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Chemical changes to nonaggregated particulate soil organic matter following grassland-to-woodland transition in a subtropical savanna

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[1] Encroachment of thorn woodlands into grasslands of southern Texas has resulted in greater aboveground and belowground biomass and greater soil organic carbon (SOC) stocks. Our previous studies showed that a large percentage of the SOC accrued under invading woody clusters was not stabilized within protective soil aggregates or on mineral-surfaces. Here we evaluated lignin and cutin- and suberin-derived substituted fatty acid (SFA) chemistry to determine if the accrual of nonaggregated particulate organic matter (POM) in woodlands was promoted by inherently greater recalcitrance of tissues from woody versus grass species, and if there was selective input of aboveground versus belowground plant carbon to POM. Woody clusters exhibited reduced concentrations of cutin-derived SFA and cinnamyl phenols within surface litter compared to fresh aboveground plant material. However, root litter exhibited relatively minor changes in biopolymer chemistry compared to fresh root tissue, suggesting it was either more stable or was refreshed at a greater rate. Between 14 and 105 years of woody plant encroachment, SFA in free POM fractions appeared to be consistently derived from root material while SFA within intraaggregate POM were increasingly derived from cutin sources. In addition, the shift from herbaceous to woody input was accompanied by enrichment in the amount of cutin and suberin-derived aliphatics with respect to lignin in both root and surface litter as well as nonaggregated POM. Woody plant encroachment at this site results in the rapid accrual of POM pools that are biochemically recalcitrant, providing a mechanism by which soil organic carbon can accumulate in this sandy soil system. Our results also lend further credence to the hypothesis that aliphatic biopolymers, particularly root-derived suberin, are important components of long-term soil organic carbon stabilization.

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1. Introduction

[2] Woody plant encroachment (WPE) into grasslands and savannas is a widespread phenomenon that alters soil organic carbon (SOC) dynamics through changes to the mode, chemistry, and rate of litter inputs [*Connin et al.*, 1997; *Hibbard et al.*, 2001; *Archer et al.*, 2001; *McCulley et al.*, 2004]. This land cover change has occurred in many grassland regions around the world over the last 100–150 years and is caused largely by livestock grazing and fire suppression, which favor the establishment and expansion of woody plants [*Van Auken*, 2000]. At the global level, impacts to SOC resulting from this phenomenon are of great interest given that grasslands and savannas account for ~35% of the global terrestrial net primary productivity [*Field et al.*, 1998]. Controls on the magnitude and direction of the impact of WPE on SOC stocks remain controversial; field observations demonstrate a variety of responses ranging from accrual to losses of SOC compared to pre-encroachment levels [e.g., *Archer et al.*, 2001; *Jackson et al.*, 2002].

[3] For a soil to accrue SOC over the long-term, organic matter inputs must exceed the rate of decomposition and the system must possess mechanisms to stabilize plant detritus in pools with relatively slow turnover times [*Sollins et al.*, 1996; *Six et al.*, 2002]. It has been shown that SOC is stabilized by a combination of three main protection mechanisms: (1) inherent or acquired biochemical resistance of

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organic matter to microbial decay (i.e., biochemical recalcitrance), (2) physical protection of organic matter by occlusion in soil aggregates or within pores, and (3) strong physicochemical association of organic matter with mineral surfaces, [*Tisdall and Oades*, 1982; *Baldock and Skjemstad*, 2000; *Krull et al.*, 2003]. For example, particulate organic matter (POM) occluded within soil aggregates (particularly microaggregates 50–250 μm diameter) is comparatively more protected than nonaggregated POM because the microenvironment limits air, water, and nutrient exchanges, extracellular enzyme action, and decomposer access to POM. Nonaggregated POM, however, may be in various degrees of exchange with the aggregated fractions depending upon the mean life time of the aggregates [*Oades*, 1984; *Golchin et al.*, 1994; *Jastrow and Miller*, 1998; *Six et al.*, 2000]. Biochemical recalcitrance is often attributed to slowly degrading lignin or aliphatic components (such as cutin, suberin, or waxes) that differ in abundance and chemistry between plant tissues and between plant species [*Baldock and Skjemstad*, 2000; *Kogel-Knabner*, 2002; *Rasse et al.*, 2005; *Lorenz et al.*, 2007]. Many studies have now shown that aliphatic biopolymers, in particular, make up an important subset of recalcitrant material in soils [e.g., *Nierop et al.*, 2003; *Mikutta et al.*, 2006] and may form part of the core of materials tightly bound to mineral surfaces. WPE, because it is accompanied by changes in the relative amounts and types of detritus inputs to soil (e.g., root, shoot, bark and foliar tissue) as well as input chemistry, is likely to change the relative importance of biochemical recalcitrance and affect the stability of nonaggregated or mineral associated organic matter [*Gill and Burke*, 1999; *Connin et al.*, 1997].

[4] Our recent investigations in the savannas of the Rio Grande Plains of southern Texas showed SOC increased linearly with woody plant stand age in areas where woodlands have replaced grasslands [*Archer et al.*, 2004; *Liao et al.*, 2006a; *Boutton et al.*, 2008]. Additionally, WPE resulted in increased carbon storage in plant biomass and, in particular, resulted in increased surface litter and shallow root inputs that exceeded litterfall [*Gill and Burke*, 1999; *Hibbard et al.*, 2001; *McCulley et al.*, 2004; *Liao et al.*, 2006a, 2006b]. Soil organic carbon that accrued under woody clusters was found mostly in nonaggregated and macroaggregated POM fractions, which occurred in fundamentally different proportions than in the native grasslands, where SOC was predominantly associated with microaggregates and silt- and clay-sized particles. In the woody clusters silts and clays and microaggregates outside of macroaggregates were bound together to form new macroagregates [*Liao et al.*, 2006a, 2006b]. This shift in accrual pattern, however, represented a change from the mechanisms controlling SOC stabilization in the grassland, where physical protection in aggregates and on silts and clays dominated stabilization, to a system where a significant POM pool has accrued without the benefit of physical and mineral surface stabilization. *Liao et al.* [2006a, 2006b] speculated that this change in the nature of SOC storage following WPE was driven primarily by the rate of new plant carbon input exceeding the relatively limited capability of this sandy loam system to stabilize organic matter within microaggregate structures. However, the biopolymer composition of the new plant inputs was also suggested as a factor that might be slowing the rate of microbial alteration and thus affecting the nature of SOC storage. In general, the chemical composition of detritus may play a greater role in SOC stabilization in sandy soils which have a low potential for microaggreate formation and mineral interactions [*Six et al.*, 2002].

[5] The purpose of this study was to determine if increased SOC storage in this subtropical savanna could be linked to specific changes in the sources (i.e., root versus litter) and biopolymer chemistry (i.e., lignin, suberin, and cutin) of woody plant inputs. We focused this investigation on POM fractions external to water-stable aggregate structures within remnant grasslands and in tree/shrub clusters of increasing age because these SOC pools exhibited the greatest proportional gain in mass with WPE [*Liao et al.*, 2006a].

2. Methods

2.1. Field Site and Soil, Litter, and Plant Sampling

[6] The samples used in this investigation were a subset of the larger study by *Liao et al.* [2006a, 2006b] conducted at the Texas Agricultural Experiment Station, LaCopita Research Area (27°40′N, 98°12′W) in the Rio Grande Plains of southern Texas. The site has a subtropical climate, with a mean annual temperature of 22.4°C and mean annual precipitation of 716 mm. Surface soils are sandy loams (Typic and Pachic Argiustolls). Discrete clusters of woody plants with a central colonizing *Prosopis glandulosa* [Torr.] var. *glandulosa* (honey mesquite) occur in patches within the remnant C4 grassland matrix. An understory of woody plants develops beneath *Prosopis* trees older than about 15–20 years. For a complete description of the ecology of the site, see *Archer et al.* [1988] and *Boutton et al.* [1998].

[7] Soil and litter samples were collected from seven sites - including three grasslands dominated by the C4 grass *Chloris cucullata* Bisch. and four mesquite clusters established 14, 40, 80, and 105 years ago - all located within an area of approximately 1 km^2. These sites are a subset of the 40 sites investigated by *Liao et al.* [2006a], and detailed soil, litter, and plant sampling methods are provided therein. Briefly, one composite sample for each site was created from four soil cores (0–15 cm depth) taken from each cardinal direction within 0.5 m of the *P. glandulosa* bole in the clusters or the base of a large *C. cucullata* in the remnant grasslands. Root litter was quantified for well-mixed subsamples (~100 g) from each pooled soil sample. Surface litter was collected from a 0.25 m^2 plot located within 0.5 m of the bole of the largest *P. glandulosa* in wooded areas, or around a clump of *C. cucullata* in the grasslands. Litter samples were washed with water over a 2 mm sieve to remove adhering soil particles.

[8] Live tissues (leaves, stems, and roots <0.5 cm in diameter) from 3 grass species dominant in the grassland (*Tridens muticus* (Torr.) Nash, *Bouteloua rigidista* (Steud.) Hitchc., and *C. cucullata*) and 2 tree species dominant in woody clusters (*P. glandulosa* and *Zanthoxylum fagara* (L.) Sarg.) were sampled to provide a limited survey of lignin and cutin- and suberin-derived hydroxyl and carboxyl-substituted fatty acid (SFA) sources to SOC. Samples were

collected from 5 live plants from each species and pooled before being ground.

2.2. Soil Physical Fractionation

[9] Homogenized, field-moist soils were sieved at 8 mm, air-dried, and fractionated according to density and size following a procedure detailed by *Liao et al.* [2006a]. Briefly, eleven fractions were isolated from whole soil and included water-stable macro- and microaggregates, free and aggregate-occluded POM, and silts and clays with a mass balance of carbon and nitrogen following wet-sieving of 98% and 92% of that in whole soil, respectively. The fractions investigated herein were: (a) free, nonaggregated, POM with density <1.0 g/cc (termed free light fraction or FLF); (b) the nonaggregated POM of size 53–250 μm and density <1.85 g/cc (termed micro free POM or mfPOM); and (c) nonaggregated POM of size >250 μm and density <1.85 g/cc (termed macro free POM or MfPOM). Density separations were achieved by flotation in water (<1.0 g/cc) or sodium polytungstate solution (<1.85 g/cc).

2.3. Biopolymer and Elemental Chemical Analysis

[10] Alkaline cupric-oxide (CuO) oxidation was used to assess lignin [*Hedges and Mann*, 1979] and SFA [*Goñi and Hedges*, 1990a, 1990b, 1990c] in the POM fractions, surface and root litter, and fresh plant tissues. Alkaline CuO provides a simple means to asses SFA composition similar to other techniques that extract SFA from plants and soils using base hydrolysis and solvent extraction [e.g., *Riederer et al.*, 1993; *Nierop et al.*, 2003; *Otto and Simpson*, 2006]. These base hydrolysis techniques, however, provide a more inclusive distribution of aliphatic compounds that include SFA with mid chain epoxides which are unfortunately completely hydrolyzed to dihydroxyl species by the CuO process [*Goñi and Hedges*, 1990a].

[11] Prior to analysis, fractions were powdered to a flour consistency with a liquid N_2 SPEX CertiPrep (Metuchen, NJ, USA) freezer mill to ensure homogeneity in analysis. Samples were reacted and extracted in Monel reaction vessels (Prime Focus, Inc. Seattle, WA, USA). Gas chromatography mass spectrometry and extracted ion internal calibration curves were used to assess compound concentrations [*Filley et al.*, 2008]. The trimethylsilyl (TMS) derivatives of vanillyl (V) -based (i.e., vanillin, acetovanillone, vanillic acid), syringyl (S) -based (i.e., syringealdehyde, acetosyringone, syringic acid), and cinnamyl (C) -based (i.e., *p*-hydroxycinnamic acid and ferulic acid) lignin were quantified and the total yields of lignin phenols is denoted as "SVC-lignin. "Additionally, the TMS derivatives of nine SFA peaks were quantified and included 16-hydroxyhexadecanoic acid, hexadecanoic diacid, 18-hydroxyoctadec-9-enoic acid, a coelution of 9,16- + 10,16- dihydroxyhexadecanoic acid, 9-octadecene-1,18-dioic acid, 7&8-hydroxyhexadecane dioic acid, 9,10,18-trihydroxyoctadec-12-enoic acid, and 9,10,18-trihydroxyoctanoic acid. Compound concentration is given as mg compound/100 mg organic carbon (C_{org}). All POM, plant, and litter fractions were analyzed for total carbon and nitrogen concentrations using a Carlo Erba EA-1108 elemental analyzer. Because no carbonates were present in the POM fractions evaluated in this study, total carbon is equivalent to organic carbon.

2.4. Statistical Treatment of Data

[12] Differences in the concentrations of SFA and SVC-lignin in litter, roots, and POM fractions between grassland sites (N = 3) versus cluster sites (N = 4) were evaluated using two-tailed Student's t-tests [*SAS Institute*, 2003].

3. Results

3.1. Lignin and SFA Characteristics Among Selected Plant Species

[13] The SFA from grasses and their roots were comparatively lower than the leaves and roots of woody plants (Table 1). Leaf tissue of *P. glandulosa* had the highest SFA content among the plants, containing more than 3x that of leaves of *Z. fagara* and nearly 60x that of the grasses *B. rigidiseta* and *C. cucullata*. Consistent with known distributions, the cutin-SFA in the leaf tissue of the woody plants were particularly abundant in C_{16} chain lengths with respect to C_{18} chain lengths [e.g., *Kollattukudy*, 1980; *Riederer et al.*, 1993]. In particular, the coeluting compounds, 9,16- and 10,16-dihydroxy hexadecanoic acid (diOH-C_{16}) were dominant in leaf tissues, but they were absent or at trace levels in the roots of all analyzed species as well as the grasses. This is in contrast to the relatively high concentrations of diOH-C_{16} in live wheatgrass reported by *Otto and Simpson* [2006]. The C_{18} chain lengths were relatively dominant in grass root suberin aliphatics, as was also found for grasses by *Otto and Simpson* [2006]. Because suberin and cutin contain very similar monomer compounds, specific SFA ratios are often used to discern input from these two sources [e.g., *Kogel-Knabner et al.*, 1989]. A simple ratio of two compounds, diOH-C_{16} to 18-hydroxyoctadec-9-enoic acid (ω-OH-$C_{18:1}$) proved to be a good indicator of woody plant cutin (i.e., foliar aliphatic tissue) versus suberin (i.e., root aliphatic tissue) in this system; we utilize this proxy to track relative input of woody plant root and leaf material to POM fractions with WPE. However, grass root and shoot could not be distinguished in this manner (Table 1).

[14] The SVC-lignin content of grass roots and shoots (ranging from 12.50 to 17.65 mg/100 mg C_{org}) were higher than the woody roots and leaves (ranging from 4.22 to 9.06 mg/100 mg C_{org}) (Table 1). Roots, on average, contained higher contents of SVC-lignin than shoots and leaves although this was not the case for *P. glandulosa* due to its high cinnamyl content in leaves. Hard wood tissue of the tree and shrubs had the highest SVC-lignin content ($\bar{x}$ = 25.95 mg/100 mg C_{org}). The relative amount of cinnamyl phenols to total SVC-lignin (the ratio of monomers C/SVC) in grass roots ($\bar{x}$ = 0.33) was dramatically higher than roots from the woody plants species ($\bar{x}$ = 0.04) and this was also reflected in the C/V ratio. The C/SVC ratio among the woody plant leaves and grass shoots were comparable, with both being higher than their roots, and wood tissue had the lowest values (<0.01). These differences in overall lignin phenol composition are consistent with previous findings that demonstrated the potential of phenol ratios to distinguish among fresh herbaceous and woody tissues [*Hedges and Mann*, 1979], although it should be noted that it is not uniquely possible to assign the cinnamyl components of ferulic and p-coumaryl to lignin as they are also present in significant quantities in suberin.

Table 1. Concentration and Molecular Parameters of Lignin Phenols and Substituted Fatty Acids for Five Common Grass, and Two Dominant Woody Plant Species Found at the LaCopita Research Area[a]

Samples		SFA	diOH-C_{16}	OH-$C_{18:1}$	SV-Lignin	SVC-Lignin	diOHC_{16}/ OH-$C_{18:1}$	C/V	S/V	C/SVC	SVC/SFA	Ac/Al(v)	Ac/Al(s)
Roots													
Tridens muticus	Grass	0.92	0.00	0.04	11.90	17.65	0.00	0.79	0.68	0.33	19.18	0.27	0.18
Bouteloua rigidiseta	Grass	0.40	0.00	0.19	11.10	15.90	0.00	0.75	0.71	0.30	39.75	0.35	0.24
Chloris cucullata	Grass	0.92	0.00	0.27	9.52	14.89	0.00	0.95	0.69	0.36	16.18	0.20	0.14
Prosopis glandulosa	Tree	2.38	0.00	0.72	7.39	7.76	0.00	0.11	1.09	0.05	3.26	0.19	0.15
Zanthoxylum fagara	Shrub	1.11	0.00	0.16	6.27	6.44	0.00	0.06	1.20	0.03	5.80	0.20	0.14
Leaves/shoots													
Tridens muticus	Grass	1.28	0.23	0.73	7.27	12.70	0.37	1.85	1.50	0.43	9.92	0.17	0.26
Bouteloua rigidesta	Grass	0.51	0.08	0.24	7.30	12.50	0.44	1.45	1.01	0.42	24.51	0.22	0.26
Chloris cucullata	Grass	0.52	0.18	0.27	8.00	13.80	0.49	1.69	1.37	0.42	26.54	0.19	0.27
Prosopis glandulosa	Tree	27.17	22.51	0.21	5.89	9.06	106.00	1.42	1.67	0.35	0.29	0.31	0.18
Zanthoxylum fagara	Shrub	8.12	5.60	0.20	2.40	4.22	69.00	1.44	0.85	0.43	0.52	0.36	0.18
Wood													
Prosopis glandulosa	Tree	0.00	0.00	0.00	25.00	25.20	0.00	0.02	2.05	0.01	0.00	0.09	0.07
Zanthoxylum fagara	Shrub	0.00	0.00	0.00	26.50	26.70	0.00	0.03	2.03	0.01	0.00	0.05	0.06

[a]Abbreviations diOH-C_{16} and OH-$C_{18:1}$ refer to 9,16- + 10,16-dihydroxyhexadecanoic acid and 18-hydroxyoctadec-9-enoic acid, respectively. The C/SVC value is the relative amount of cinnamyl phenols. Units are in mg/100 mg C_{org}.

[15] The vanillic acid to vanillin [Ac/Al(v)] ratio and syringic acid to syringealdehyde [Ac/Al(s)] ratio are often presented as lignin degradation proxies as these ratios tend to increase with plant decay. For roots, Ac/Al(v) ranged from 0.19 to 0.35, and Ac/Al(s) ranged from 0.14 to 0.24. Similar ranges were found for leaves and shoots. Wood tissue had the lowest values (<0.1) in keeping with previous findings for undegraded tissue [*Hedges et al.*, 1988; *Opsahl and Benner*, 1995].

3.2. Chemistry of Surface and Root Litter Among Grasslands and Woody Clusters

[16] The SV-lignin content, a value that excludes cinnamyl phenols, of root litter was lower in woody clusters ($\bar{x}$ = 5.91 mg/100 mg C_{org}) than remnant grasslands ($\bar{x}$ = 7.86 mg/100 mg C_{org}). In contrast, the opposite pattern was exhibited for surface litter where SV-lignin content is significantly greater in woody clusters ($\bar{x}$ = 8.66 mg/100 mg C_{org}) than remnant grasslands ($\bar{x}$ = 5.65 mg/100 mg C_{org}) (Table 2). The C/V and C/SVC values for surface and root litter were higher in grasslands than woody clusters (Table 2). There was no discernable difference in S/V ratio among surface litters, while root litter from grasslands had lower S/V values ($\bar{x}$ = 0.63) than the clusters ($\bar{x}$ = 0.86). The C/SVC values for grass litter ($\bar{x}$ = 0.38) were greater than those of litter from clusters ($\bar{x}$ = 0.05). The C/SVC ratio of root litter from clusters exhibited a wide range in values from 0.01 to 0.11, with the lowest values from the two oldest clusters. The C/SVC ratio of root litter from grasslands ($\bar{x}$ = 0.19) was significantly higher than all clusters ($\bar{x}$ = 0.07). The Ac/Al(v) values for roots and litter were within ranges expected for fresh plant materials throughout all sites [*Hedges et al.*, 1988] and were actually lower in some cases than living plant tissues (Table 1).

[17] As illustrated in *Liao et al.* [2006a] large differences in the elemental organic carbon to total nitrogen ratio (C_{org}/N) were observed between the grassland and woody cluster for both surface litter and roots with woody clusters exhibiting lower C_{org}/N values (Table 2). In general, for each landscape element, surface litter had higher C_{org}/N ratios than root litter.

3.3. Apportionment of Soil Organic Carbon Among Physical Fractions

[18] Figure 1, replotted from data presented by *Liao* [2005], shows the relative distribution of SOC among nonaggregated POM and all remaining soil fractions combined (i.e., nonaggregated silts and clays plus all aggregated pools). Grasslands (T = 0 years) contained less than 16.5% of SOC as nonaggregated POM. In contrast, woody clusters exhibited a trend from 14 years to 80 years of an increasing proportion of SOC stored within nonaggregated POM - up to 65% of total SOC by 80 years. Additionally, *Liao et al.* [2006b] calculated, through stable isotope modeling, that these nonaggregated POM fractions have relatively short mean residence times with FLF, MfPOM, and mfPOM at 24, 18, and 19 years, respectively. These values are consistent with the rapid accumulation accompanying WPE at LaCopita. This subset of samples from *Liao et al.* [2006a, 2006b] is consistent with the overall trend of a linear increase in SOC among all physical fractions with WPE at LaCopita, but with a particularly dramatic shift in the apportionment of SOC to nonaggregated POM [*Liao et al.*, 2006a].

3.4. Chemistry of Nonaggregated POM Among Grasslands and Woody Clusters

[19] There were large differences in the composition and concentration of lignin and SFA among the nonaggregated POM fractions at all sites (Table 3). For grassland samples, the lignin concentration decreased in the order FLF > MfPOM > mfPOM. This trend was not apparent in the clusters as the MfPOM had the highest lignin content in the 14, 80, and 105 yr old sites but not for the 40 yr site (individual data not shown). Lignin content in the FLF exhibited a progressive decrease with age of woody cluster for each time point, similar to that observed for the root isolates. Similarly, the molecular composition of lignin also showed a decrease in the proportion of cinnamyl phenols (C/SVC) for each of the fractions with increasing cluster age (Figure 2). These composition changes are also reflected in the commonly used source proxies, C/V and S/V values, where C/V values generally decrease and S/V increases with

Table 2. Mean Concentration and Molecular Parameters of Lignin Phenols and Substituted Fatty Acids for Surface Litter and Isolated Roots From Soil (0–15 cm) in Three Remnant Grasslands and Woody Clusters of Increasing Age (14, 40, 80, 105 Years) at the LaCopita Research Area[a]

Sample	SFA	$diOHC_{16}$	SV-Lignin	SVC-Lignin	$diOHC_{16}/OH\text{-}C_{18:1}$	C/V	S/V	C/SVC	SVC/SFA	Ac/Al(v)	C_{org}/N
Grassland Surface Litter	0.70 (0.07)	0.25 (0.02)	5.65 (0.06)	8.89 (0.40)	2.18 (1.58)	1.32 (0.03)	1.25 (0.16)	0.38 (0.02)	12.92 (1.9)	0.14 (0.0)	38.40 (0.94)
Cluster Surface Litter	3.38 (0.88)	2.39 (0.48)	8.66 (1.37)	9.13 (1.34)	30.19 (12.39)	0.11 (0.03)	1.12 (0.17)	0.05 (0.012)	2.92 (1.13)	0.22 (0.03)	24.41 (5.0)
Probability of a greater t-value (surface litter grass vs. cluster)	0.004	0.001	0.013	0.985	0.013	<.0001	0.36	<.0001	0.00	0.006	0.02
Grassland Root Litter	1.09 (0.13)	0.10 (0.05)	7.86 (0.33)	9.71 (0.38)	0.43 (0.17)	0.39 (0.09)	0.63 (0.03)	0.19 (0.02)	9.09 (0.95)	0.18 (0.0)	31.06 (6.7)
Cluster Root Litter	1.91 (0.83)	0.38 (0.24)	5.91 (0.58)	6.34 (0.58)	1.30 (0.72)	0.13 (0.08)	0.86 (0.15)	0.07 (0.04)	3.79 (1.5)	0.27 (0.06)	18.5 (2.4)
Probability of a greater t-value (roots grass vs. cluster)	0.156	0.112	0.004	0.002	0.101	0.009	0.061	0.008	0.003	0.060	0.05

[a]Elemental organic carbon to nitrogen (C_{org}/N) ratios obtained from *Liao et al.* [2006a]. Values in parentheses represent standard error of values among sites. Abbreviations diOH-C_{16} and OH-$C_{18:1}$ refer to 9,16- + 10,16-dihydroxyhexadecanoic acid and 18-hydroxyoctadec-9-enoic acid, respectively. Comparisons of the means of grassland (T = 0) and woody clusters (mean of all ages) by Student's t-test are also given. Units are in mg/100 mg C_{org}.

WPE (Table 3). The mean Ac/Al(v) value was higher for FLF and mfPOM in woody clusters with respect to grasslands. (Table 3). Within each cluster age, the mfPOM fraction, the smallest sized fraction, had the highest Ac/Al(v) values. In both grasslands and woody clusters all POM fractions exhibited an elevated Ac/Al(v) relative to the surface litter and roots (Table 2).

[20] The mean SFA content among the fractions was generally higher for woody clusters compared to grasslands (Tables 2 and 3), although the difference between mean cluster and grassland values was only significant for mfPOM. There were strong compositional changes in SFA through cluster development that were evident in comparisons of the diOH-C_{16}/ω-OH-$C_{18:1}$ ratio (a potential discriminator between woody leaf-cutin and root-suberin input in this system) among the fractions (Figure 3). In general, MfPOM and mfPOM fractions in clusters exhibited comparatively high values with respect to grasslands after 40 years of encroachment while cluster FLF had low values. This suggests that with cluster development leaf litter cutin is selectively added to mfPOM and MfPOM while FLF receives root suberin.

[21] Only the MfPOM sample showed a statistical difference in C_{org}/N values among grassland (18.5) and clusters (14.7) (Table 3). In both systems, the lowest C_{org}/N was found mfPOM, and woody clusters exhibited a decrease in C_{org}/N from FLF > MfPOM > mfPOM.

4. Discussion

4.1. Source and Degree of Alteration of Plant Biopolymers in Litter

[22] When comparing the potential plant litter sources to soil organic matter at LaCopita it is important to consider that plant species composition as well as the relative proportion of leaf, wood tissue, and root will change with cluster age [*Archer et al.*, 1988], thus shifting plant biopolymer inputs over time. Equally important, however, are

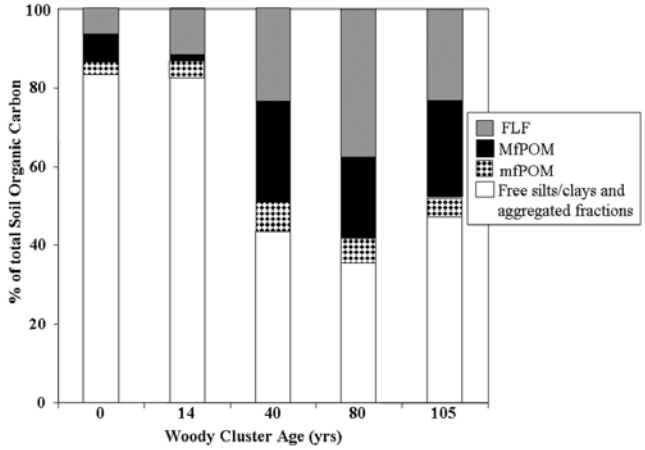

Figure 1. Apportionment of total soil organic carbon among nonaggregated particulate organic matter (POM) pools and all remaining pools combined (nonaggregated silts and clays and all aggregated fractions) in soils (0–15 cm) within grasslands (T = 0 years) and woody clusters of increasing age (14, 40, 80, and 105 years) at the LaCopita Research Area. Values taken from *Liao* [2005].

Table 3. Mean Concentration and Molecular Parameters of Lignin Phenols and Substituted Fatty Acids for Nonaggregated Particulate Organic Matter Fractions (POM) From Soil (0–15 cm) in Three Remnant Grasslands and Woody Clusters of Increasing Age (14, 40, 80, 105 Years) at the LaCopita Research Area[a]

Sample	SFA	DHC_{16}	$DHC_{16}/18HC_{18}$	C/V	S/V	SV-Lignin	SVC-Lignin	%C-Lignin	SVC/SFA	Ac/Alv	Ac/Als
Surface Litter (mean grass values)	0.65 (0.02)	0.28 (0.02)	3.02 (0.66)	1.32 (0.01)	1.15 (0.03)	5.65 (0.06)	9.11 (0.13)	37.9 (0.24)	13.92 (0.51)	0.14 (0.0)	0.24 (0.0)
Surface Litter (mean cluster values)	3.38 (0.88)	2.39 (0.48)	30.19 (12.39)	0.11 (0.03)	1.12 (0.17)	8.66 (1.37)	9.13 (1.34)	5.22 (1.25)	2.92 (1.13)	0.22 (0.03)	0.18 (0.02)
Prob ability of a greater t-value (surface litter grass vs. cluster)	0.015	0.004	0.043	<.0001	0.74	0.043	0.985	<.0001	0.00	0.017	0.013
Roots (mean grass values)	1.09 (0.13)	0.13 (0.05)	0.52 (0.08)	0.35 (0.05)	0.63 (0.01)	7.86 (0.33)	9.52 (0.18)	17.5 (1.92)	8.89 (0.85)	0.17 (0.0)	0.17 (0.0)
Roots (mean cluster values)	1.91 (0.83)	0.38 (0.24)	1.30 (0.72)	0.13 (0.08)	0.86 (0.15)	5.91 (0.58)	6.34 (0.58)	6.71 (4.0)	3.79 (1.5)	0.27 (0.06)	0.22 (0.03)
Prob ability of a greater t-value (roots grass vs. cluster)	0.262	0.230	0.221	0.026	0.106	0.015	0.002	0.030	0.016	0.010	0.078

[a]Elemental organic carbon to nitrogen (C_{org}/N) ratios obtained from *Liao et al.* [2006a]. Values in parentheses represent standard error of values among sites. Abbreviations diOH-C_{16} and OH-$C_{18:1}$ refer to 9,16- + 10,16-dihydroxyhexadecanoic acid and 18-hydroxyoctadec-9-enoic acid, respectively. Comparisons of the means of grassland (T = 0) and woody clusters (mean of all ages) by Student's t-test are also given. Units are in mg/100 mg C_{org}.

the changes in chemical signatures brought about by decomposition and conversion of live plant to litter [*Riederer et al.*, 1993; *Opsahl and Benner*, 1995]. Microbial decay [e.g., *Riederer et al.*, 1993; *Opsahl and Benner*, 1995], macroinvertebrate activity [e.g., *Rawlins et al.*, 2006; *Filley et al.*, 2008], and variations in plant growth stage, such as fresh versus senescent [e.g., *Goñi and Hedges*, 1990c; *Dalzell et al.*, 2005], have been indicated as factors that change biomarker ratios (such as C/V and S/V) and SFA composition. Nonetheless, there were clear distinctions in chemistry for above- and belowground litter among the clusters and grassland samples that can be related to changing plant biopolymer chemistry, and which can be distinguished from alterations due to biopolymer decay.

4.1.1. Plant Source-Surface Litter Dynamics

[23] In the progression from woody plant tissue to surface litter in the clusters, large decreases were observed in the yields of cinnamyl phenols and SFA, the biopolymer components most susceptible to hydrolysis (Figure 2 and Tables 1 and 2). For example, the concentration of diOH-C_{16} in the cluster litter (2.39 mg/100 mg C_{org}) was much lower than that observed for the dominant *P. glandulosa* (22.51 mg/100 mg C_{org}). This relative difference was also present in the total yield of SFA. Similarly, the relative abundance of cinnamyl phenols also exhibited a large difference between source and surface litter but only in the clusters. For example, there was little difference in the C/V ratio between grass shoots ($\sim$ 1.66) and grass litter (1.32) while the same comparison between *P. glandulosa* (1.42) and cluster litter (0.11) showed a dramatic change. The relative abundance of cinnamyl phenols in the grassland litter versus cluster litter is important because these components are thought to be predominantly ester linked and joined directly to sugars, and therefore more easily

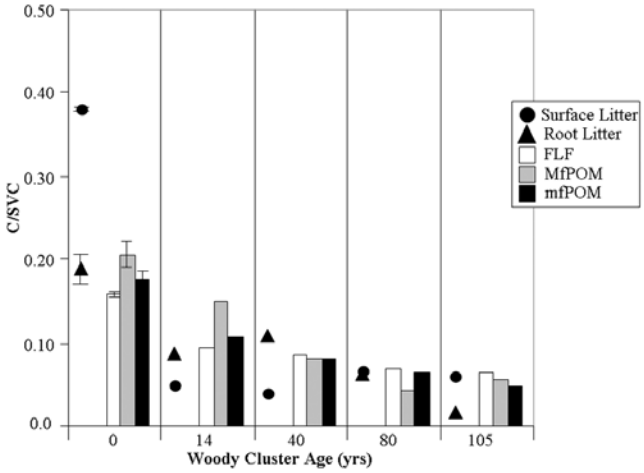

Figure 2. The relative proportion of cinnamyl (C) phenols of the total syringyl (S), vanillyl (V), and cinnamyl (C) phenols recovered by CuO analysis of surface and root litter and nonaggregated particulate organic matter (POM) fractions from soil (0–15 cm). Samples are taken from two grassland areas (T = 0 years) and different age woody clusters of (14, 40, 80, and 105 years) at the LaCopita Research Area. Error bars represent standard error in values for three grassland samples.

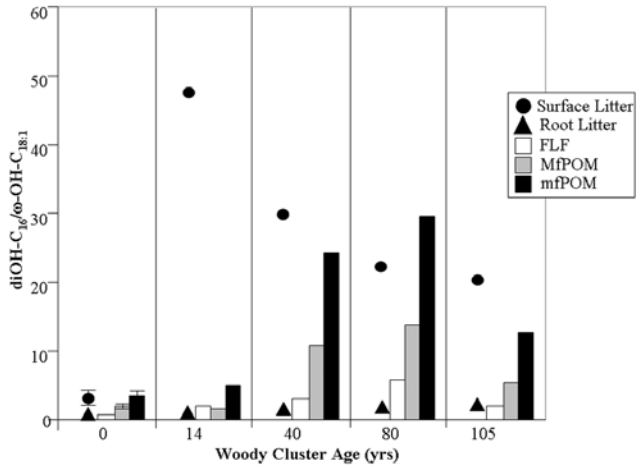

Figure 3. The relative concentration of 9,16 and 10,16-dihydroxyhexadecanoic acid (diOH-C_{16}) to 18-hydroxyoctadec-9-enoic acid (ω-OH-$C_{18:1}$) determined by the alkaline CuO analysis of nonaggregated particulate organic matter (POM) fractions as well as surface litter and root litter (0–15 cm). Samples are taken from three grassland areas (T = 0 years) and different age woody clusters of (14, 40, 80, and 105 years) at the LaCopita Research Area. Error bars represent standard error in values for three grassland samples.

liberated from the lignin backbone and lost to leaching during decay than the S- and V-based lignin components [*Hedges and Weliky*, 1989; *Haddad et al.*, 1992]. In addition, hydrolyzable aromatic compounds are abundant in suberin and might be expected to track proportional SFA changes to some degree. It is also possible that the proportional decrease in SFA and cinnamyl phenols is due to a dilution effect from stem wood input of *P. glandulosa and Z. fagara*, which are high in SV-lignin (~26.0 mg/100 mg C_{org}) and low in cinnamyl phenols (C/SVC = 0.01) and SFA (0.0) (Table 1), given that surface litter has a higher SV-lignin than *P. gladulosa* leaves. However, selective loss of hydrolysable compounds is consistent with other studies showing a decrease in total cutin and suberin hydrolysis products in the conversion from plant tissue to litter [*Riederer et al.*, 1993; *Otto and Simpson*, 2006]. In both the grassland and clusters, the ratio of SVC-lignin to SFA decreased from source to litter, and the litter became progressively more aliphatic with age of cluster (Figure 4).

[24] Changes were also apparent in the relative abundance of cutin and suberin monomers moving from source to surface litter. For example, the relative abundance of diOH-C_{16} and ω-OH-$C_{18:1}$ in woody cluster litter (30.2) was lower than observed in live woody plants (106.0 for *P. glandulosa* and 69.0 for *Z. fagara*) suggesting either a selective loss of diOH-C_{16} or an input of plant matter with a greater ω-OH-$C_{18:1}$ content in some clusters. Such changes in SFA chemistry from source to litter could be indicative of the relative stability of the cutin and suberin monomers. Few studies have specifically tracked cutin monomer composition during leaf or needle decay, and there is no clear consensus as to whether some components are more resistant to decay. *Goñi and Hedges* [1990c] observed that diOH-C_{16} was comparatively less stable than ω-OH-$C_{18:1}$ in buried conifer needles; however, their study was conducted on Dabob Bay (Washington) sediments, wholly different environmental conditions than the LaCopita litter layer. In a far more relevant environmental comparison, *Riederer et al.* [1993] demonstrated that ω-OH-$C_{18:1}$ and diOH-C_{16} decayed at relatively similar rates during decomposition of *Fagus sylvaticus* leaves in a forest floor decay experiment. In apparent contrast to the finding of *Riederer et al.* [1993], *Dignac and Rumpel* [2006] in a study of maize input to mineral soils and *Nierop et al.* [2003] investigating sandy soils under oak found that there was selective decay of ω-OHC$_{18:1}$ with respect to diOH-C_{16} in the organic matter of the mineral soil. For the present study, we believe that the observed progression to lower ω-OH-$C_{18:1}$/diOH-C_{16} values in the cluster litter with age (Figure 3) is a function of the declining contribution of *P. glandulosa* litter, which has a high ratio value of 106, and an increase in contribution of understory plants such as *Z. fagara,* which have relatively lower ω-OH-$C_{18:1}$/diOH-C_{16} values. Therefore, if this ratio is not driven by a selective decomposition of ω-OH-$C_{18:1}$ its value in the POM fractions can be used as a tracer for surface litter input to SOC.

4.1.2. Plant Source-Root Litter Dynamics

[25] In contrast to cluster surface litter dynamics, smaller differences in chemistry were observed between the live plant roots and root litter. For example, root litter in both cluster and grassland (Table 2) had total SFA values similar to that of live plants from the sites and no significant changes in S/V ratio. Curiously, root litter contained far more diOH-C_{16} than any of the fresh roots where it was below detection limits. This discrepancy could be due to adsorption of cutin SFA onto roots or input of roots from species that contain this compound. Additionally, the CuO oxidation processes may not efficiently extract SFA from roots, but once they are partially degraded in soils they

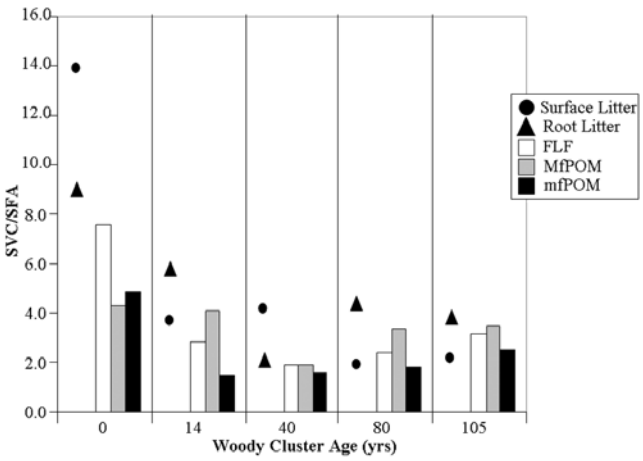

Figure 4. The ratio of the concentrations of total lignin phenols (SVC) and substituted fatty acids (SFA) released by the alkaline CuO analysis of nonaggregated particulate organic matter (POM) fractions as well as surface litter and root litter (0–15 cm). Samples are taken from three grassland areas (T = 0 years) and different age woody clusters of (14, 40, 80, and 105 years) at the LaCopita Research Area.

might be easier to extract. The C/SVC ratio exhibited little difference between live plant roots and root litter in clusters, but for grasslands the accumulated root litter value (0.19) was somewhat lower than the isolated plants (0.33). Likewise, the SV-lignin of root litter in grassland exhibited lower values than root sources, while cluster root litter showed little change from source tissue. Consequently, grass root litter progresses to a more aliphatic nature (i.e., lower SVC/SFA) compared to the plant source than do woody plants.

4.2. Evolution of Litter Biopolymer Chemistry With Woody Encroachment

[26] Overall, with WPE surface and root litter became relatively enriched in aliphatic biopolymers (cutin or suberin) with respect to lignin (Figure 4), and the accumulated lignin was proportionately enriched in SV-lignin versus cinnamyl phenols (Figure 2). This suggests that a more refractory litter accumulated with WPE. Such changes have importance within the context of carbon accrual rates determined for WPE at LaCopita, where surface and root litter accrued at 1.9 and 10.0g C m^2 yr^1, respectively [*Liao et al.*, 2006a; *Boutton et al.*, 2008]. Increased rates of above- and belowground productivity, relative to grasslands, and the potentially more refractory chemical nature of woody plant tissue were proposed as causal factors to explain the accrual. The large increase in root litter, with its lower SVC/SFA values and minimal alteration from live roots seen herein, would have a particularly significant bearing upon SOC accrual at LaCopita. This finding is in keeping with those of *Rasse et al.* [2005] who demonstrated that roots represented a source of highly recalcitrant carbon delivered directly to the soil matrix and, therefore, was a more important source to SOC than surface litter. The shift to litter with a low SVC/SFA ratio is driven primarily by plant source at LaCopita. However, such changes have also been observed with depth and with decreasing soil particle size in numerous grassland and forest systems - a change driven presumably by selective chemical decay of lignin [*Nierop and Verstraten*, 2003; *Rumpel et al.*, 2004; *Feng and Simpson*, 2007]. These and other observations in mineral soils have lead to the hypothesis that greater input of aliphatic biopolymers from cutin, suberin or waxes will promote greater stabilization and accrual of SOC in mineral soils [e.g., *Lorenz et al.*, 2007].

4.3. Impact of Woody Encroachment on the Accrual of Nonaggregated POM

[27] Similar to the observations for above- and belowground litter, nonaggregated POM exhibited a relative accumulation of SFA compared to lignin following WPE (Table 2). The most aliphatic composition occurred midway through the chronosequence at 40 years of encroachment (Figure 4). This middle-age aliphatic maximum may be a function of plant species dynamics occurring in the understory [*Archer et al.*, 1988] and consequent shifts in the chemical nature of the inputs. For example, *Z. fagara* is lower in cutin and suberin content (Table 1), and becomes more important as an understory species after about 40 years. Importantly, however, we observed through comparison of the diOH-C_{16}/ω-$OHC_{18:1}$ ratio that, by a cluster age of 40 years, denser free POM fractions (especially mfPOM) selectively accrue cutin SFA from woody plant leaves, while the FLF continued to receive a significant proportion of its new aliphatic material from roots (Figure 3). We believe this is the first time that selective apportionment or accrual of cutin and suberin aliphatics has been observed in different POM fractions. It is possible that macroinvertebrates residing in the clusters may selectively pelletize and translocate litter cuticle and wax material below ground into the mfPOM and MfPOM fractions, and that this process is of greatest importance in more developed clusters near 40 years of age. The specific changes observed in Ac/Al(s,v), C_{org}/N, and SFA chemistry may also be promoted by similar phenomena, as has been demonstrated by others investigating the role of macroinvertebrates in litter [e.g., *Rawlins et al.*, 2006; *Filley et al.*, 2008] and soil transformations [e.g., *Bossuyt et al.*, 2005].

[28] Many recent studies have shown that the relative abundance of suberin-SFA with respect to cutin-SFA and extractable lignin increases with soil depth [e.g., *Kogel-Knabner et al.*, 1989; *Nierop et al.*, 2003; *Feng and Simpson*, 2007]. In these studies it was difficult to differentiate between the selective, progressive decay of cutin and lignin [e.g., *Nierop and Verstraten*, 2003] versus the direct and continual addition of suberin from fresh root material at depth [*Otto and Simpson*, 2006] as a cause of the relative enrichment in suberin. When patterns opposite to the trend of increasing suberin/cutin were observed in soils it was presumed that the soil system experienced high rates of illuviation of surface material [*Feng and Simpson*, 2007]. At LaCopita, we believe that the different diOH-C_{16}/ω-$OHC_{18:1}$ ratio response of the POM fractions to WPE is a primary input signal. Over the first 80 years of cluster development the FLF, which we believe is selectively receiving root material, is accruing at a greater rate than the other nonaggregated POM fractions (Figure 1). This is consistent with our suggestion that root litter is relatively more refractory than surface litter, and that root litter is accumulating at a greater rate than surface litter in this system [*Boutton et al.*, 2008]. The exact role that these three nonaggregated POM fractions play in long-term carbon storage at LaCopita is unknown.

4.4. Controls on Lignin Decomposition State of Litter and Nonaggregated POM

[29] Given the large variation in the Ac/Al(s,v) values of native plants, it is difficult to state unequivocally that a greater Ac/Al ratio in litter and POM in this system is indicative of greater lignin side chain oxidation. However, the increase in Ac/Al(v) ratios for the three POM fractions, relative to values for surface and root litter, suggests that these nonaggregated fractions are undergoing some degree, albeit at a low level, of lignin decomposition and thus loss. Lower lignin recovery and an increase in Ac/Al(s,v) with decreasing soil fraction size or within aggregate gradients, have been demonstrated in numerous forest, grassland, and agricultural environments [*Guggenberger et al.*, 1994; *Amelung et al.*, 1999; *Kiem and Kogel-Knabner*, 2003; *Rumpel et al.*, 2002]. This is also true at LaCopita where mfPOM has a comparatively higher Ac/Al(v) than the larger MfPOM fraction. Trends in lignin chemistry with particle size and age have also been shown to coincide with changes in the values of C_{org}/N. At LaCopita, we also observed a

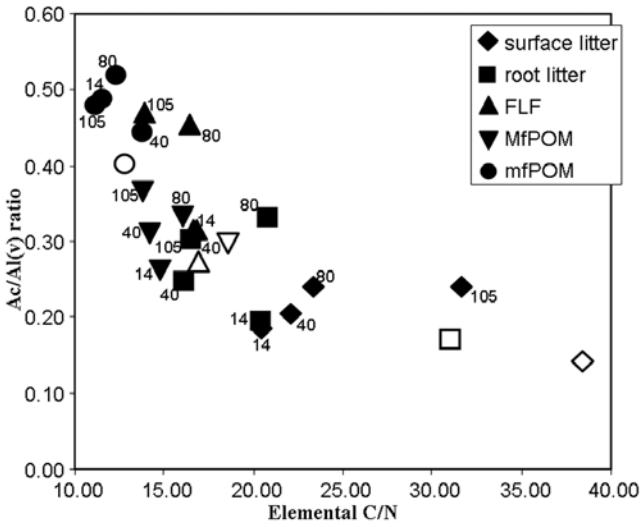

Figure 5. Relationship between elemental C/N ratio [from *Liao*, 2005] and the Ac/Al(v) lignin phenol ratio from surface litter, root litter, and nonaggregated POM (0–15 cm) fractions (FLF, MfPOM, and mfPOM). Solid symbols are for woodland clusters of age (14, 40, 80, and 105 years) and open symbols are for grasslands at the La Copita Research Area.

concomitant shift to lower C_{org}/N and higher Ac/Al(v) moving from above- and belowground litter to FLF and MfPOM and finally to the mfPOM fraction (Figure 5). Other studies have observed that small-sized (<250 μm) and/or dense (>$\sim$ 1.8 g/cm^3) mineral-associated soil fractions are comprised of lignin that has undergone a relatively greater degree of oxidation and soil organic matter with narrower C_{org}/N ratios than sand-sized or light (<1.6 g/cm^3) fractions from the same soil [e.g., *Oades and Waters*, 1991; *Baldock and Skjemstad*, 2000; *Sollins et al.*, 2006]. At LaCopita, grassland litter had broader C_{org}/N ratios than cluster litter and thus exhibited the greatest difference between litter and POM fractions suggesting a greater incorporation of microbial N as litter is transformed into soil organic carbon. But lignin in the grassland fractions had slightly lower Ac/Al(v) than the most "decomposed" lignin in the cluster fractions. The controlling factor governing the general trend of higher lignin oxidation state and lower C_{org}/N moving from litter to POM fractions with WPE appears to be a function of differences in both inherent plant source chemistry and decay dynamics at the sites.

[30] Our conclusion that the accrued nonaggregated POM is progressively shifted to a more recalcitrant chemical nature is also consistent with the observation that the ratio of soil microbial biomass carbon to soil organic carbon decreases following woody plant encroachment [*McCulley et al.*, 2004; *Liao and Boutton*, 2008]. This suggests that SOC in the wooded areas is comprised of more recalcitrant materials that are less suitable as microbial substrate compared to remnant grasslands. Additionally, these findings support the growing battery of work which has demonstrated that plant aliphatic materials including lipids, cutin, and suberin can be significant components of longer-term SOC storage in grassland, agricultural, and forest soils, whereas lignin appears to be relatively less refractory [e.g., *Bull et al.*, 2000; *Rumpel et al.*, 2002; *Dignac and Rumpel*, 2006; *Rasse et al.*, 2006]. Many forest and agricultural soils exhibit selective enrichment of aliphatic components with respect to lignin along gradients of decreasing particle size, increasing decay, or with mineral association [*Baldock and Skjemstad*, 2000; *Nierop and Verstraten*, 2003; *Schöning et al.*, 2005; *Mikutta et al.*, 2006], even without presumed shifts in plant chemical composition facilitating such changes, as seen herein.

5. Conclusions

[31] In the Rio Grande Plains of Texas, high rates of above- and belowground plant productivity in woody clusters developing in grassland have resulted in accrual of SOC primarily as POM external to soil aggregate structures. Hence, the proportion of unprotected carbon has increased from only about 15% of total SOC in remnant grasslands to as high as 65% in the 80-year-old woody cluster. During the conversion of fresh plant material to surface litter in woody clusters, there were significant losses in cutin-derived SFA and cinnamyl phenols. In contrast, root tissue exhibited only minor changes in biopolymer chemistry, suggesting root litter was either more stable or was refreshed chemically at a greater rate. Compositional differences in SFA between roots and leaves of woody plants showed that mfPOM and MfPOM became enriched with cutin-derived SFA while the FLF was enriched with root-derived SFA, indicating distinct source input paths to SOC pools as clusters matured. We also found that the shift from herbaceous to woody input was accompanied by an enrichment in the amount of cutin and suberin-derived aliphatic biopolymers with respect to lignin for above- and belowground litter as well as nonaggregated POM fractions. Given the developing knowledge of the important role of aliphatic biopolymers in long-term storage potential of soil organic matter, particularly root-derive suberin, we believe that succession from grassland to woodland in this subtropical savanna rapidly produces nonaggregated POM pools that are considered, at least by their chemical signature, more recalcitrant in nature. This is significant as SOC accrues in the woody cluster system primarily without mineral or aggregate-associated physical protection. These data reveal an important mechanism by which productivity-driven increases in litter and root input can result in accrual of unprotected carbon in this ecosystem.

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