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Biological, chemical and thermal indices of soil organic matter stability in four grassland soils

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ABSTRACT

The various ecosystem functions of soil organic matter (SOM) depend on both its quantity and stability. Numerous fractionation techniques have been developed to characterize SOM stability, and thermal analysis techniques have shown promising results to describe the complete continuum of SOM in whole soil samples. However, the potential link between SOM thermal stability and biological or chemical stability has not yet been adequately explored. The objective of this study was to compare conventional chemical and biological methods used to characterize SOM stability with results obtained by thermal analysis techniques. Surface soil samples were collected from four North American grassland sites along a continental mean annual temperature gradient, each with a native and cultivated land use. Soil organic C concentrations ranged from 6.8 to 33 g C kg^{-1} soil. Soils were incubated for 588 days at 35 °C, and C mineralization rates were determined periodically throughout the incubation by measuring CO_2 concentration using an infrared gas analyzer (IRGA) to calculate biological indices of SOM stability. Hot-water extractable organic C (HWEOC) contents were determined before and after incubation as chemical indices. Finally, samples from before and after incubation were analyzed by simultaneous thermal analysis (i.e., thermogravimetry (TG) and differential scanning calorimetry (DSC)) to determine thermal indices of SOM stability. Long-term incubation resulted in the mineralization of up to 33% of initial soil C. The number of days required to respire 5% of initial soil organic carbon (SOC), ranged from 27 to 115 days, and is proposed as a standardized biological index of SOM stability. The number of days was greater for cultivated soils compared to soils under native vegetation, and generally decreased with increasing site mean annual temperature. HWEOC (as % of initial SOC) did not show consistent responses to land use, but was significantly lower after long-term incubation. Energy density (J mg^{-1} OM) was greater for soils under native vegetation compared to cultivated soils, and long-term incubation also decreased energy density. The temperatures at which half of the mass loss or energy release occurred typically showed larger responses to land use change than to incubation. Strong correlations demonstrated a link between the thermal and biogeochemical stability of SOM, but the interpretation of the thermal behavior of SOM in bulk soil samples remains equivocal because of the role the mineral component and organo-mineral interactions.

1. Introduction

Soil organic matter (SOM) stability can be defined in terms of how easily carbon and nitrogen in the SOM can be mineralized. The identification, isolation and characterization of SOM fractions with short versus long residence times have received a great deal of scientific interest because of their implications in the permanence of soil organic C (SOC) during sequestration (e.g., Smith, 2005) and vulnerability of SOC stocks in response to disturbance or climate change (e.g., Schuur et al., 2008). Though SOM stability is often equated to recalcitrance, the concept must go beyond biochemical composition and encompass all of the various mechanisms known to stabilize SOM. A large number of SOM fractionation procedures have been developed that seek to distinguish between SOM that is more easily decomposed (i.e., relatively low stability, high-quality) or less easily decomposed (i.e., relatively high stability, low-quality) by the soil microbial population and its enzymes. These procedures have been reviewed previously (e.g., Denef et al., 2009; von Lützow et al., 2007) and include physical fractionation by size or density, and various chemical fractionation methods that separate SOM by

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solubility, hydrolysability, or resistance to oxidation. Biological fractionation, typically by laboratory incubation, separates SOM into labile and resistant components through microbial mineralization, assuming that microbes consume primarily high-quality, labile SOM and leave behind low-quality, resistant SOM.

Special emphasis has been placed on SOM fractions with rapid turn over because these respond more rapidly to changes in management or environmental conditions, and might serve as early indicators for total SOM change (Haynes, 2005). Determination of labile SOM using potentially mineralizable C (PMC) methods can require extended incubation times, and therefore more rapid fractionation- or extraction-based methods such as light fraction, particulate organic matter, microbial biomass C and N, and water soluble or hydrolysable C and N are frequently used. McLauchlan and Hobbie (2004) directly compared four common methods for measuring labile SOM (microbial biomass C, acid-hydrolysable C, respired C during 12-day incubation, and light fraction C) and found that all were positively correlated to one another but generated significantly different pool sizes.

To date, no single biological, physical, or chemical fractionation technique has been developed that adequately describes the complete continuum of SOM that exists in nature (Paul et al., 2006). However, application of thermal analysis techniques to characterize soil humic substances in the past (e.g., Schnitzer and Hoffman, 1966; Schnitzer et al., 1964; Shurygina et al., 1971) and whole soils or physical fractions more recently (e.g., De la Rosa et al., 2008; Lopez-Capel et al., 2005; Plante et al., 2005) have shown promise in measuring differences in the complete SOM stability continuum. The goal of the current study was to compare two conventional methods to characterize SOM stability with results of thermal analyses. The underlying assumption of many interpretations of SOM thermal analyses is that thermal stability is directly linked to biological or chemical stability. The current study seeks to provide preliminary results to test the validity of this assumption on whole soil samples.

2. Materials and methods

In a previous study, surface (0–20 cm) soil samples were collected from four sites along a continental mean annual temperature gradient, each with a native and adjacent cultivated land use (Table 1; Haddix et al., 2011). Collected soil samples were air-dried, passed through a 2-mm sieve, and stored at room temperature until incubations began. From these initial samples, four 80-g aliquots from each site and treatment combination were rewetted and incubated at 60% water filled pore space and 35 °C for 588 days. Samples were placed in sealed canning jars fitted with septa, along with scintillation vials containing 20 mL of water to maintain humidity. Soils were pre-incubated for three days at 25 °C and then four days at 35 °C prior to measurements to account for and avoid the "Birch effect" in subsequent measurements. Headspace gas samples were analyzed for CO_2 concentration using an LI-COR LI-6252 infrared gas analyzer (IRGA) (LI-COR Biosciences Lincoln, NE, USA). Jars were flushed with compressed tank air before CO_2 concentrations reached 5% to prevent CO_2 concentration from inhibiting microbial activity. CO_2 measurements were taken at 36 sampling times over the course of 588 days. Further details of the incubation experiment are found in Haddix et al. (2011). Once the incubation was completed, incubated samples were air-dried and archived for further analyses. Total organic C concentrations of samples before and after incubation were determined by dry combustion in a Carlo-Erba NC1500 elemental analyzer.

2.1. Biological indices of SOM stability

We used cumulative CO_2 respiration during the long-term incubation to calculate two biological indices of SOM stability in the current study. The first, and most conventional, index is to express the total amount of CO_2 respired as a proportion of initial SOC. We also determined the number of incubation days required to respire fixed proportions (5 and 10%) of initial SOC. The numbers of incubation days were determined by fitting cumulative respiration data to fourth-order polynomial functions and solving for time given fixed amounts of CO_2 respired. We selected the fourth-order polynomial model because of goodness of fit rather than any implied mechanistic expression of SOM decomposition because our goal was strictly to achieve the best estimate for incubation durations.

2.2. Chemical indices of SOM stability

Hot-water extractable organic carbon (HWEOC) was measured on air-dry samples before and after incubation using a method modified from Sparling et al. (1998) as a chemical means of assessing SOM stability. Approximately one gram of soil was mixed with 10 mL of water in 16 × 100-mm glass culture tubes and shaken for 30 min. Tubes were transferred to a hot water bath at 70 °C. After 18 h, tubes were removed from the bath and mixed on a vortex shaker for 3 s, and suspensions were vacuum filtered. Solutions were analyzed for total organic carbon (TOC) using a persulfate digestion and spectrophotometric method (Method 10173, Hach Company, Loveland CO). Results are reported as a concentration (i.e., μg C g^{-1} soil) or as a proportion of initial SOC.

2.3. Thermal indices of SOM stability

Several thermal indices of SOM stability were calculated using simultaneous thermogravimetry (TG) and differential scanning

Table 1
Characteristics of the sites and sampled soils used in the long-term incubation and subsequent analyses.

Site	Location	MAT/MAP[a]	Soil taxonomy	Treatment	Vegetation	Clay (g 100 g^{-1})	Initial SOC concentration (g C kg^{-1} soil)
Indian Head, SK	50.533 N −103.517 E	2 °C 421 mm	Udic Boroll	Native	Cool-season grass dominant grassland	50	33.1
				Cultivated	Spring wheat dominant crop rotations	61	21.0
Mandan, ND	46.767 N −100.917 E	5 °C 402 mm	Typic Argiboroll	Native	Warm, mixed-grass prairie	28	28.1
				Cultivated	Continuous spring wheat	33	23.8
Akron, CO	40.150 N −99.143 E	9.2 °C 420 mm	Aridic Paleustoll	Native	C_4 grass dominant grassland	23	12.0
				Cultivated	Continuous wheat	28	6.8
Vernon, TX	33.939 N −99.143 E	17 °C 665 mm	Typic Paleustoll	Native	Mixed C_4 and C_3 grassland	31	8.2
				Cultivated	Continuous wheat	44	7.1

[a] MAT, mean annual temperature; MAP, mean annual precipitation.

calorimetry (DSC) data from samples before and after incubation. Thermal analyses were performed using a Netzsch STA 409PC Luxx simultaneous thermal analyzer equipped with a type-S (Pt/PtRh) TG–DSC sample carrier (Netzsch–Gerätebau GmbH, Selb, Germany). Prior to analysis, air-dry soils were lightly ground in a mortar and pestle to pass a 500 μm sieve. Samples (30 mg) were placed in a Pt/Rh crucible (with an identical and empty crucible used as the reference) and heated from ambient to 700 °C at 10 °C min^{-1}under an oxidizing atmosphere of 30 mL min^{-1} of synthetic air (20% O_2 and N_2 balance) and 10 mL min^{-1} of N_2 as a protective gas. The instrument was previously calibrated for temperature and enthalpy sensitivity using the melting points of five salts, and calcium oxalate was periodically used as a check. DSC data were corrected for baseline drift using instrumental correction runs, as well as *a posteriori* corrections. During *a posteriori* baseline correction, the region >600 °C was defined as baseline because SOM oxidation has generally ended by this point and minimal mass loss was observed. An inflection in the raw DSC data was generally observed near 190 °C, suggesting a transition between endothermic water loss and exothermic organic matter oxidation. For this reason, the region 180–200 °C was also set as baseline. The remaining baseline of DSC curves was determined using the non-parametric baseline fitting function of Peakfit software (Systat Software Inc., Chicago IL).

Total exothermic energy content (in mJ) was determined by integrating the DSC heat flux (in mW) over the exothermic region 190–600 °C, which was found to represent the temperature range in which SOM is oxidized. Thermogravimetric mass loss was determined for the same range. Energy density (J mg^{-1} OM) was thus determined by dividing energy content by thermogravimetric mass loss, *sensu* Rovira et al. (2008). We also calculated two additional thermal indices of SOM stability suggested by Rovira et al. (2008), the temperature at which half of the exothermic mass loss has occurred (TG-T_{50}) and the temperature at which half of the exothermic energy has been released (DSC-T_{50}).

2.4. Data analysis

Results of analyses performed on samples before laboratory incubation are reported as means without measures of variability. Only the mean is reported because the four aliquot samples on which analyses were performed were pulled from the same original sample, representing pseudo-replication. Conversely, respiration results from the incubation and analyses performed on samples after the incubation are reported with standard errors of the mean because the aliquot samples underwent the incubation separately and thus represent independent replicates. Relationships between biological and chemical versus thermal indices of SOM stability were tested by correlation. Pearson product moment correlation coefficients and associated probabilities were determined using SigmaPlot11 software (Systat Software Inc., San Jose CA). Thermal indices calculated for samples before incubation were tested against HWEOC (as % initial SOC) measured on samples before incubation, and against respiration data from the incubations.

3. Results

The total amount of C respired as CO_2 during the long-term incubation was significantly different ($P < 0.001$ by paired *t*-test) from the change in measured SOC before and after incubation (data not shown); such that the measured change in SOC generally underestimated the amount of CO_2 respired. Amounts of CO_2 respired during the incubation as a proportion of initial SOC concentration ranged from 13.3 to 33.5%, and were generally greater for native compared to cultivated soils at each site (Table 2).

Table 2
Initial soil organic carbon concentration of native and cultivated samples from four sites, and amount of CO_2 respired during 588 days of laboratory incubation at 35 °C (mean ± standard error, $n = 4$).

Site	Treatment	Total CO_2 respired		Days to respire *x*% of initial C	
		(μg C g^{-1} soil)	(% initial SOC)	5%	10%
Indian Head, SK	Native	5326 ± 86	16.1 ± 0.3	74 ± 4	226 ± 9
	Cultivated	2985 ± 114	14.2 ± 0.5	103 ± 5	327 ± 25
Mandan, ND	Native	4869 ± 938	17.3 ± 3.3	109 ± 16	318 ± 88
	Cultivated	3175 ± 297	13.3 ± 1.2	115 ± 8	357 ± 38
Akron, CO	Native	3509 ± 278	29.2 ± 2.3	34 ± 3	79 ± 3
	Cultivated	1667 ± 179	24.5 ± 2.6	47 ± 1	116 ± 6
Vernon, TX	Native	2738 ± 49	33.5 ± 0.6	28 ± 1	62 ± 2
	Cultivated	2360 ± 163	33.2 ± 2.3	27 ± 3	56 ± 5

The number of days required to respire a fixed proportion of initial soil C was also less for native soils compared to cultivated soils, but the difference decreased as site mean annual temperature increased, to the point where the treatments at Texas were not statistically different (Table 2). Overall, the proportion of initial SOC respired increased, and the number of days to respire a fixed proportion of initial soil C decreased, as the mean annual temperature of the sites increased, which the exception of the Mandan site (Table 2).

Amounts of HWEOC (in μg C g^{-1} soil) were significantly greater in native compared to cultivated soils at each site, both before and after incubation (Table 3), as might be expected due to greater SOC concentrations. When expressed as a proportion of initial SOC concentration values ranged from just under 2% up to 11%. Differences between native and cultivated treatments were not consistent, but HWEOC proportions before incubation were significantly greater than those determined after incubation in both native and cultivated samples from all sites. A trend in HWEOC proportions across the mean annual temperature gradient of sites was not evident in samples before incubation, but increased with increasing mean annual temperature in samples after incubation.

Differences in SOM concentrations between native versus cultivated samples and between samples before and after incubation are reflected in the heights and areas of the exothermic DSC peaks (Fig. 1). Interestingly, qualitative differences in the DSC curves were greater at lower temperatures (<400 °C) compared to smaller differences (if any) at higher temperatures. The exothermic region 190–600 °C was selected to represent SOM even though the thermal behavior observed in the TG and DSC curves can be attributable to both the organic and mineral components of the soil. While several soil mineral components may lose significant amounts of mass during TG analysis (e.g., Nieto et al., 2008), these mass losses appear to be relatively minor in the current study as indicated by relationships with sample organic C concentration. Thermogravimetric mass losses and energy contents calculated for the exothermic region 190–600 °C were highly correlated to sample organic C concentrations as measured by elemental analysis ($r > 0.95$; Fig. 2). The slope of the regression between TG mass loss and sample C concentration was found to be 2.06. This estimate is greater than the "van Bemmelen factor" typically used to convert soil organic C to soil organic matter, suggesting that more than SOM mass was lost. However, in an extensive review, Pribyl (2010) suggested the conversion factor of 2 would be more accurate than the conventional factor of 1.724. These results indicate that TG mass loss in the exothermic region is dominated by SOM with little contribution from the mineral fraction. Calculated energy densities ranged from 4.70 to 12.01 J mg^{-1} OM, and showed statistically significant differences between land uses and incubation treatments (Table 4). Values of TG-T_{50} and DSC-T_{50} also differed between

Table 3
Hot-water extractable organic carbon (HWEOC) of native and cultivated samples from four sites, before and after 588 days of laboratory incubation at 35 °C (mean ± standard error, $n = 4$).

Site	Treatment	HWEOC before incubation		HWEOC after incubation	
		(μg C g^{-1} soil)	(% of total SOC)	(μg C g^{-1} soil)	(% of total SOC)
Indian Head, SK	Native	1860	5.7	593 ± 11.8	1.9 ± 0.07
	Cultivated	610	2.8	298 ± 12.5	1.6 ± 0.15
Mandan, ND	Native	1770	6.2	628 ± 67.0	2.8 ± 0.35
	Cultivated	1730	7.1	476 ± 20.3	2.2 ± 0.05
Akron, CO	Native	965	8.2	391 ± 14.6	4.0 ± 0.20
	Cultivated	580	8.5	298 ± 49.9	4.8 ± 0.85
Vernon, TX	Native	960	11.2	288 ± 19.6	4.4 ± 0.34
	Cultivated	515	7.4	235 ± 26.7	4.4 ± 0.53

land use and incubation treatments (Tables 5 and 6), though the sign and magnitude of the change differed between the two indices.

4. Discussion

4.1. Conventional indices of SOM stability

Significant decreases in SOC concentrations were found in cultivated soils compared their native counterpart at each site, which is typical of land use conversions (e.g., Davidson and Ackerman, 1993). However, changes in SOM quantity provide insufficient information to assess changes in SOM stability, thus requiring additional analyses. Potentially mineralizable C is frequently reported as a measure of SOM stability. Values of PMC can be reported as either the total amount of CO_2 respired during the incubation or as the proportion of initial SOC respired. In the current study, native soils from each site respired more C than cultivated soils in absolute (μg C g^{-1} soil) and relative (% initial SOC) terms. This suggests the presence of a greater proportion of labile SOM in the native soils. Potentially mineralizable C can account for a wide range of total SOC (0.8–12%; Haynes, 2005). This wide range reflects a lack of consensus for the duration of incubations, which is a significant barrier to using this index for comparing SOM stability across studies. We therefore propose an alternative expression of SOM stability that reports the number of days of incubation required to respire a fixed proportion of initial SOC representative of the labile pool. Even though the number of days required to respire 10% of initial SOC was less than one year in all cases (Table 2), we propose the number of days required to respire 5% of initial SOC as a simple and practical index of the stability of SOM. Microbial biomass carbon has been found to be closely related to HWEOC (Sparling et al., 1998). Both fractions have been used to represent a labile SOM pool, and they generally represent 1–5% of total SOC (Haynes, 2005). In the current study, native soils required significantly shorter incubation periods than cultivated soils to

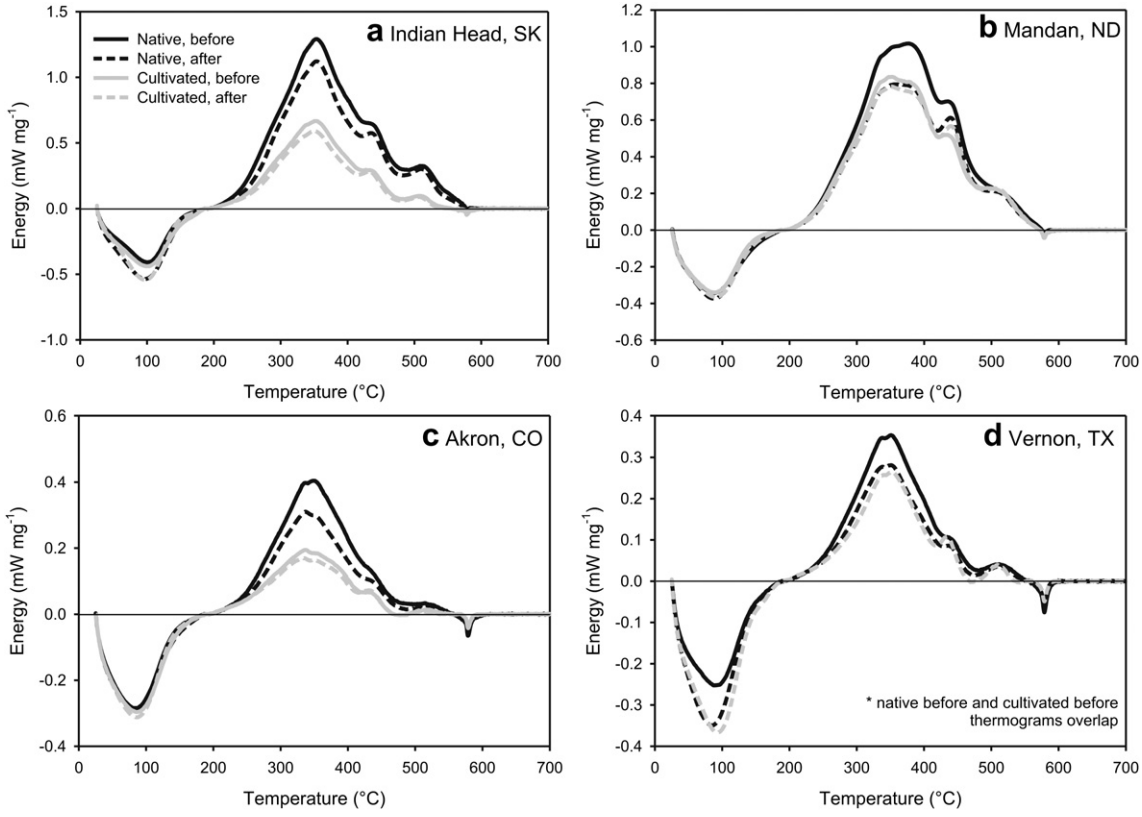

Fig. 1. Results of differential scanning calorimetry (DSC) of native and cultivated samples from four sites, before and after 588 days of laboratory incubation at 35 °C.

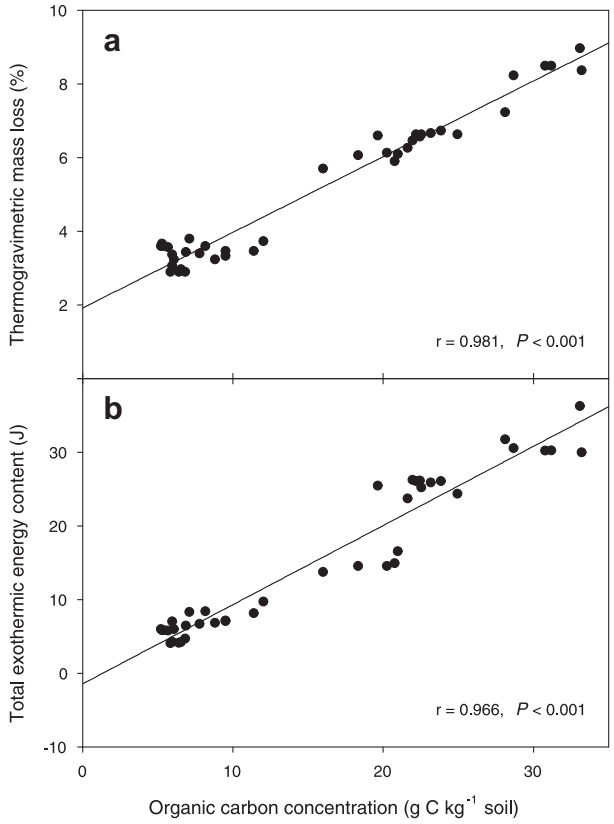

Fig. 2. Correlationsbetween soil organic carbon concentration and a) thermogravimetric mass loss and b) total exothermic energy content in the exothermic region (190–600 °C), for native and cultivated samples from four sites, before and after 588 days of laboratory incubation at 35 °C.

respire 5% of initial SOC; further support for a greater proportion of labile SOM in the native soils. The disadvantage of using days to respire 5% of initial SOC is that it requires potentially longer incubation times than those used for PMC (typically 30–90 days), particularly since the number of days reported in this study was from incubations performed at 35 °C rather than the usual 20 or 25 °C. The warmer temperature for these incubations was used to maximize C mineralization to achieve greater resolution in the subsequent thermal analyses.

As a whole, results of the biological indices of SOM stability generally support the hypothesis that SOM in the native soils had a greater proportion of more easily decomposable compounds than SOM in the cultivated soils (i.e., greater proportion of SOC respired,

Table 4
Energy density of native and cultivated samples from four sites, before and after 588 days of laboratory incubation at 35 °C. Energy density is the integral of the differential scanning calorimetry (DSC) signal (in J) divided by the thermogravimetric (TG) mass loss of organic matter (in mg), both determined over the exothermic region 190–600 °C (mean ± standard error, $n = 4$).

Site	Treatment	Energy density before incubation (J mg^{-1} OM)	Energy density after incubation (J mg^{-1} OM)
Indian Head, SK	Native	13.48	12.01 ± 0.12
	Cultivated	9.05	8.10 ± 0.11
Mandan, ND	Native	14.64	13.04 ± 0.28
	Cultivated	12.91	12.77 ± 0.08
Akron, CO	Native	8.68	7.21 ± 0.22
	Cultivated	5.37	4.70 ± 0.02
Vernon, TX	Native	7.79	6.50 ± 0.18
	Cultivated	6.40	5.42 ± 0.05

Table 5
Temperature at which one half of the thermogravimetric (TG) mass loss has occurred (TG-T_{50}, °C) for native and cultivated samples from four sites, before and after 588 days of laboratory incubation at 35 °C (mean ± standard error, $n = 4$).

Site	Treatment	Before	After
Indian Head, SK	Native	384.4	387.9 ± 0.5
	Cultivated	410.9	417.1 ± 1.0
Mandan, ND	Native	382.5	386.5 ± 0.7
	Cultivated	382.8	386.2 ± 1.2
Akron, CO	Native	392.5	396.3 ± 0.5
	Cultivated	415.7	418.3 ± 1.1
Vernon, TX	Native	405.7	412.5 ± 1.1
	Cultivated	425.7	429.2 ± 0.4

fewer days required). The results also suggest that soils from sites with greater mean annual temperature had a greater proportion of more easily decomposable SOM than those from sites with cooler mean annual temperatures.

Hot-water extractable organic carbon has been identified as a rapid means of isolating a pool of labile SOM (Davidson et al., 1987; Ghani et al., 2003; Sparling et al., 1998). Several biochemical analyses have revealed the composition of HWEOC is dominated by bioavailable carbohydrates and N-containing compounds, with few aromatic compounds (Balaria et al., 2009; Landgraf et al., 2006; Leinweber et al., 1995), indicating a significant contribution from the soil microbial biomass (Sparling et al., 1998). The results of using HWEOC (as % initial SOC) as a chemical index of SOM stability did not support our hypothesis that SOM in the native soils had a greater proportion of more decomposable compounds than SOM in the cultivated soils (i.e., greater proportion of HWEOC). This may be due to the highly dynamic and heterogeneous properties of HWEOC itself. However, significant decreases in the proportion of HWEOC after laboratory incubation suggest that a significant amount of this fraction may be considered labile SOM because a significant proportion of the pool was mineralized, or at least transformed to the point of no longer being extractable, after 588 days of incubation.

4.2. Thermal indices of SOM stability

Results of DSC analyses (Fig. 1) showed significant differences in the exothermic reactions in the region <400 °C between land uses and incubation treatments, suggesting enhanced mineralization after land use conversion and mineralization during incubation consumed thermally labile SOM. Though differences in the region >400 °C were small, it should be noted that this region contributed a greater proportion of the DSC curves from SK and ND soils compared to curves from the CO and TX soils. Previously published thermal indices of SOM stability have primarily used ratios of TG mass loss in various temperature regions, referred to as Exo_1 and Exo_2 (e.g., Dell'Abate et al., 2002; Kristensen, 1990; Lopez-Capel

Table 6
Temperature at which one half of the energy release has occurred (DSC-T_{50}, °C) for native and cultivated samples from four sites, before and after 588 days of laboratory incubation at 35 °C (mean ± standard error, $n = 4$).

Site	Treatment	Before	After
Indian Head, SK	Native	365.4	365.2 ± 0.5
	Cultivated	355.0	353.8 ± 0.3
Mandan, ND	Native	375.0	374.6 ± 1.1
	Cultivated	370.3	374.0 ± 0.7
Akron, CO	Native	350.7	347.5 ± 0.6
	Cultivated	343.5	342.1 ± 0.5
Vernon, TX	Native	351.4	349.5 ± 0.3
	Cultivated	350.7	350.1 ± 0.3

et al., 2005). Weight losses and the associated exothermic reactions reflected in DSC curves at temperatures <400 °C have typically been attributed to labile SOM, while losses and reactions at higher temperatures are attributed to more stable SOM or potential condensation products derived from thermal reactions during the analysis. However, the temperature limits for separating thermally labile (Exo_1) from thermally resistant (Exo_2) regions have not always been rigorously defined and can vary significantly (Plante et al., 2009) due to organo-mineral interactions leading to deterministic, but difficult to predict, effects on thermal decomposition. Prior to their analyses, Duguy and Rovira (2010) demineralized their samples by hydrofluoric (HF) acid pre-treatment and were thus able to identify unambiguous minima in their bimodal DSC signals to define two thermal regions. The DSC curves in the current study did not show such distinct bimodal patterns because samples were not pre-treated (Rovira, *personal communication*). Indeed, the shape of the exothermic region and the relatively low energy densities observed (4.7–12 J mg^{-1} OM) are likely due to energy absorption by the mineral matrix and energy consumption for the desorption of the SOM from the mineral phase.

Fig. 3 provides a summary of the differences in thermal indices for native versus cultivated land uses, before versus after incubation and with site mean annual temperature, and illustrates the relationships between energy density and the TG-T_{50} and DSC-T_{50}

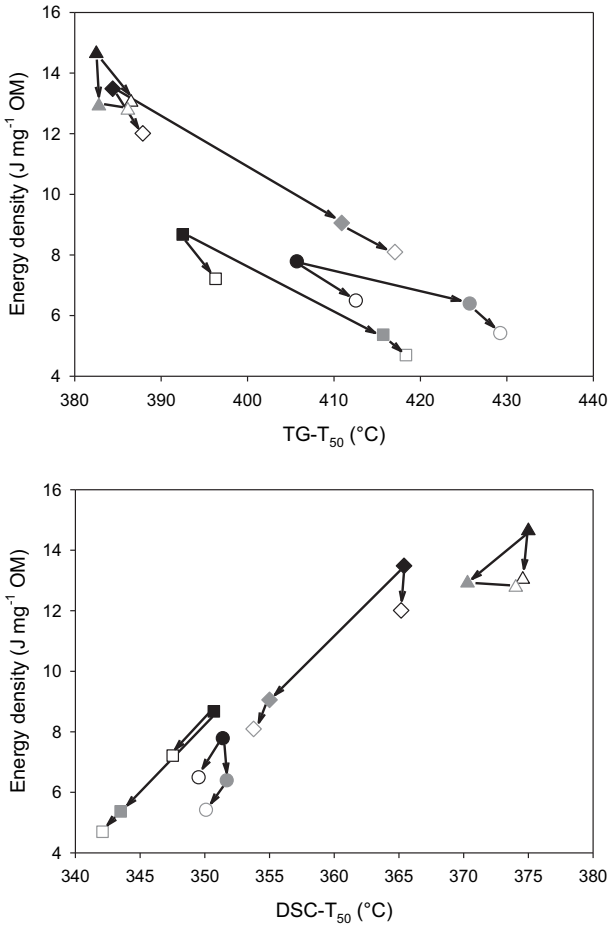

Fig. 3. Changes in thermal indices of soil organic matter stability. Black points represent native samples before incubation, gray points represent cultivated samples before incubation, and open points represent native or cultivated (black or gray outline) samples after incubation. (●, Vernon, TX; ■, Akron, CO; ▲, Mandan, ND; ◆, Indian Head, SK).

indices. We observed three general trends in DSC energy density data (Table 4). Greater energy densities were observed in native versus cultivated samples, in samples before versus after incubation, and in samples from sites with lower versus greater mean annual temperature. Several studies have reported increasing caloric contents (i.e., energy density) in plant residues and litters during decomposition using bomb calorimetry (De la Cruz and Gabriel, 1974; Uvarov, 1990; Van Cleve, 1971) and DSC (Rovira et al., 2008), though Malone and Swartout (1969) provided a potential exception when they observed decreasing caloric content with decreasing particulate organic matter size in old-field and forest soils. We speculate that the conflicting observations of energy density between most previous studies and the current study may be attributed to the nature of the organic matter being tested. Some of these studies examined litter materials while our study examines SOM in bulk soil samples, where a significant proportion of SOM is mineral associated, is likely of greater maturity, and therefore has a significantly different composition. The contribution of thermal reactions of the mineral phase is relatively small compared to that of SOM on an equivalent mass basis (Langier-Kuźniarowa, 2002), but the composition of mineral associated SOM has been shown to be significantly different from uncomplexed organic matter (Lopez-Capel et al., 2005). At a minimum, homogeneity of the mineralogical composition in the native and cultivated samples within sites allows straightforward comparisons because the presumable effect of minerals should represent a consistent background.

While trends in energy density were contrary to Rovira et al. (2008), general trends observed for TG-T_{50} and DSC-T_{50} were consistent. Lower TG-T_{50} temperatures were observed in native versus cultivated samples, before versus after incubation, and lower versus greater mean annual temperature (Table 5). Individual differences were sometimes small, but they were statistically significant overall. Greater DSC-T_{50} temperatures were generally observed in native versus cultivated samples, before versus after incubation, but differences across the mean annual temperature gradient were not consistent, and the differences were not found to be statistically significant overall (Table 6).

4.3. Linking biological, chemical and thermal indices of SOM stability

Bosatta and Ågren defined SOM quality as the number of enzymatic steps required to mineralize SOM to CO_2, such that the larger the number of steps the lower the quality of the SOM, and conversely, the higher the quality of SOM the more rapidly the microbial community can consume and grow on this SOM (Ågren and Bosatta, 1996; Bosatta and Ågren, 1999). In addition, SOM quality is thought to decrease as decomposition proceeds (Ågren and Bosatta, 1996). The authors saw this as a means to connect SOM stability to thermodynamics. Rovira et al. (2008) expanded on these concepts by separating and defining the terms recalcitrance and quality, where *recalcitrance* was equated to the energy input required to assimilate or mineralize a substrate (consistent with the generally accepted definition of quality) and where *quality* was redefined and equated to the net energetic benefit obtained from such assimilation or mineralization.

This conceptual framework provides an important theoretical rationale to link SOM stability in the biogeochemical sense with some measure of system energy (i.e., by thermal analysis), but the link is not simple and self-evident because SOM stability is not exclusively a function of quality (as defined above). SOM stability goes beyond biochemical composition and encompasses several stabilization processes: i) selective biodegradation of readily decomposable biomass constituents and the accumulation of recalcitrant

biomacromolecules, ii) physico-chemical interactions with clay surfaces and aluminum and iron oxides, and iii) decreased accessibility to microbes due to physical occlusion within soil aggregates (Sollins et al., 1996; von Lützow et al., 2006). Mineral interactions are increasingly recognized as the primary mechanism for long-term SOM stabilization in upland soils (Marschner et al., 2008), and are of particular importance in thermal assessments of SOM stability.

Regression analysis was used to compare the new thermal indices of SOM stability to the more conventional indices (Table 7). The selected chemical index of SOM stability (HWEOC as % initial SOC) was not well correlated with any of the thermal indices: energy content, energy density, TG-T_{50} or DSC-T_{50}. The lack of correlation is consistent with the lack of general trends in HWEOC observed among treatments and sites. Three of the four thermal indices were well correlated with the two biological indices of SOM stability: CO_2 respired as % initial SOC and days to respire 5% of initial SOC. TG-T_{50} was not well correlated with the biological indices. The strong relationships between energy content and the biological indices were consistent with expectations; as energy content increased, the proportion of respired SOC decreased and the number of days to respire 5% of initial SOC increased (Table 7). However, it is important to note that energy content is inherently a function of SOC quantity (see Fig. 2). Conversely, energy density is normalized for SOC concentration of the sample and thus might better characterize SOM stability. DSC-$T_{50}$was similarly correlated to the biological indices; as DSC-T_{50} increased, the proportion of respired SOC decreased and the number of days to respire 5% of initial SOC increased. Taken together, the results of the correlations suggest that high energy content, high energy density and high DSC-T_{50} represents high SOM stability.

The current study provided two opportunities to directly test for changes in SOM stability: comparisons between native and cultivated samples, and comparisons before and after long-term laboratory incubation (in the case of thermal indices). In these tests, both biological and thermal indices of SOM stability generated expected results. Soil organic matter in native samples before incubation consists of SOM with low activation energy (as measured by greater relative respiration) and high energy yield (as measured by greater DSC energy density), while SOM in cultivated samples before incubation consists of higher activation energy and lower energy yield. During incubation, activation energy increases and energy yield decreases, thus making the decrease in SOM quality consistent with definitions provided by both Bosatta and Ågren (1999) and Rovira et al. (2008). However, a significant proportion of the variance explained by the observed correlations originates from the relative large differences in the number of days to respire 5% of initial SOC across sites rather than the relatively smaller differences in energy density between native and cultivated soils within sites (Fig. 4). Among other environmental variables, mean annual temperature of the sites is believed to be a significant contributor to the differences. Soil organic matter from sites with low mean annual temperature appear to consist of high activation energy and energy yield, while SOM from sites with high mean annual temperature consists of low activation energy as well as low energy yield. Therefore biological indices show the decreasing activation energy with increasing mean annual temperature consistent with the definition of SOM quality provided by Bosatta and Ågren (1999), while thermal indices show the decreasing energy yield consistent with the definition of SOM quality provided by Rovira et al. (2008).

Table 7
Pearson product moment correlation coefficients (and *P*-value) for regressions between thermal and conventional indices of soil organic matter stability. Thermal indices and hot-water extractable organic C (HWEOC) are from samples before incubation. Values in bold are statistically significant, with $P \leq 0.05$.

	HWEOC (% initial SOC)	CO_2 respired (% initial SOC)	Days to respire 5% initial SOC
Energy content (J mg^{-1} sample)	−0.497 (0.21)	**−0.762 (0.03)**	**0.740 (0.04)**
Energy density (J mg^{-1} OM)	−0.396 (0.33)	**−0.709 (0.05)**	**0.755 (0.03)**
TG-T_{50}	0.129 (0.76)	0.583 (0.13)	−0.579 (0.13)
DSC-T_{50}	−0.371 (0.36)	**−0.703 (0.05)**	**0.809 (0.01)**

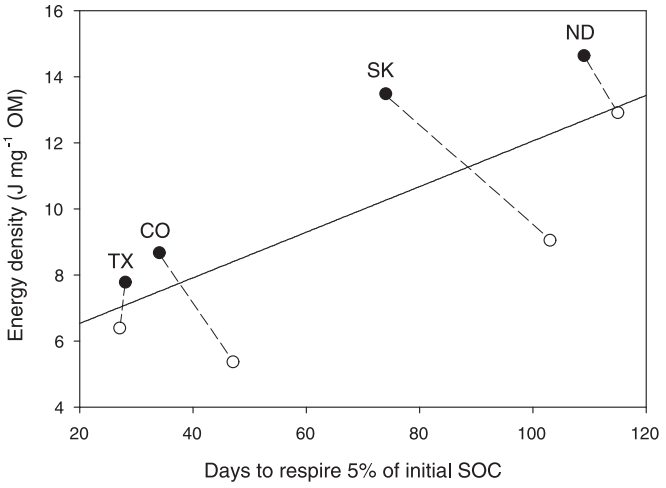

Fig. 4. Correlation between the numbers of days required to respire 5% of initial soil organic carbon (SOC) and energy density of samples before incubation. Native (closed symbols) and cultivated (open symbols) samples from the same sites (label) are connected by dashed lines (TX = Vernon, TX; CO = Akron, CO; ND = Mandan, ND; SK = Indian Head, SK).

5. Conclusions

Biological fractionation using long-term incubation might be considered the gold standard for identifying SOM that is more or less easily decomposable because it is a direct measure of the capacity of the microbial population and its enzymes to mineralize the SOM present. However, incubation durations used to measure PMC vary widely in the literature, making comparisons between studies difficult. We propose the number of days required to respire 5% of initial SOC as a simple and quantitative index of SOM stability. This will likely require longer durations of incubations than typically used, which represents an important potential impediment to adoption. Simpler and more rapid indices of SOM stability are increasingly favored. DSC-derived thermal indices such as energy density and DSC-T_{50} were strongly correlated with incubation-derived indices such as % initial C respired and days to respire 5% of initial C. These correlations explain a significant proportion of the variability in biological activity of soil during incubation, to an extent that could be used for forecasting SOM stability. The strong correlation between energy density and respiration during incubation is particularly interesting as it demonstrates a potential link between the thermal stability of SOM and its biogeochemical stability, but the interpretation of the thermal behavior of SOM in bulk soil samples remains equivocal because of the role the mineral component and organo-mineral interactions.

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