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Degree of woody encroachment into grasslands controls soil carbohydrate and amino compound changes during long term laboratory incubation

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ABSTRACT

Up to 50% of organic C and 80% of organic N within soil can exist as amino acids, amino sugars and carbohydrates. To determine how potential microbial accessibility and turnover of these compounds is impacted by encroachment of woody plants into grasslands, we investigated changes in evolved CO₂ during thermal analysis and in carbohydrate and amino compound chemistry after long term laboratory incubation of sandy loam grassland woodland soils from southern Texas, USA. Thermal analysis showed that incubation increased the amount of soil organic matter (SOM) released at higher temperatures and that evolved CO₂ profiles correlated with increases in amino C. During incubation, total carbohydrate C decreased slightly faster than bulk soil C, with preferential loss of plant-derived carbohydrates and/or production of microbial carbohydrates most strongly expressed in grassland and younger woodland soils. Total N content did not change during incubation, so the reduction in extractable amino N in older woodland soils suggested that N became more resistant to extraction during incubation. These data, along with previous measurements of respired CO₂, indicate that changes in carbohydrate C and amino C did not predict mineralized CO₂ yields and that amino compounds and microbial carbohydrate C were not selectively lost during incubation. The differing response in SOM loss (or enrichment) during incubation of the older woodland soils revealed a system with altered SOM dynamics due to woody encroachment, confirming that the short term 'lability' or 'recalcitrance' of SOM components is dependent on a number of interacting variables.

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1. Introduction

Woody plant encroachment into grass-dominated ecosystems is a globally extensive land cover change that has been occurring at a rapid rate over the past 100–200 years (Maestre et al., 2009; Van Auken, 2009; Barger et al., 2011; Eldridge et al., 2011). The phenomenon appears to be driven by a number of factors, including livestock grazing (Roques et al., 2001), reduced fire frequency (Scholes and Archer, 1997), changes in atmospheric N deposition (Köchy and Wilson, 2001), rising atmospheric CO₂ concentration (Polley et al., 1994; Bond and Midgley, 2000), and climate change (Knapp et al., 2008).

In the Rio Grande Plains region of southern Texas, woody plant assemblages dominated by N-fixing woody plants are encroaching into areas that were once open grassland (Archer et al., 1988;

Archer, 1995; Boutton et al., 1998). Following woody plant invasion in southern Texas, above and below ground primary productivity increase as soil C and N accumulate (Boutton et al., 1999; Liao et al., 2006b; Boutton and Liao, 2010), predominantly in more physically unprotected free light and particulate organic matter (POM) fractions (Liao et al., 2006a, 2006b). The amount of C held within the more physically unprotected free light and macroaggregate sized fractions increases from ca. 28% of total soil C in grassland soils to ca. 61% of total soil C in woodland soils after 40 years of woody stand development (Liao et al., 2006a, 2006b; Creamer et al., 2011). Soil OM (SOM) under the encroaching woody stands also reflects the changing plant input chemistry, with a buildup of woodland-derived lignin and aliphatic biopolymers in non-mineral bound soil fractions (Filley et al., 2008). Long term incubation of these soil samples indicated that the potential for C degradation was altered by woody stand establishment, with soil samples from older woody tree clusters (34–86 years) respiring a significantly greater proportion of soil organic carbon (SOC; 17 ± 3%) after 1 year of incubation than recently established,

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younger woody tree clusters (14–23 years) and the remnant grassland ($11 \pm 2\%$ and $9 \pm 3\%$ of SOC, respectively; Creamer et al., 2011). Stable carbon isotope evidence gathered from CO_2 respired during the incubation was unable to identify precisely the C sources which contributed to greater SOC loss from older woody clusters, although the data did suggest that after the initial few weeks, newer (C_3 woodland derived) and physically accessible C in the free light and macroaggregate sized fractions was respired preferentially from the older woody clusters due to an increase in the proportions of whole soil C held within these fractions.

SOM is a complex mixture of plant and microbial biopolymers at various stages of alteration, and preservation or accrual can result from a number of different stabilization mechanisms (Baldock and Skjemstad, 2000). Several approaches, such as soil incubation, chemical extraction, soil fractionation, thermal analyses, and spectroscopic methods, have been developed to assess the relative stability and composition of SOM with the aim of finding a rapid, consistent and quantitative index for determining the mean residence time of SOM across a wide range of environmental conditions (Kögel-Knabner, 2000; von Lützow et al., 2007; Plante et al., 2009). However, the turnover of SOM is dependent on a wide array of factors, which are often difficult to separate and are related to the properties of different ecosystems (Schmidt et al., 2011).

Organic geochemical perspectives of the potential “lability” of SOM usually focus on the relative proportions of holocellulose, lignin, and amino compounds within different fractions (Poirier et al., 2005). Cellulose and hemicellulose are important for SOM cycling as they are the most abundant plant biopolymers (Kögel-Knabner, 2002), are generally considered to be easily hydrolyzed by microorganisms, and may represent 10–30% of SOC (Guggenberger et al., 1994; Martens et al., 2004; Derrien et al., 2006) although values as high as 50% have been reported. Amino sugars and amino acids (AAs) can account for up to 15–20% of SOC (Martens et al., 2004) but, more importantly, have been shown to comprise between 30% and 80% of total soil N (Knicker et al., 1993; Amelung et al., 2001; Martens et al., 2006; Olk et al., 2008). As these compounds represent potentially important sources of C and N for microorganisms, their input rate and turnover can affect the rate of C and N cycling and help facilitate or prevent SOM accrual (Fontaine et al., 2004).

Thermal analysis uses a different perspective from chemical extraction to gain insight into the makeup of soil. It has recently been used to link thermal stability with the biological and chemical stability of SOM and, as such, may be able to provide a broader picture of SOM composition (Grisi et al., 1998; Plante et al., 2009, 2011). However, the degree and nature of mineral–OM interaction is often a difficult analytical challenge that interferes with the ability to compare potentially complementary tools used to assess SOM stability, such as thermal analysis and wet chemical extraction. The soils examined here, with low clay contents (<10%) and low proportions of whole soil C held on free silt and clay particles in the grassland (ca. 30%) and woodland (5–16%), allow utilization of different methods for assessing SOM stability (laboratory incubation, thermal analysis, wet chemical extraction) with minimal impact from soil minerals.

The purpose of this study was to document changes in SOM composition during incubation of sandy loam soils with different proportions of grass and woody tissues. Our objective was to determine if degradation of amino compounds and carbohydrates was responsible for the unexpectedly high proportion of SOC lost from older cluster soils relative to younger cluster and grassland soils during a previous laboratory incubation (Creamer et al., 2011). We used thermal analysis as a preliminary means of assessing overall changes in SOM as a result of incubation and then determined changes in relative concentrations of individual carbohydrate and amino compounds to examine whether or not specific

compounds were preserved or degraded during the incubation in response to changing OM input following woody plant encroachment.

2. Material and methods

2.1. Site description, and soil sampling and incubation

Soil samples were from the Texas AgriLife La Copita Research Area in the Rio Grande Plains of southern Texas (Blair, 1950). In this region, fire suppression combined with livestock overgrazing has resulted in progressive encroachment of subtropical C_3 thorn woodlands into the remnant C_4 -dominated grassland over the past 150 years (Archer, 1990; McLendon, 1993; Boutton et al., 1998). After initial establishment of the N-fixing *Prosopis glandulosa* (honey mesquite), other shrub/tree species, such as *Zanthoxylum fagara* (lime prickly ash) and *Diospyros texana* (persimmon), enter the understory and form woody clusters (Archer et al., 1988). Thus, woody clusters are composed of a single mesquite tree with up to 15 other tree/shrub species beneath its canopy. Soils under these woody clusters and the remnant grassland are sandy loams (fine-loamy mixed hyperthermic Typic Argiustolls of the Runge series) with 80% sand, 10% silt and 10% clay. More information on the soils is presented in Table 1. Mean annual temperature is 22.4°C and average annual rainfall 716 mm. For more detailed site descriptions, see Scifres and Koerth (1987), Archer (1995) and Boutton et al. (1998).

Cores were taken from the upper 30 cm of the mineral soil from 15 woody clusters and 15 grassland patches adjacent to each of the woody clusters in October 2006. For each woody cluster site, four cores (one in each cardinal direction) were taken within 50 cm of the base of the largest mesquite tree, divided into 10 cm depth increments, and pooled. From the adjacent grassland, four cores were taken near the base a randomly selected C_4 plant (one in each cardinal direction). The 15 woody cluster sites ranged in age from 14–86 years and established a chronosequence of woody encroachment. The basal diameter of the mesquite tree in each woody cluster was measured and used in site-specific regression equations developed by Stoker (1997) to determine the age of the tree and therefore the age of the woody cluster. After sampling, the uppermost 10 cm of the field-moist soil were passed through an 8 mm sieve and oven-dried at 50°C until constant weight. Ten of these woody cluster soils (3 younger woody cluster soils and 7 older woody cluster soils) and their corresponding grassland soils were subjected to a year incubation as described by Creamer et al. (2011). Briefly, 2 mg of soil was mixed in equal weight with ashed quartz sand and incubated at 30°C at constant moisture after addition of an inoculum. Respired CO_2 was measured periodically, and at the end of the year the samples were oven dried at 50°C for 2 days. The dried incubated soil and a sub-sample of each dry unincubated soil were ground to a fine powder using a steel ball mill (Retsch, Haan, Germany). C and N concentrations were determined for the ground soil prior to and after incubation using an elemental analyzer (EA) interfaced to a PDZ Europa 20/20 isotope ratio mass spectrometer (Sercon, Crewe, UK). Prior to and after incubation, celluloses, hemicelluloses, AAs, and amino sugars were extracted from the ground soils from all ten woody cluster soils (3 younger woody cluster sites and 7 older woody cluster sites) and three of the ten grassland soils as described below. Three replicates of the unincubated soils and the three biological replicates of the incubated soils were extracted.

2.2. Thermal analysis

Changes in SOM composition were assessed using evolved gas analysis (EGA) of CO_2 (CO_2 EGA) during the thermal analysis

Table 1

Soil properties. Grasslands represent time 0, while younger clusters are 14–23 years and older clusters 34–86 years.

	pH	mg C g ⁻¹ soil	mg N g ⁻¹ soil	Whole soil carbon (%)			
				FLF ^a	Macros ^b	Micros ^c	Free silt + clay ^d
Grassland	7.0	5.5 ± 0.13	0.56 ± 0.14	11	17	41	31
Young clusters	7.1	8.0 ± 3.2	0.80 ± 0.35	18	20	46	16
Old clusters	6.0	18 ± 2.0	1.59 ± 0.19	25	36	34	5

^a Free light fraction (<1.0 g cm⁻³).^b Macroaggregate fraction (>250 μm).^c Microaggregate fraction (52–250 μm).^d Free silt and clay fraction (<53 μm).

combustion of SOM. Preliminary results showed that patterns of CO₂ EGA and differential scanning calorimetry (DSC) were highly correlated, making both well suited to SOM characterization. DSC is strongly affected by the changing heat capacity of the mineral matrix during analysis, so requires significant correction for baseline drift. This is particularly problematic for low SOM samples. Due to the low SOM content of the samples, we elected to use CO₂-EGA data to characterize changes in SOM.

A subset of incubated and unincubated soils taken along the chronosequence was subjected to thermal analysis by heating 30 mg of the soil and sand mixture from 30 to 700 °C at 10 °C min⁻¹ (with a 15 min isothermal stop at 105 °C to drive off moisture) using a Netzsch STA 409PC Luxx TG-DSC thermal analyzer equipped with a type-S (Pt–Rh) sample carrier (Netzsch-Gerätebau GmbH, Selb, Germany). Samples were held in Pt crucibles, with an empty reference crucible under an oxidizing atmosphere of 30 ml min⁻¹ with synthetic air (20% O₂ and N₂ balance) and 10 ml min⁻¹ with N₂ as protective gas. CO₂ EGA was measured using a LI-840A CO₂/H₂O infrared gas analyzer (LI-COR Biosciences, Lincoln, Nebraska) coupled to the thermal analyzer. Due to the low clay content of the soils (<10%) and the uniformity of their texture along the chronosequence, differences in CO₂ release during thermal analysis should be primarily a result of differences in the degradability and thermal stability of SOM rather than differences in soil mineralogy. This provided an exceptional opportunity to examine, via both thermal analysis and wet chemical extraction, the impact of changing SOM chemistry in response to woody encroachment and microbial decay during incubation.

2.3. Biochemical analyses

2.3.1. Cellulose and hemicellulose

Monosaccharides derived from cellulose and hemicellulose were extracted and analyzed as described by Martens and Frankenberg (1990) and Martens and Loeffelmann (2002). Briefly, 800 μl of 6 M H₂SO₄ was added to 100 mg whole soil in a glass culture tube. After mixing, the solution was allowed to sit (30 min) at room temperature and then diluted to 1 M H₂SO₄. The solution was autoclaved for 30 min at 121 °C, centrifuged, and the supernatant was removed following quantitative rinsing of the remaining residue with distilled water. This residue was saved and sequentially processed for cellulose (strong-acid extractable carbohydrates) as described below. The supernatant containing the hemicellulose (mild acid-extractable carbohydrates) was adjusted with NaOH to pH 5.5–6.5, diluted, and an aliquot was injected into a Dionex (Sunnyvale, CA) DX-500 anion chromatograph equipped with a CarboPac PA-10 column (2 mm × 250 mm). Carbohydrates were detected using triple-pulse amperometry (Johnson et al., 1993; Olk, 2008). The stronger acid (cellulose) extraction, which isolated hydrolyzed glucose, was performed on the dried residue from the weaker acid extraction using 300 μl of 18 M H₂SO₄, which was then diluted to 1.5 M after sitting for 30 min.

2.3.2. AAs and amino sugars

The extraction and analysis of AAs and amino sugars was identical to the procedure reported by Creamer et al. (2012), and described by Martens and Loeffelmann (2003) and modified by Olk et al. (2008). Briefly, 4 M methanesulfonic acid containing 0.2% (wt.) tryptamine [3-(2-aminoethyl)indole] was added to 250 mg soil and autoclaved at 136 °C and 248 kPa for 90 min. The solution was neutralized, diluted, centrifuged and injected into a Dionex (Sunnyvale, CA) DX-500 anion chromatograph equipped with a Dionex AminoPac PA-10 column and detected with triple-pulse amperometry (Johnson et al., 1993; Olk, 2008). With this method, asparagine and glutamine are converted to glutamic acid and aspartic acid, respectively, and cysteine converted to cystine. In addition, tryptophan is not recovered, although the other AAs plus two amino sugars (glucosamine, galactosamine) and two non-essential AAs (hydroxyproline, ornithine) can be identified.

2.4. Calculations and statistical analysis

Student t-tests were used to compare measurements of extracted carbohydrates, AAs, and amino sugars before and after incubation. One-way ANOVA was used to test for differences in extracted compounds in response to stand age or landscape element. Natural groupings of respiration data obtained from the incubation, as described by Creamer et al. (2011), justified the grouping of the samples into landscape elements of younger clusters (woody stands 14–23 years), older clusters (woody stands 34–86 years) and grasslands (0 years).

Principal component analysis (PCA) was performed on CO₂ EGA data from 125–765 °C for samples before and after incubation using the NIPALS algorithm. Additionally, partial least squares regressions (PLSs) were computed, with CO₂ EGA data as the explanatory variable and chemical analysis data as response variable. PLS were performed using the NIPALS algorithms along with leave-one-out cross-validation. The PLS II method was used for amino compounds, as it allows analysis of all amino groups in a single regression run, while PLS I was applied separately for carbohydrates and glucose. PLS II was particularly suited for amino groups because it allowed accounting of the strong co-linearity between amino compounds in the calculation. The CO₂ EGA data were normalized for sample C concentration prior to PCA and PLS analysis to reflect changes in SOM quality and not SOM quantity. PCA and PLS analyses were performed using Unscrambler 10.1 software (Camo, Norway).

3. Results

3.1. Thermally evolved CO₂ gas analysis

CO₂ EGA curves for all samples displayed significant qualitative differences before and after incubation, where incubated samples had a relative decrease in CO₂ released between 330 °C and 470 °C and a relative increase in CO₂ released around 470 °C and 620 °C

(Fig. 1). PCA of these CO₂ evolution curves showed that samples before and after incubation were differentiated on both PC1 and PC2 (Fig. 2). PC1 explained 90% of the total variance and was most positively correlated with CO₂ released at 365 °C, but also with CO₂ released at 430 °C, as indicated by the shoulder visible on PC1 loadings (Fig. 2). Samples after incubation were shifted toward the left of the PCA plane vs. samples before incubation, reflecting depletion of CO₂ evolved at 365 °C during incubation. PC2 correlated most positively with CO₂ evolved at 325 °C and 510 °C, and most negatively with CO₂ evolved at 400 °C. PC2 more clearly discriminated samples before and after incubation than PC1, but also explained less of the total variance (6%). Samples before incubation were situated at the bottom of the PCA plane, whereas samples after incubation were situated at the top, reflecting enrichment in compounds

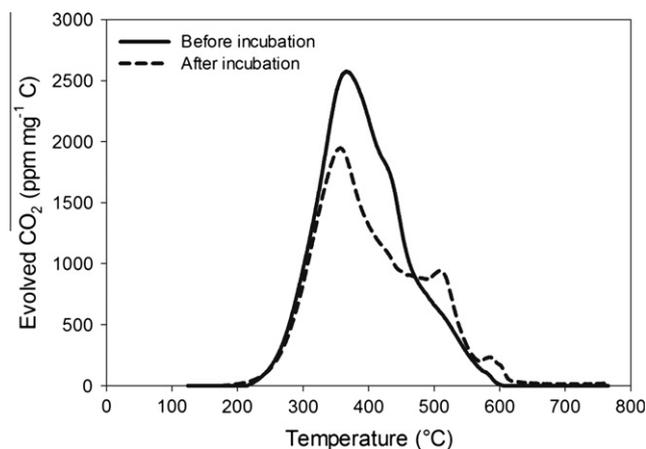


Fig. 1. CO₂ release (ppm mg C⁻¹) during thermal analysis of a grassland soil. Changes are representative of those seen in response to incubation for all landscape elements.

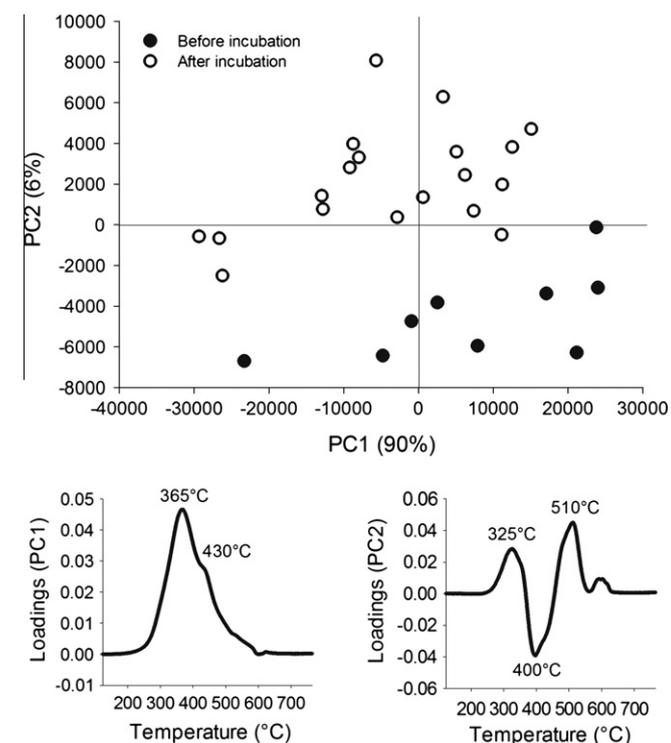


Fig. 2. PCA of CO₂ released during thermal analysis (ppm mg C⁻¹). The variance explained by each PC is shown in parentheses. Loadings are shown for PC 1 and PC2.

producing CO₂ at 325 °C and 510 °C and a depletion in compounds producing CO₂ at around 400 °C for incubated samples.

3.2. Biochemical analyses

3.2.1. Carbohydrates

For the three landscape elements (grassland, younger woody cluster, older woody cluster) the concentration of extractable carbohydrate C dropped slightly from ca. 22% of SOC to ca. 20% of SOC after incubation. However, the loss was only significant for grassland and younger cluster soils (Table 2); the loss from older cluster soils was not significant ($P = 0.30$). Younger clusters exhibited the greatest drop in carbohydrate content, shifting from 230 ± 38 mg carbohydrate-C g⁻¹ SOC before incubation (the highest among the three landscape elements) to 187 ± 33 mg carbohydrate-C g⁻¹ SOC after incubation (the lowest post-incubation values).

Among the three landscape elements, the proportion of SOC held within total extractable carbohydrates was generally consistent for both sampling times, although small differences did exist, especially between older woody clusters and the other two landscape elements (Table 2). Generally, older woody clusters had less fucose, mannose and xylose, although only some of these relationships were significant.

Glucose, calculated as the sum of the material obtained from the mild and strong acid extractable fractions, was the largest contributor to total carbohydrates in all soils, on average comprising $38 \pm 4\%$ of total carbohydrates prior to incubation and $36 \pm 3\%$ after incubation. Glucose was also the only carbohydrate compound lost significantly during incubation of all samples, although there was a decreasing trend for all carbohydrate compounds except fructose. Galactose, xylose, and arabinose were lost significantly from the soils from at least one of the landscape elements: galactose was lost from the younger woody cluster and grassland soils, xylose from both woody cluster soils, and arabinose from younger woody cluster soils only. Fucose was the only carbohydrate that increased and, although it increased for all landscape elements, the increase was significant only for grassland soils.

The ratios of mannose/xylose and (galactose + mannose)/(arabinose + xylose), which are used as general markers for ratios of microbial/plant sugars (Oades, 1984; Hu et al., 1995; Glaser et al., 2000), increased significantly for younger woody cluster soils (Table 2). Increases for other landscape elements were not significant, although the increase in the mannose/xylose did show a strong increasing trend for older cluster soils ($P = 0.07$).

3.2.2. AAs and amino sugars

The total SOC-normalized amino content (mg amino C g⁻¹ SOC) increased significantly in the grassland and younger cluster soils but remained constant in older cluster soils during incubation (Table 3). The largest increase was in the acidic AAs, whose concentration nearly doubled for grassland and younger cluster soils but increased just slightly for older cluster soils. A number of other polar and nonpolar AAs increased significantly for younger cluster and grassland soils, but many of the trends appeared to be landscape element specific. All landscape elements experienced significant increases in glycine, aspartate, and cystine and significant decreases in methionine. However, the dynamics of amino C changes seemed to differ substantially between older cluster soils vs. younger cluster and grassland soils. Grassland and younger cluster soils showed increasing trends (≥ 1 mg amino C g soil C⁻¹) in 13 and 19 out of 21 extracted amino compounds, respectively, of which 7 (grassland soils) and 12 (younger cluster soils) out of 21 extracted amino compounds showed a significant increase. For older cluster soils, however, only 5 out of 21 amino compounds showed an increasing trend, and only 3 of these were significant.

Table 2

SOC normalized concentrations of carbohydrates in native grasslands, younger woody clusters (14–23 years) and older woody clusters (34–86 years) before and after incubation. Asterisks indicate a significantly altered carbohydrate concentration in a particular landscape element (* $P < 0.05$, ** $P < 0.01$). Different symbols indicate significant differences in carbohydrate concentrations among the landscape elements ($P < 0.05$).

	Grasslands (mg carbohydrate C g ⁻¹ SOC)		Young clusters (mg carbohydrate C g ⁻¹ SOC)		Old clusters (mg carbohydrate C g ⁻¹ SOC)	
	Before	After	Before	After	Before	After
Fucose	[†] 6 ± 1.6	[†] 8 ± 2.9*	[†] 6 ± 1.8	[†] 7 ± 2.6	[§] 5 ± 1.3	[§] 6 ± 1.7
Arabinose	34 ± 5	[†] 31 ± 7	38 ± 7	[†] 31 ± 7**	36 ± 8.6	[§] 38 ± 7
Rhamnose	26 ± 10	24 ± 11	28 ± 11	23 ± 13	24 ± 9.8	24 ± 12
Galactose	35 ± 4	30 ± 3**	38 ± 6	31 ± 4**	36 ± 6.7	34 ± 6
Mannose	[†] 33 ± 7	30 ± 5	[†] 37 ± 8	33 ± 3	[§] 29 ± 4.4	26 ± 5
Xylose	[†] 24 ± 4	[†] 21 ± 5	[†] 24 ± 5	[§] 14 ± 4**	[§] 17 ± 4.8	[§] 12 ± 3*
Glucose ^a	87 ± 9	74 ± 9**	86 ± 15	67 ± 15*	86 ± 22.2	73 ± 13*
Total carbohydrates	227 ± 21	201 ± 24*	230 ± 38	187 ± 33*	216 ± 52	201 ± 34
M/X ^b	1.4 ± 0.2	1.5 ± 0.5	1.6 ± 0.4	2.4 ± 0.5*	1.8 ± 0.4	2.4 ± 1.2
(G + M)/(A + X) ^c	1.3 ± 0.2	1.4 ± 0.4	1.3 ± 0.09	1.6 ± 0.3*	1.2 ± 0.2	1.2 ± 0.1

^a Extracted in hemicellulose and cellulose fraction.

^b Mannose/xylose.

^c (Galactose + mannose)/(arabinose + xylose).

Table 3

Amino C (mg g⁻¹ of SOC) extracted before and after incubation. Asterisks indicate significant differences between amino C before and after incubation (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$).

Amino compound	Grasslands (mg amino C g ⁻¹ soil C)		Young clusters (mg amino C g ⁻¹ soil C)		Old clusters (mg amino C g ⁻¹ soil C)	
	Before	After	Before	After	Before	After
Hydroxyproline	1.3 ± 0.5	1.1 ± 0.4	2.1 ± 0.4	4.1 ± 3.2	4.1 ± 0.8	4.1 ± 0.9
Ornithine	0.1 ± 0.06	0.2 ± 0.1*	0.05 ± 0.02	0.1 ± 0.1*	0.04 ± 0.02	0.04 ± 0.04
Amino sugars	28 ± 3.4	29 ± 4.5	25 ± 4.2	33 ± 9.7*	22 ± 4.5	22 ± 4.0
Galactosamine	6.5 ± 0.7	7.2 ± 0.8	6.6 ± 0.8	9.0 ± 2.4*	5.3 ± 1.2	5.5 ± 0.9
Glucosamine	21 ± 2.8	22 ± 3.8	19 ± 3.4	24 ± 7.5	17 ± 3.3	16 ± 3.1
Acidic	17 ± 4.8	35 ± 13**	19 ± 8.5	37 ± 11**	16 ± 4.3	19 ± 7.6
Glutamate	7.5 ± 2.1	14 ± 5.5**	8.5 ± 3.8	15 ± 5.3**	7.8 ± 2.0	8.4 ± 1.0
Aspartate	9.1 ± 2.8	21 ± 7.9**	10 ± 4.8	21 ± 6.2**	8.3 ± 2.3	11 ± 4.6*
Basic	26 ± 5.1	30 ± 7.2	23 ± 6.2	31 ± 6.5*	19 ± 4.4	21 ± 4.8
Lysine	6.0 ± 1.0	6.2 ± 1.1	5.6 ± 1.0	7.5 ± 1.9*	5.0 ± 1.1	5.1 ± 1.0
Arginine	17 ± 3.7	20 ± 5.1	14 ± 4.5	20 ± 3.6*	11 ± 2.6	12 ± 3.1
Histidine	3.1 ± 0.8	3.1 ± 1.2	3.3 ± 0.9	4.2 ± 1.8	3.4 ± 0.8	3.5 ± 0.9
Polar	17 ± 2.1	20 ± 3.3*	17 ± 1.9	26 ± 7.0**	18 ± 3.2	18 ± 3.0
Threonine	6.4 ± 0.6	6.5 ± 1.1	6.7 ± 0.6	9.3 ± 2.9*	6.7 ± 1.2	6.7 ± 1.1
Serine	4.7 ± 0.7	5.1 ± 1.0	5.0 ± 0.6	7.4 ± 2.5*	5.4 ± 1.0	5.5 ± 0.9
Tyrosine	5.5 ± 0.9	8.2 ± 1.5**	5.6 ± 0.9	9.4 ± 1.8***	5.6 ± 1.2	6.1 ± 1.0
Nonpolar	48 ± 5.9	52 ± 13	52 ± 5.0	68 ± 23	52 ± 9.1	51 ± 10
Phenylalanine	2.3 ± 2.3	8.5 ± 6.0*	2.3 ± 0.9	4.3 ± 4.1	3.3 ± 1.3**	1.9 ± 1.4
Proline	6.5 ± 0.8	6.1 ± 1.0	7.3 ± 0.7	10 ± 3.4*	7.4 ± 1.4	7.5 ± 1.1
Alanine	10 ± 1.0	9.5 ± 1.8	11 ± 1.2	13 ± 4.5	9.7 ± 1.8	9.8 ± 2.2
Glycine	9.2 ± 1.2	12 ± 2.4*	8.9 ± 1.0	14 ± 2.9**	7.5 ± 1.3	9.5 ± 2.6**
Valine	8.5 ± 2.1	6.1 ± 3.0	9.0 ± 1.5	9.7 ± 4.9	8.6 ± 1.3	8.5 ± 1.9
Isoleucine	3.6 ± 0.5	3.1 ± 0.7	4.5 ± 0.6	5.5 ± 2.4	5.0 ± 0.9	4.7 ± 0.9
Leucine	6.7 ± 0.9	5.8 ± 1.1	7.7 ± 0.8	9.8 ± 4.1	9.1 ± 1.5**	7.8 ± 1.3
Methionine	0.3 ± 0.1*	0.18 ± 0.07	0.4 ± 0.2	0.4 ± 0.2	0.9 ± 0.3***	0.3 ± 0.07
Cysteine	0.7 ± 0.2	1.3 ± 0.6*	0.9 ± 0.1	1.8 ± 0.6**	0.8 ± 0.2	1.1 ± 0.3*
Total	136 ± 18	167 ± 37*	138 ± 22	199 ± 56**	132 ± 25	134 ± 26

In contrast to amino C, which increased significantly for younger cluster and grassland soils, total amino N increased slightly but not significantly for these landscape elements ($P=0.3$ and 0.14 , respectively). For older cluster soils, total amino N actually decreased significantly, predominantly through significant losses of polar and nonpolar AAs and amino sugars but also through non-significant loss of basic AAs (Table 4). Although grassland soils experienced significant losses of the nonpolar AAs proline, valine, isoleucine, leucine, and methionine, this was more than offset by a large significant increase in acidic AAs and increasing but non-significant trends in basic AAs and a few polar and nonpolar AAs. A similarly large (60%) increase in acidic AAs, driven predominantly by aspartate, occurred in younger cluster soils, but these

compounds remained unchanged for older cluster soils. Cystine and tyrosine also increased significantly for both younger cluster and grassland soils.

4. Discussion

4.1. Contribution of carbohydrate and amino compounds to respired CO₂

Despite significantly higher SOC losses from older cluster soils (17 ± 3%) relative to younger cluster (9 ± 3%) and grassland soils (11 ± 2%) (Creamer et al., 2011), total carbohydrate C was not

Table 4
Milligrams of amino N g⁻¹ soil N extracted before and after incubation. Asterisks indicate significant differences between amino-N before and after incubation (**P* < 0.05, ***P* < 0.01 ****P* < 0.0001).

Amino compound	Grasslands (mg amino N g ⁻¹ soil N)		Young clusters (mg amino N g ⁻¹ soil N)		Old clusters (mg amino N g soil ⁻¹ N)	
	Before	After	Before	After	Before	After
Hydroxyproline	2.9 ± 1.1	2.4 ± 0.9	4.9 ± 1.3	7.8 ± 6.5	10 ± 1.5**	8.9 ± 2.1
Ornithine	0.5 ± 0.3	1.0 ± 0.4*	0.2 ± 0.1	0.5 ± 0.5	0.2 ± 0.1	0.2 ± 0.2
Amino sugars	52 ± 5.1	53 ± 7.3	50 ± 9.6	52 ± 17	48 ± 10**	40 ± 6.2
Galactosamine	12 ± 1.3	13 ± 1.5	13 ± 1.9	14 ± 4.2	12 ± 2.7*	10 ± 1.4
Glucosamine	40 ± 4.0	40 ± 6.1	37 ± 7.6	38 ± 13	37 ± 7.4**	30 ± 4.9
Acidic	43 ± 13	86 ± 29**	50 ± 23	80 ± 29*	48 ± 14	47 ± 17
Glutamate	17 ± 5.0	30 ± 10**	20 ± 9	29 ± 11	20 ± 5.8	18 ± 7.0
Aspartate	26 ± 8.2	56 ± 19**	30 ± 14	51 ± 17*	27 ± 8.4	29 ± 11
Basic	169 ± 31	188 ± 49	155 ± 47	167 ± 38	138 ± 33	125 ± 25
Lysine	23 ± 3.6	23 ± 4.1	22 ± 4.3	24 ± 6.6	22 ± 4.9*	18 ± 2.8
Arginine	128 ± 27	149 ± 40	113 ± 38	123 ± 26	94 ± 23	87 ± 19
Histidine	18 ± 4.4	17 ± 6.0	19 ± 5.7	20 ± 9.5	22 ± 5.6	19 ± 4.7
Polar	43 ± 3.9	46 ± 7.1	47 ± 6.7	55 ± 18	53 ± 9.7**	45 ± 6.4
Threonine	18 ± 1.2	18 ± 2.3	20 ± 2.4	22 ± 7.2	22 ± 3.7**	18 ± 2.6
Serine	18 ± 2.0	18 ± 3.3	20 ± 3.1	23 ± 8.4	24 ± 4.3**	20 ± 3.0
Tyrosine	7.0 ± 1.1	10 ± 1.9**	7.4 ± 1.4	9.9 ± 2.1*	8.1 ± 1.8	7.4 ± 1.0
Nonpolar	153 ± 15	156 ± 34	166 ± 21	177 ± 60	175 ± 31**	146 ± 19
Phenylalanine	3.0 ± 2.9	11 ± 7.6*	2.9 ± 1.1	4.6 ± 4.6	4.7 ± 1.8***	2.3 ± 1.7
Proline	15 ± 1.6*	13 ± 2.2	18 ± 2.2	19 ± 6.6	20 ± 3.7**	16 ± 2.1
Alanine	39 ± 3.7	34 ± 6.4	42 ± 6.0	40 ± 1.5	42 ± 7.9**	34 ± 4.8
Glycine	53 ± 5.7	63 ± 12*	53 ± 7.4	65 ± 15	49 ± 8.8	48 ± 5.8
Valine	20 ± 4.8*	13 ± 6.3	22 ± 4.2	19 ± 9.9	22 ± 3.5**	18 ± 3.4
Isoleucine	6.8 ± 0.7*	5.6 ± 1.3	9.0 ± 1.4	8.7 ± 4.0	11 ± 1.9**	8.6 ± 1.5
Leucine	13 ± 1.4*	11 ± 2.0	15 ± 1.9	15 ± 6.7	20 ± 3.0***	14 ± 2.2
Methionine	0.6 ± 0.1**	0.4 ± 0.1	0.9 ± 0.6	0.7 ± 0.4	2.2 ± 0.7***	0.7 ± 0.2
Cysteine	2.8 ± 0.7	4.4 ± 1.9*	3.6 ± 0.7	5.6 ± 2.0*	3.7 ± 1.0	3.8 ± 0.9
Total	464 ± 62	533 ± 114	474 ± 97	540 ± 155	473 ± 9.6*	413 ± 67

significantly lost from older cluster soils (Table 2) and, more importantly, the carbohydrate loss was proportionally greatest for younger clusters and grassland soils. Therefore, a higher rate of carbohydrate C degradation was not the reason for higher proportional CO₂ losses from older woody cluster soils. In fact, assuming all decreases in soil extractable carbohydrate C were mineralized to CO₂, a significantly (*P* < 0.0001) smaller proportion of the respired CO₂ was derived from carbohydrate C for older cluster soils (12 ± 2.8%) compared with younger cluster (20.6 ± 4.9%) and grassland soils (24.9 ± 9%). These values should be considered the maximal carbohydrate C contribution to CO₂, since a portion of the “lost” carbohydrate C was likely recycled into other metabolites or rendered non-extractable. Despite these limitations, the calculation does highlight the fact that carbohydrate C likely contributed less to respired CO₂ for older woody cluster soils than in the other landscape elements.

As amino compound contributions to SOC remained constant in older cluster soils and increased in younger cluster and grassland soils (Table 3), it does not appear that they represented a substantial source of respired CO₂ in any of the landscape elements. However, contributions from amino C to SOC increased to a greater extent in younger cluster and grassland soils than in older cluster soils, suggesting that the stabilization or microbial production of amino compounds differed between the landscape elements. In particular, there is the potential for greater stabilization of amino C on silt and clay particles in younger cluster and grassland soils as a result of the higher proportion of whole soil C held within this fraction (Table 1).

Interestingly, the amino C increases for grassland and younger cluster soils was driven predominantly by an increase in acidic AAs (glutamate and aspartate), although a number of polar and nonpolar AAs also showed significant increases in these landscape elements. Although studies have shown modest increases in aspartate during litter decomposition and sediment digestion,

glutamate is typically lost during these processes as it is assumed to be more labile (Cowie and Hedges, 1992; Dauwe and Middelburg, 1998; Keil et al., 2001; Tremblay and Benner, 2006). Aspartate and glutamate are found in higher concentration in grass tissue at this site than in woody tissue (Creamer et al., 2012) and there is a possibility that microbial access to these tissues in younger cluster and grassland soils during incubation may have resulted in some of the observed differences. Alternatively, acidic AAs can be protected through binding to Al or Fe oxides (Sowden et al., 1976). As a higher proportion of total soil C is held in the free silt and clay fraction in grassland and younger cluster soils than older cluster soils (Table 1), stabilization of acidic AAs on Fe oxides could be greater in grassland and younger cluster soils, potentially resulting in the observed relative enrichment.

4.2. Loss of carbohydrate C

The loss of bulk carbohydrate C differed between the landscape elements, whereby carbohydrate C was degraded either slightly faster than bulk SOC (younger clusters and grasslands) or slightly slower than bulk SOC (older clusters). The results are similar to studies that have reported a slightly slower than expected turnover of carbohydrate C (Gleixner et al., 2002), which is on par with the turnover of bulk SOC (Derrien et al., 2006). Decreased turnover of carbohydrate C in older cluster soils could potentially result from greater production of microbial carbohydrate C or from differences in carbohydrate C stabilization.

The turnover rate of different carbohydrate C compounds can vary greatly, and carbohydrates of microbial origin are thought to be preserved to a greater extent than those of plant origin due to microbial recycling of these compounds and their greater dominance in finer soil fractions, affording them greater physical and chemical protection from degradation (Kiem and Kögel-Knabner, 2003; Nacro et al., 2004; Derrien et al., 2006). Additionally, the

slower decay of microbial carbohydrates could be partially facilitated by their structure (e.g. glycoproteins) and their importance in binding to soil minerals (Sollins et al., 2006; Kleber et al., 2007). During incubation, there was an increase in the ratios of microbial/plant derived carbohydrates, such as mannose/xylose and (galactose + mannose)/(arabinose + xylose), for all landscape elements, although the increase was only significant for younger woody clusters (Table 2). The increase in these ratios, which can be used to indicate microbial vs. plant contributions to soil carbohydrates (Oades, 1984; Hu et al., 1995; Glaser et al., 2000), resulted from a greater loss of the plant-derived sugars (predominantly xylose) relative to galactose and mannose. This suggests there was production of microbial sugars during incubation or that carbohydrates derived from microbial biomass were preserved to a greater extent than plant-derived carbohydrates. Fucose, a microbially derived carbohydrate (Cheshire, 1979), was the only extracted carbohydrate compound that increased significantly (grassland soils only), further suggesting that microbial sugars were produced during the incubation.

4.3. Apparent stabilization of amino C

In contrast to carbohydrate C, amino C was preserved relative to bulk SOC during the incubation as it increased for all landscape elements by 2–44% (Table 3), suggesting that amino compounds were either selectively preserved relative to other forms of SOC or were generated by microbial processes during incubation. Other studies have revealed the apparent stabilization of amino compounds in soil (Fan et al., 2004; Miltner et al., 2009; Knicker, 2011), often attributed to physical protection from degradation or to microbial recycling of the compounds. There is potential for amino C stabilization on silt and clay particles in grassland and younger woody cluster soils, although the low proportion of whole soil C held within this fraction in older woody cluster soils (ca. 5%; Table 1) suggests that physical or physiochemical protection may be limited for this particular landscape element. As microbial biomass has recently been shown to contribute substantially to slower-cycling SOM pools (Kiem and Kögel-Knabner, 2003; Simpson et al., 2007; Liang and Balsler, 2011), there is also the possibility that amino compounds are recycled into microbial biomass and subsequently incorporated into structures that are less accessible to microbial degradation.

Amino sugars, such as glucosamine and galactosamine, are considered to be derived from microorganisms and have been used to estimate contributions from microbial biomass to SOM (Amelung, 2001; Glaser, 2004; He et al., 2011). Although there was a general increase in amino sugars during incubation, it was significant only for younger woody clusters (Table 3), providing evidence for the reworking of amino C into microbial compounds and for enhancement of amino C stabilization.

4.4. Accrual of non-hydrolysable N for older woody cluster soils

In contrast to amino C, the total amount of extractable amino N decreased significantly for older woody cluster soils and increased slightly but not significantly for the other landscape elements (Table 4). During incubation, total soil N did not change significantly for grassland soils (0.56–0.51 mg N g⁻¹ soil), young cluster soils (0.80–0.76 mg N g⁻¹ soil) or older cluster soils (1.59–1.64 mg N g⁻¹ soil). Therefore, either greater production of other forms of organic and inorganic N occurred in older woody clusters during incubation or amino N became less extractable by acid hydrolysis. Greater N mineralization rates have been documented in woody clusters at La Copita, although the proportion of N mineralized is lower in woody clusters relative to grasslands (McCulley et al., 2004). Unless the dynamics of N mineralization into

inorganic forms was substantially altered by the incubation, this is likely not the reason for lower extractable amino N recovery in this study for older woody clusters after incubation. Therefore, the formation of non-hydrolysable N during incubation is a likely mechanism for recovery of N as amino N for older woody cluster soils. For older woodland soils, non-extractable amino-N increased from 53% to 59% of total N, while for younger cluster and grassland soils non-extractable amino N decreased from ca. 53% to ca. 46% of total soil N. This non-hydrolysable N, which in some cases has been shown to consist of heterocyclic material as well as protein (Knicker and Hatcher, 1997; Leinweber and Schulden, 1998, 2000), could be formed through condensation and coupling reactions of proteinaceous compounds with phenolic and polyphenolic compounds (Kraus et al., 2003; Schmidt-Rohr et al., 2004; Olk et al., 2009) or through encapsulation by hydrophobic biomolecules (Knicker and Hatcher, 1997; Knicker, 2011). In response to woody encroachment in the region, lignin and substituted fatty acids accumulate in the POM and free light fractions (Filley et al., 2008), fractions which also store the majority of accumulating C (Liao et al., 2006a, 2006b) and were the dominant sources of respired C during incubation, and thus were substantially degraded (Creamer et al., 2011). Amino N in the older woody stands is therefore potentially more susceptible to conversion to non-extractable forms in older woody stands in response to incubation. This is consistent with published work for the region suggesting N sources in woodland soils become progressively more complex and harder to decompose as woody stands develop (Creamer et al., 2012).

4.5. Relationship between CO₂ EGA and chemical data

The increase in SOM released at higher temperatures during thermal analysis after incubation (Fig. 1) supports the notion that thermal stability can be related to SOM stability in this system. The CO₂ evolution profiles were also well-correlated with the data obtained from the amino compound analysis. Although individual peaks from thermal analysis cannot be attributed solely to the combustion of a particular compound, certain compound classes may be associated more with particular temperature regions than others (Dell'Abate et al., 2003; Strezov et al., 2004; Plante et al., 2009). PLS II analysis performed between CO₂ EGA and soil C held in all amino groups (hydroxyproline, ornithine, amino sugars, and acidic, basic, polar and nonpolar AAs) revealed that amino compounds distinguished between incubated and unincubated soils (Fig. 3). The temperature regions of CO₂ release during thermal analysis that explained 21% of the variance in amino C data (Fig. 3; 325, 400, 510 and 600 °C), were also predictive for separating incubated vs. unincubated soils (Fig. 2). Therefore, the relative increase in amino groups during incubation, as measured via acid hydrolysis (Table 3), was reflected in the CO₂ evolution profiles of SOM during thermal analysis. Additionally, the positive correlation between amino groups and CO₂ evolved at high temperature suggests these compounds were relatively stable, confirming the hypothesis that they were selectively preserved or were produced by microorganisms during incubation.

Although carbohydrate C was lost to the greatest extent of the compounds extracted, its correlation with CO₂ EGA was not sufficient to produce a meaningful PLS regression model. However, the temperature with the largest coefficient of determination was around 365 °C (data not shown), which interestingly decreased during incubation (Figs. 1 and 2) and has also been previously correlated with carbohydrate C loss (Dell'Abate et al., 2003). These data show that thermal analysis was able to provide a rapid assessment of SOM quality that reflected increases in amino C observed during the incubation.

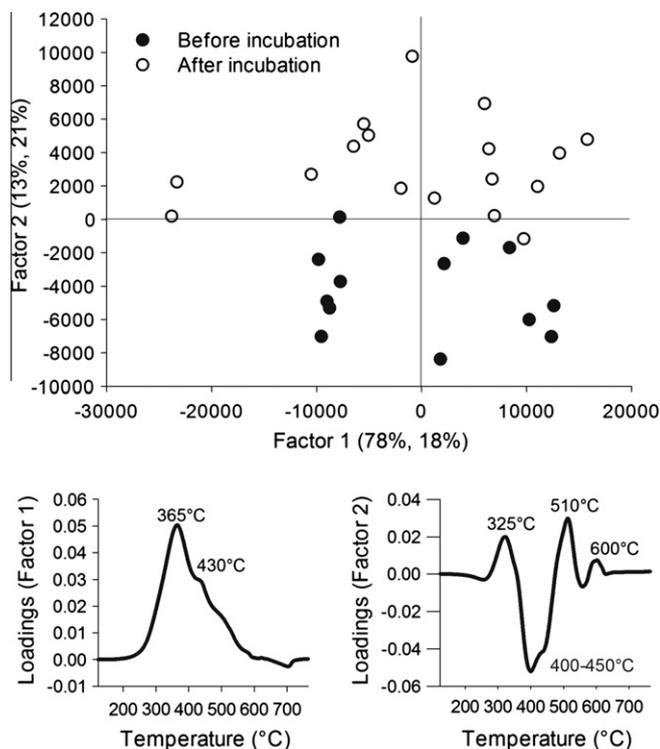


Fig. 3. Partial least squares II (PLS II) of CO₂ released during thermal analysis (ppm mg C⁻¹) and C held within amino groups (hydroxyproline, amino sugars, and acidic, basic, polar and nonpolar AAs). For each factor, the proportion (%) explained of the X-variance (CO₂ released during thermal analysis) and Y-variance (amino groups) is shown in parentheses. The PLS factors are designed to explain as much of the Y-variance as possible. Loadings are shown for Factors 1 and 2.

5. Conclusions

Due to the major role of soil organic C in the global C cycle, efforts to characterize the mechanisms that regulate the stability and turnover of this pool have intensified. However, these efforts have not yet clarified the role of SOM chemistry in SOC storage and dynamics. Changes in the abundance of amino compounds before and after incubation revealed that, in general, amino compounds will not be preferentially degraded, and will be selectively enriched or produced by microbial processes during incubation. This was confirmed from thermal analysis data, which also showed that the soils responded to incubation through an increase in SOM released at higher temperatures and a decrease in SOM released at lower temperatures.

However, the changes in SOM chemistry during incubation differed greatly between older cluster soils and younger cluster and grassland soils. Amino compounds were selectively enriched in younger cluster and grassland soils during incubation due to the loss of alternative C sources or production of amino C by microorganisms. Decreases in extractable amino N in older woody clusters suggest that, in response to woody encroachment and with enhanced decomposition, organic N may become more inaccessible to microorganisms. The smaller proportional loss of carbohydrate C from older woody clusters confirms that the dynamics of SOM turnover are different between older woody cluster soils and younger woody cluster and grassland soils. The greater loss of plant vs. microbially-derived C in the landscape elements supports studies suggesting the production and recycling of carbohydrate C by microorganisms can aid in its persistence in soil, and that fresher plant-derived material can be utilized during incubation. Together, these data show that in these sandy loam soils, the

stability of SOM during incubation was strongly driven by properties of the landscape elements (younger clusters and grasslands vs. older clusters) in addition to the less important impact of pre-defined notions of SOM 'lability' on its turnover.

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