in the case of the native soils, the cultivated soils were dominated by plant-derived sterols and β-sitosterol was the most abundant sterol in all the soils except Brazil which was dominated by stigmasterol. Triterpenoids were only detected in the Indian Head-Saskatchewan and Costa Rica cultivated soils which were dominated by α-amyrin and ursolic acid, respectively. Small amounts of the $C_{16:1}$ monoacylglyceride and the phenolic compound ferulic acid were detected in some soils. Carbohydrates, including glucose, mannose and sucrose, were most abundant at the warmest site (Brazil), but showed no clear trend with MAT. The disaccharide trehalose was most abundant in the soil from Brazil.

The relative abundance of microbial (short-chain saturated and unsaturated alkyl compounds ($<C_{20}$); the sterols cholesterol and ergosterol) and plant-derived (long-chain alkyl compounds ($\geq C_{20}$); the sterols campesterol, stigmasterol and β-sitosterol; the triterpenoids β- and α-amyrin, lupeol and ursolic acid; ferulic acid) solvent-extractable compounds was calculated in relation to the total concentration of free lipids measured in the native and cultivated soils (Fig. 1). The native soils are dominated by plant-derived compounds (Fig. 1) although there is no significant trend with increasing MAT ($r^2 = 0.18$; $P = 0.34$). The relative abundance of microbial-derived compounds showed a slight increasing trend with increasing MAT ($r^2 = 0.37$; $P = 0.15$) and a significantly higher relative abundance of microbial-derived compounds was found in the native soils with higher MAT ($P \leq 0.05$; Fig. 1). The cultivated soils are also dominated by plant-derived compounds with the exception of the Texas soil which has equal amounts of microbial and plant-derived SOM and the Brazil soil which is dominated by microbial-derived material. The relative abundance of microbial-derived compounds typically increases from the native to the cultivated soils and shows a larger increase at warmer temperatures (up to 17% increase). The relative abundance of plant-derived compounds typically decreases from the native to the cultivated soils, with the largest decrease occurring between the Alberta (native) and Melfort (cultivated) soils (up to 64% decrease).

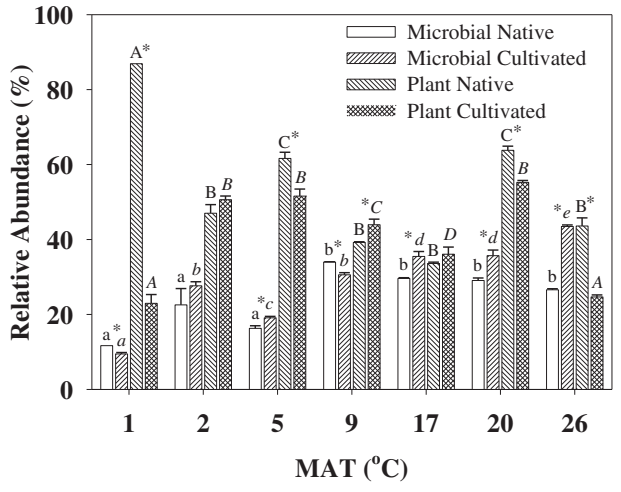

Fig. 1. The relative abundance (%) of the total solvent-extractable lipids of microbial and plant origin in the native and cultivated soils collected along the mean annual temperature (MAT) gradient. The microbial components include: short-chain saturated and unsaturated alkyl compounds ($<C_{20}$) and the sterols cholesterol and ergosterol. Plant components include: long-chain alkyl compounds ($\geq C_{20}$); the sterols campesterol, stigmasterol and β-sitosterol; the triterpenoids β- and α-amyrin, lupeol and ursolic acid; ferulic acid. Bars are averages of triplicate analyses and the error bars indicate standard error. Significant differences ($P \leq 0.05$) in microbial or plant components between sites for the native and cultivated soils are indicated by different letters: Microbial components between the native soils (lowercase), microbial components between the cultivated soils (lowercase italics), plant components between the native soils (uppercase) and plant components between the cultivated soils (uppercase italics). Significant differences ($P \leq 0.05$) in microbial or plant components between native and cultivated soils at each site are indicated by an asterisk.

The difference in microbial and plant-derived solvent-extractable compounds since the year of land conversion was calculated and used as an estimate for the rate of SOM decomposition or accumulation (Eq. 1). On a per year basis, the solvent-extractable microbial-derived components generally decreased with cultivation (Fig. 2a). At the cooler sites (Alberta and Indian Head-Saskatchewan) and at the warmest site (Brazil), microbial-derived SOM components increased with cultivation. The solvent-extractable plant-derived SOM also decreased from the time of land-use conversion (with the only exception of Indian Head-Saskatchewan) and the warmest sites showed the largest decreases of plant-derived SOM. The variation in the response of compound classes in the soils collected along the MAT gradient are likely due to the different agricultural management practices which vary in disturbance type.

The major compound classes identified in the base hydrolysis extracts of the native and cultivated soils collected along the MAT gradient consisted of alkyl compounds (n-alkanols, *n*-alkanoic acids, branched *n*-alkanoic acids, *n*-alkane-α,ω-dioic acids, ω-hydroxyalkanoic acids, α-hydroxyalkanoic acids and mid-chain hydroxy and epoxy acids) and small amounts of benzyls, phenols and steroids (Supplementary Tables S3 and S4). The most abundant alkyl components in the native soils were generally the *n*-alkanoic acids which ranged from C_{12} to C_{32} and had a low molecular weight C_{max} (C_{16} or $C_{18:1}$). The native soils from the cooler sites, Alberta and Indian Head-Saskatchewan, had a C_{max} at C_{28} and C_{24}, respectively. Short-chain branched *n*-alkanoic acids (iso- and anteiso-C_{16} and C_{18}) were detected in all the native soils and were most abundant in the sample from Colorado. The *n*-alkane-α,ω-dioic acids ranged from C_{16} to C_{28} with a long-chain C_{max} (C_{22}, C_{24} or C_{26}) at all the sites except at the two warmer sites, Costa Rica and Brazil, which had a short-chain C_{max} (C_{16}). The ω-hydroxyalkanoic acids ranged from C_{16} to C_{26} and typically had a long-chain C_{max} (C_{24} or C_{26}). A short-chain C_{max} ($C_{16:1}$) was observed for the North Dakota, Costa Rica and Brazil soils. Mid-chain hydroxy acids were most abundant in the warmer, native forest soils and their distribution was dominated by 7- or 8-hexadecane-1,16-dioic acid in Costa Rica and dihydroxy-methoxyoctadecanoic acid in Brazil. These compounds were found either at low concentration or were not detected in the low MAT native grassland soils. The observed benzyls and phenols were major components of the native soil base extracts (Table S3).

The cultivated soils were dominated by *n*-alkanoic acids ranging from C_{12} to C_{32} and a short-chain C_{max} (C_{16} or $C_{18:1}$). Branched alkanoic acids of microbial origin ranged from C_{15} to C_{18} and were detected in all the cultivated soils except North Dakota. The *n*-alkane-α,ω-dioic acids ranged from C_{16} to C_{28} and had a long-chain C_{max} (C_{22} or C_{24}), except the soil from Brazil which had a short-chain C_{max} (C_{16}). The ω-hydroxyalkanoic acids ranged from C_{16} to C_{26} and typically had a long-chain C_{max} (C_{22}, C_{24} or C_{26}) except for the Indian Head-Saskatchewan cultivated soil ($C_{18:1}$) and the soils from Texas and Brazil ($C_{16:1}$). As in the case of the native soils, mid-chain hydroxy acids were most abundant in the cultivated soils from the warmer sites. Benzyl compounds were typically not very abundant in the cultivated soils while phenols were most abundant in soils from the warmer sites.

Based on the suberin to cutin ratio (S/C), the cooler, native grassland soils are dominated by root inputs, except for the Alberta soil which had a lower S/C value (Fig. 3a). The warmer, native forest soils from Costa Rica and Brazil have the lowest S/C ratios, indicating dominant inputs from leaf cuticles. Upon cultivation, the S/C ratio generally increased (up to 94% at the North Dakota site) suggesting either a change from aboveground to belowground inputs or enhanced degradation of cutin. At the Indian Head-Saskatchewan and Colorado sites, the cultivated soils had a lower S/C ratio compared to the native soils (up to 49% decrease). For both the native and cultivated soils, the S/C ratio showed a linear decrease with increasing MAT despite their different mineralogy and other environmental characteristics. For the native and cultivated

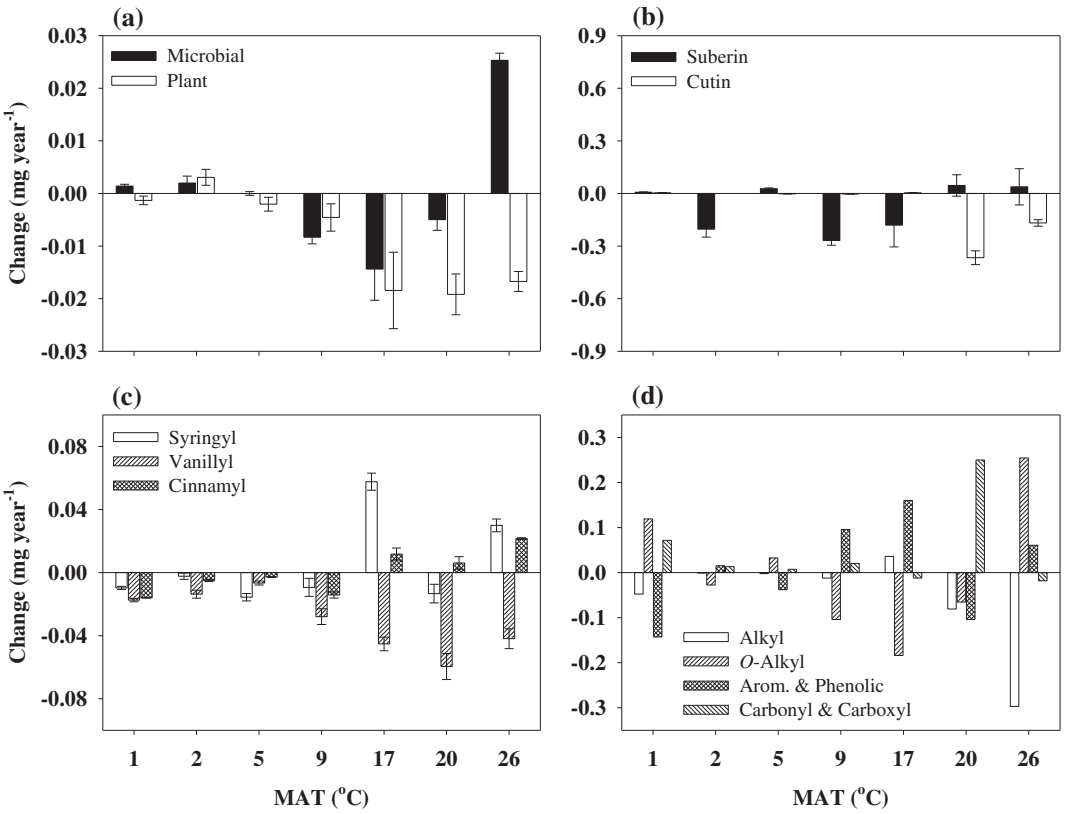

Fig. 2. Changes in the amount of molecular and bulk SOM components since the time of conversion from native grassland/forest to cultivated/pasture land-use calculated using Eq. (1). Changes in: (a) free microbial- (short-chain saturated and unsaturated alkyl compounds($<C_{20}$); the sterols cholesterol and ergosterol) and plant-derived compounds (long-chain alkyl compounds ($\geq C_{20}$); the sterols campesterol, stigmasterol and β-sitosterol; the triterpenoids β- and α-amyrin, lupeol, ursolic acid; ferulic acid), (b) bound suberin- (C_{20}–C_{32} ω-hydroxyalkanoic acids; C_{20}–C_{32} α,ω-dioic acids) and cutin-derived lipids (C_{16} mono-, dihydroxy acids and diacids), (c) syringyl, vanillyl and cinnamyl lignin phenols and (d) bulk SOM components from ^{13}C CP-MAS NMR integration data. Bars are averages of triplicate analyses and the error bars indicate standard error.

soils (Fig. 3b) the lower ω-$C_{16}/\Sigma C_{16}$ values obtained for the soils at the warmer sites may indicate fresh cutin inputs. Generally, the native soils had a higher ω-$C_{16}/\Sigma C_{16}$ ratio compared to the cultivated soils, suggesting a more degraded state of the cutin in the native soils. This trend may be the result of continuous fresh cutin inputs from crop rotation at the cultivated sites. At the two warmer sites, the difference in cutin degradation between the native and cultivated soils is much smaller (up to 6% increase in the ω-$C_{16}/\Sigma C_{16}$ ratio) than at the colder sites and likely due to the longer growing seasons. The ester-bound suberin-derived compounds decreased at some of the grassland sites (Indian Head-Saskatchewan, Colorado and Texas) since the year of land-use conversion (Fig. 2b) while these compounds increased at one grassland site (North Dakota) and at the two warmer sites (Costa Rica and Brazil). Relatively small changes were observed in cutin-derived compounds except at the two warmer sites where cutin-derived compounds decreased, likely due to changes in vegetation and a shift from aboveground to belowground inputs.

Upon CuO oxidation, eight lignin-derived phenols were released and identified which belonged to the vanillyl (vanillin, acetovanillone and vanillic acid), syringyl (syringaldehyde, acetosyringone and syringic

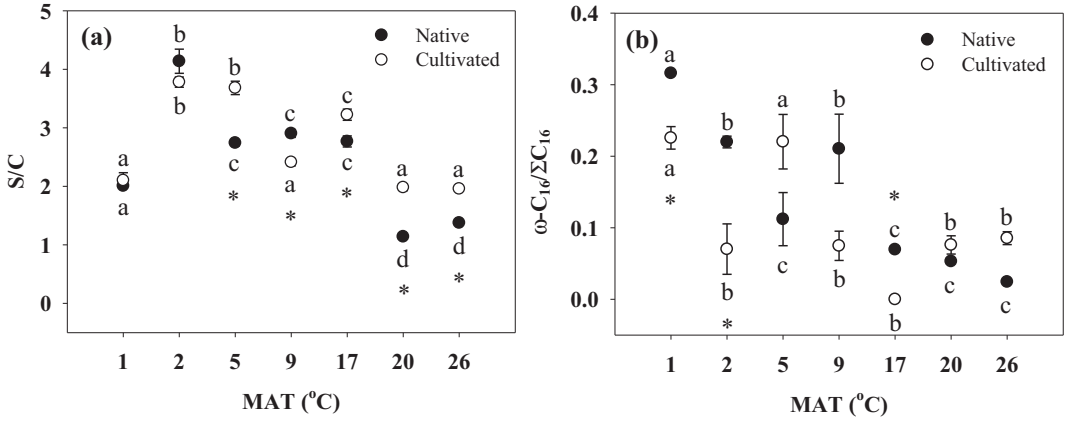

Fig. 3. Cutin and suberin inputs to the native and cultivated soils collected along the mean annual temperature (MAT) gradient: (a) The suberin to cutin ratio (S/C) and (b) the cutin source and degradation parameter ω-$C_{16}/\Sigma C_{16}$. Both ratios were calculated according to Otto and Simpson (2006a). Data points are averages of triplicate analyses and the error bars indicate standard error. Significant differences ($P \leq 0.05$) between sites for the native and cultivated soils are indicated by different lowercase letters. Significant differences ($P \leq 0.05$) between native and cultivated soils are indicated by an asterisk.

acid) and cinnamyl (*p*-coumaric acid and ferulic acid) groups (Supplementary Table S5). The native soils are dominated by vanillyl-type monomers, except for the North Dakota soil which is dominated by syringyl-type units. The total amount of lignin-derived phenols did not show an increasing trend with MAT ($r^2 = 0.02$; $P = 0.73$). In the cultivated soils, the most abundant lignin monomers were generally the syringyl units. As in the case of the native soils, the total lignin phenols did not show an increasing trend with MAT ($r^2 = 0.20$; $P = 0.33$). Overall, the lignin-derived phenols decreased in most of the soils since the year of land-use conversion (Fig. 2c) with vanillyl monomers decreasing to a greater extent than cinnamyl and syringyl monomers. In the Texas and Brazil soils, syringyl units increased while cinnamyl units increased in the three warmer soils.

A plot of S/V and C/V (Fig. 4a) shows differences between lignin residues in grassland and forest sites. The native grassland sites have significantly higher C/V ratios ($P \le 0.05$; Table S5) compared to the native forest soils which are clearly dominated by gymnosperm inputs. Similarly, the S/V values are significantly higher at the northern grassland sites ($P \le 0.05$; Table S5) compared to the forest soils. In addition to lignin sources, phenolic monomers were used to determine changes in lignin oxidation along the MAT gradient and with soil cultivation. A plot of Ad/Al ratios for the native and cultivated soils (Fig. 4b) shows that the native grassland soils from the colder sites (Alberta, Indian Head-Saskatchewan and North Dakota) had the highest $(Ad/Al)_v$, indicating the highest degree of lignin oxidation. Elevated $(Ad/Al)_s$ ratios were also observed for native soils at colder MAT. However, there was no significant trend between lignin oxidation and MAT ($r^2 = 0.55$; $P = 0.06$). In general, the lignin from the cultivated soils is at a more advanced stage of oxidation compared to the native soils as indicated by elevated Ad/Al ratios of both syringyl and vanillyl units (Fig. 4b).

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

The solid-state ^{13}C NMR spectra (examples in Fig. 5) and integration results (Table 2) identified different SOM components in the native and cultivated soil samples collected along the MAT gradient. The native soils are dominated by O-alkyl structures except the Alberta native soil which has equal quantities of O-alkyl and aromatic and phenolic carbon, and the native soil from Brazil which is dominated by alkyl components. No trend was found between the different SOM carbon moieties and MAT for the native soils. The cultivated soils are also dominated by O-alkyl carbon structures except for the cultivated Texas soil which is dominated by alkyl components. Again, no significant trend was found between the different carbon moieties of the cultivated soils and MAT. The native forest soil from Brazil and the cultivated Texas soil had the highest alkyl/O-alkyl ratios, indicating that the SOM is at a more advanced stage of degradation compared to the other soils (Table 2). No significant trend was observed in the alkyl/O-alkyl ratio of the native or cultivated soils with increasing MAT.

The relative abundance of the SOM alkyl components obtained using ^{13}C NMR decreased since the year of land-use conversion at all the sites except for the Texas soil (Fig. 2d). The change in the relative abundance of O-alkyl components showed no apparent trend with increasing MAT and showed the largest increase at the Brazil site and the largest decrease at the Texas site. The relative abundance of the aromatic and phenolic carbon moieties in the SOM also showed no trend with increasing MAT, but showed the largest increase in the Texas soil and the largest decrease in the North Dakota soil. Finally, the relative abundance of the carbonyl and carboxyl carbon moieties increased at all the sites, except in Texas and Brazil.

4. Discussion

4.1. Soil organic matter composition with increasing MAT

The native soils vary in characteristics such as texture, pH, organic C content and C:N ratios (Table 1). While these characteristics provide general information on SOM quality and quantity, a more detailed characterization, through the molecular identification of source-specific biomarkers provided valuable information on SOM changes associated with MAT and land-use change. Proteins and carbohydrates are generally considered to be more easily degradable compared to macromolecular lipids and aromatic structures (Melillo et al., 1982). The carbohydrates identified in this study are of both plant and microbial origin and did not follow any significant trend with increasing MAT (Table S1), in agreement with the NMR O-alkyl signal (Table 2). However, there was no significant relationship between the relative contribution of O-alkyl components and the soil active fraction previously measured on these same soils (SOC respired; Table 2; Haddix et al., 2011), suggesting that not all of the O-alkyl components are labile and may derive from plant material that is protected within aggregates or by the mineral matrix as in the allophanic soil from Costa Rica. In addition to signals from carbohydrates and peptides, the NMR O-alkyl region also includes signals from methoxy groups in lignin (~56 ppm), causing its signal to be more abundant than the total percent of carbohydrates, peptides and N-containing compounds previously obtained using Pyrolysis-Molecular Beam Mass Spectrometry (Py-MBMS; Table 2; Haddix et al., 2011). However, a significant correlation ($r^2 = 0.76$; $P < 0.05$) was observed between the relative contribution of O-alkyl carbon to the NMR spectrum and the sum of carbohydrates, peptides and N-containing

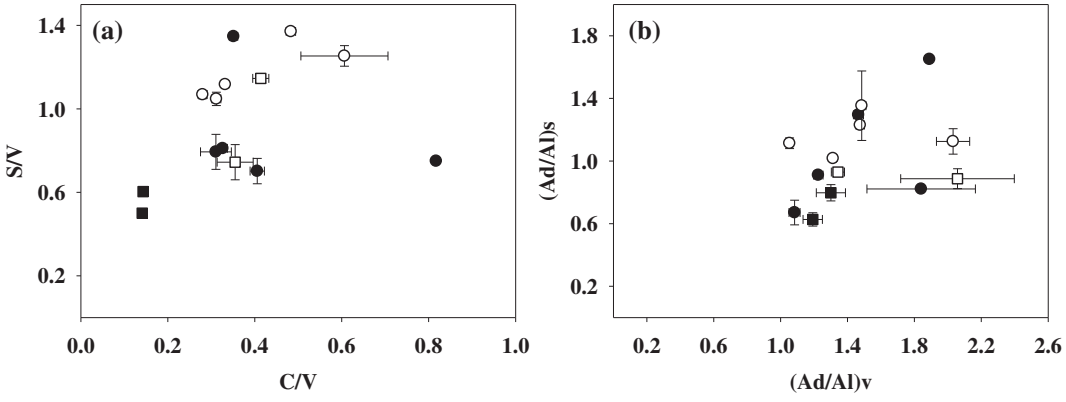

Fig. 4. Lignin source and degradation parameters for the native and cultivated soils collected along the mean annual temperature (MAT) gradient: (a) The cinnamyl/vanillyl (C/V) and syringyl/vanillyl (S/V) phenol ratios and (b) the lignin acid/aldehyde ratios for vanillyl (Ad/Al)v and syringyl (Ad/Al)s monomers. Grassland (○) and forest (□) soil samples; black = native soils, white = cultivated and pasture soils. Data points are averages of triplicate analyses and the error bars indicate standard error. Statistics for these figures are included in the Supplementary Table S5.

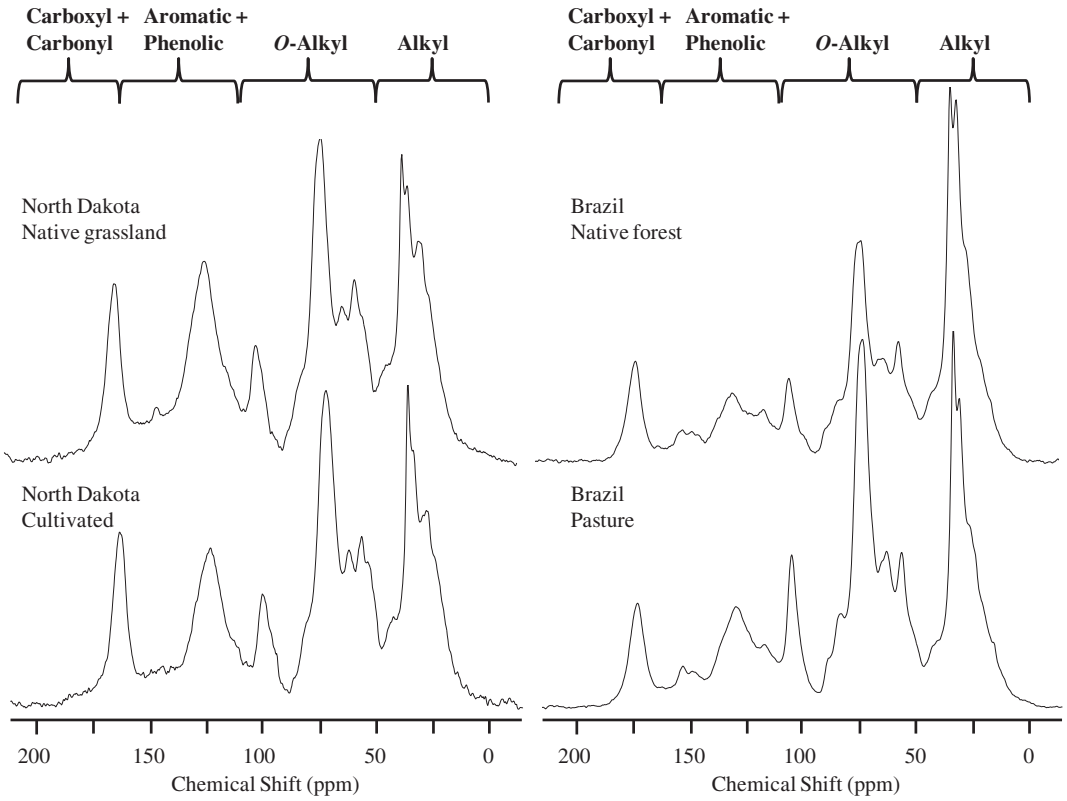

Fig. 5. Comparison of the solid-state ^{13}C cross polarization magic angle spinning (CP-MAS) nuclear magnetic resonance (NMR) spectra of a native grassland (North Dakota) and forest (Brazil) soil with the corresponding cultivated and pasture soils.

compounds detected with Py-MBMS (Fig. S1) indicating that the two techniques are comparable.

Due to its large macromolecular structure, lignin was originally believed to be stable in soils (Kögel-Knabner, 2002; Melillo et al., 2002), but studies have shown that lignin is indeed susceptible to soil priming brought on by increased soil temperatures (Feng et al., 2008; Pisani et al., 2015; Stevenson, 1982). In the native soils, the variation in the total amount of vanillyl, syringyl or cinnamyl phenols with MAT (Table S5) may be attributed to factors other than climate (e.g., the soil microbial community and soil moisture) or to a different lignin signature in the residues of plant tissues along the MAT gradient. However, no significant correlations were found between the soil lignin content and soil mineralogy (% sand, silt or clay) and pH. The cinnamyl monomers that are abundant in non-woody vascular plant tissues of grasses and angiosperm leaves (Lam et al., 2001) increased significantly with MAT only in the native grassland sites (Table S5; $r^2 = 0.87$, $P \leq 0.05$),

Table 2
Percent soil organic carbon (SOC) respired, percent of the total ion intensity (% TII) of the sum of carbohydrates, peptides and N-containing compounds detected with Pyrolysis-Molecular Beam Mass Spectrometry (Py-MBMS), the relative contribution (%) of the four main carbon structures to the solid-state ^{13}C CP-MAS NMR spectra after integration and the resulting alkyl/O-alkyl ratios of the native and cultivated grassland and forest soils.

	SOC respired (%)	Py-MBMS carb + peptides + N compounds (% TII)	Alkyl C (0–50 ppm)	O-alkyl C (50–110 ppm)	Aromatic and phenolic C (110–165 ppm)	Carboxylic and carbonyl C (165–215 ppm)	Alkyl/O-alkyl
Grassland soils							
AL-NG	na	na	27	33	33	7	0.82
AL-C	6.1	na	25	38	27	10	0.67
SK-NG	10.4	34	32	44	15	9	0.73
SK-C	7.3	26	32	41	16	11	0.77
ND-NG	8.9	33	29	37	24	10	0.78
ND-C	9.2	33	29	40	21	10	0.72
CO-NG	17.9	30	31	42	16	12	0.74
CO-C	13.0	26	29	31	26	14	0.94
TX-NG	15.0	29	31	36	20	13	0.88
TX-C	15.4	25	32	31	24	13	1.04
Forest soils							
CR-NF	4.2	42	25	52	18	5	0.47
CR-P	2.9	40	23	51	16	11	0.44
BR-NF	11.1	32	41	37	15	7	1.10
BR-P	10.6	35	31	46	17	6	0.68

na = not analyzed.
SOC respired and Py-MBMS data was taken from Haddix et al. (2011).
AL-NG ^{13}C NMR data was taken from Mitchell and Simpson (2013).

suggesting increased plant productivity at warmer temperatures. Lignin oxidation in the form of Ad/Al ratios and the intensity of the aromatic and phenolic NMR signal did not show any significant trend with increasing MAT (Table 2, Fig. 4b), suggesting that temperature alone does not control the preservation and degradation of these compounds and that there may be other environmental factors involved in the stabilization of lignin in soils (Pisani et al., 2014; Thevenot et al., 2010). Because lignin degradation was also not significantly correlated with soil mineralogy or pH, other environmental factors may be more important for lignin stabilization. In another study, grassland soils collected along a MAT gradient showed a decreasing degree of lignin degradation with increasing MAT and this was attributed to a lack of additional carbon sources, such as saccharides and root litter, which are needed for the co-metabolic biodegradation of lignin (Amelung et al., 1999). The soil nitrogen content can have a variable influence on lignin degradation as a function of local environmental conditions (Thevenot et al., 2010). Lignin oxidation may also be enhanced by abiotic mechanisms such as photo-degradation (Feng et al., 2011) which should be enhanced in grassland soils due to the lack of shading from trees. Freezing and thawing cycles may also play a role in lignin oxidation (Feng et al., 2007), especially in the cooler soils that regularly undergo repeated freezing and thawing.

Alkyl SOM structures that are abundant in the cuticles of plant leaves and the suberin of roots (Kolattukudy, 1980; Lorenz et al., 2007) are believed to be relatively stable and have been shown to accumulate in various types of soils (Feng et al., 2008; Lorenz et al., 2007; Nierop, 1998; Pisani et al., 2014; Riederer et al., 1993). The stability of these compounds in soil is typically attributed to their inherent molecular structure (Lorenz et al., 2007; Pisani et al., 2014) although their interactions with mineral surfaces through the selective sorption of polymethylene carbon (Feng et al., 2005; Lin and Simpson, 2016) and the preferential preservation of root- versus leaf-derived lipids in soils (Mueller et al., 2013) have also been reported. The S/C ratio of the soils showed a linear decrease with increasing MAT (Pisani et al., 2014) despite the different mineralogy and other environmental characteristics, suggesting that these soils may share a common set of ecological mechanisms that govern some of their biogeochemical processes (Fierer et al., 2009). Long-chain alkyl components in the soils collected along this same MAT gradient were found to accumulate with increasing MAT (Pisani et al., 2014). Due to a lack of correlation with the soil clay content and soil pH, this trend was attributed to the inherent chemical recalcitrance of these alkyl structures and to the preferential degradation of other SOM components (Pisani et al., 2014). However, 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 multiple environmental factors (Schmidt et al., 2011).

4.2. Preservation and degradation of soil organic matter components with soil cultivation

Measurement of the SOC contents (Table 1) reveals that cultivation reduced between 10 and 42% of the original carbon present in virgin, uncultivated soils. The average of 26% determined for this study is very similar to that quoted by Davidson and Ackerman (1993) and may contribute significantly to CO_2 release to the atmosphere on a global scale (Cole et al., 1997). Changes in land-use may increase the total soil microbial biomass and shift the community structure toward a fungal-dominated community, enhancing the accumulation of microbial-derived organic matter (Six et al., 2006). In this study, the relative abundance of microbial-derived solvent-extractable SOM components (short-chain alkyl compounds and microbial sterols) significantly increased with cultivation at warmer temperatures (Fig. 1). This was also the case on a per year basis (Fig. 2a) where microbial-derived compounds showed the largest increase at the warmest site. A shift toward microbial-derived SOM components with cultivation was also observed by a change in C_{max} values from long-chain to short-chain alkyl compounds (Tables S1 and S2). This large increase in microbial components may be related to the higher concentration of ergosterol, a signature lipid for soil fungi (Ruzicka et al., 2000) which have been suggested to contribute to enhanced carbon sequestration in agricultural soils (Six et al., 2006). The concentration of microbial components along the studied transect suggest that agricultural management practices differ in the type and intensity of disturbance, varying the impact on microbial biomass and microbial-derived SOM depending on the management practice employed (Plaza et al., 2013; Six et al., 2006). In addition, the degree to which microbial-derived organic matter is protected from decomposition in soil depends on their interaction with the soil mineral matrix and physical occlusion within stable soil aggregates (Six et al., 2000).

Cultivated soils generally show a lower contribution of plant-derived SOM components compared to native soils (Nierop et al., 2001; Quénéa et al., 2006) likely due to changes in vegetation, soil aggregate disruption and other disturbances associated with management practices (Elliott, 1986; Paul et al., 2004). The soil fraction that is typically associated with plant-derived material (>250 μm) has been shown to be significantly reduced in cultivated soils collected along our same MAT transect, particularly at the colder grassland sites (Table S6). In the present study, the solvent-extractable plant-derived SOM decreased from the time of land-use conversion (with the only exception of Indian Head-Saskatchewan) and the warmest sites showed the largest decreases of plant-derived SOM (Fig. 2a). Two of the major plant-derived SOM structural classes found in soils are the aromatic structures of lignin and the alkyl structures of plant waxes, cutin and suberin (Kögel-Knabner, 2002; Lorenz et al., 2007). Lignin has long been suspected to contribute to the stable carbon pool in soils (Kögel-Knabner, 2002; Melillo et al., 2002; Waksman, 1929), but studies have suggested that it may be degraded in agricultural soils (Kiem and Kögel-Knabner, 2003; Lobe et al., 2002; Stevenson, 1982). In agreement with a previous study (Lobe et al., 2002) the total lignin content was higher in the native grassland compared to the cultivated soils (Table S5), suggesting enhanced lignin degradation with land-use. Lignin oxidation may be favored with cultivation practices that result in aggregate disruption and degradation of particulate organic matter (Elliott, 1986; Paul et al., 2006). This is also shown by the higher Ad/Al ratios of both syringyl and vanillyl phenols of the cultivated soils (Fig. 4b). This was particularly the case at the warmer sites where the conversion of land from forest to pasture resulted in loss of shade and likely enhancement of lignin photo-degradation.

The contribution of the alkyl structures of cutin and suberin to SOM in cultivated soils can help elucidate the fate of above- and belowground biomass because agricultural crops have much less suberized aboveground tissues compared to woody plants (Mendez-Millan et al., 2010). In this study, soil cultivation significantly increased the S/C ratio with the exception of the Colorado site (Fig. 3a). In addition to possible changes in SOM inputs, the higher suberin content may be due to the better preservation of suberin compared to cutin compounds because suberin contains phenolic units and is embedded in root and bark tissues (Riederer et al., 1993). A significant decrease in suberin was observed in the Indian Head-Saskatchewan and Colorado cultivated soils (Fig. 2b) likely due to the particular land management practices at those sites (crop rotation and tillage; Table 1). Continuous tillage can result in soil aggregate disruption (Elliott, 1986; Paul et al., 2004) and enhance the availability of some SOM components. Cultivation resulted in cutin degradation (elevated $\omega\text{-}C_{16}/\Sigma C_{16}$ ratio) and was most pronounced in the Alberta, Indian Head-Saskatchewan and Texas soils (Fig. 3b). The differences in cutin degradation observed between sites are likely due to the varying stability of these structures in soils (Lorenz et al., 2007) or to the continuous input of fresh cutin compounds due to crop rotation. On a per year basis, the total amount of cutin decreased only at the two forest sites that were converted to pasture (Fig. 2b). This is in agreement with the reduction of the alkyl carbon NMR signal (Fig. 2d) and suggests that cutin may be susceptible to degradation with cultivation of land.

5. Conclusions

The combined use of molecular-level methods of native and cultivated soils collected along a MAT gradient revealed differences in molecularly distinct SOM components to both increasing temperature and land-use change. The variability of responses for carbohydrates and lignin to increasing MAT in the native grassland and forest soils may be attributed to other factors (e.g., the soil microbial community, moisture regime, litter quality and quantity as well as land-use). However, alkyl components from native soils collected along the same MAT gradient were found to accumulate with increasing MAT likely due to the inherent chemical recalcitrance or interactions with other soil components, such as clay mineral surfaces or other SOM constituents (Pisani et al., 2014; Feng et al., 2005; Clemente and Simpson, 2013). Increased microbial inputs and enhanced lignin degradation were the main shifts in SOM composition observed in the cultivated soils with both processes likely influenced by different site management practices (e.g., crop rotation and tillage, crop species and productivity). Soil cultivation also resulted in changes from aboveground to belowground inputs (elevated S/C ratios) and enhanced cutin degradation. These results suggest that long-term cultivation and the application of different agricultural management practices may decrease more stable SOM components, such as cutin. This carbon fraction typically accounts for a major portion of the total soil carbon pool (Li et al., 2013) suggesting that cutin-derived SOM may become a source of atmospheric CO_2 with increases in land-use change. Although our study included many soil samples collected along a bi-continental transect, future research should test the observations reported here using a wider sample range. The overarching implications of this work suggest that environmental factors, namely climate, coupled with land conversion from native to cultivated soils may decrease forms of SOM that have been previously considered to be resistant to both climate change impacts and agricultural practices.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.08.154.

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