

Lignin in Particle-Size Fractions of Native Grassland Soils as Influenced by Climate

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ABSTRACT

The turnover of soil organic carbon (SOC) in grasslands can be predicted as a function of climate, plant lignin content, texture, and kinetically defined C pools. Particle-size fractionation has been used to identify soil C pools. This study was conducted to investigate influences of climate on the dynamics of lignin in particle-size fractions. Composite samples were taken from the top 10 cm of 18 native grassland sites along temperature and precipitation transects from Central Saskatoon, Canada, to South Texas. Lignin-derived phenols were determined in the <2 μm (clay), 2- to 20- μm (silt), 20- to 250- μm (fine sand) and 250- to 2000- μm (coarse sand) size separates. With decreasing particle size the concentration of lignin-derived phenols decreased significantly from 72 g kg^{-1} SOC in the coarse sand fractions to 12 g kg^{-1} SOC in the clay fractions. Increasing phenolic acids to aldehyde ratios indicated that side chain oxidation proceeded as particle size decreased. Moreover, these ratios decreased in fractions <250 μm with increasing mean annual temperature (MAT) at the sites. This suggests that the degree of lignin decomposition decreased with increasing MAT, possibly because there was a lack of additional C sources, such as saccharides of root litter, which are needed for the cometary decay of lignin.

UNDERSTANDING THE COMPLEX INTERACTIONS of biological, chemical, and physical processes involved in the turnover of soil organic matter (SOM) is important for predicting the response of global C stocks to climate change. In mathematical models, SOM has been conceptualized as kinetically defined pools with different turnover rates (Jenkinson and Rayner, 1977; Parton et al., 1987). Experimentally, physical fractionation of soil according to particle size supported by chemical analysis of SOM constituents in the fractions proved to be one of the most useful approaches in identifying C pools with different SOM properties and turnover rates (Christensen, 1992). Still, little analytical data are available about the effect of climate on SOM constituents, such as lignin, in the C pools.

Lignin is one of the predominant components in grass and root litter (Molloy et al., 1977). Once the more labile components have been degraded, slowly decomposing lignin dominates the shape of litter decomposition curves (Minderman, 1968). The lignin concentration in litter has been used, therefore, as an indicator for predicting the litter turnover in different climates (Meentemeyer, 1978).

As intact lignin is insoluble, there are no analytical methods currently available to accurately determine the absolute lignin content in the mineral soil. Alkaline CuO oxidation, however, releases phenols from reactive sites of the lignin macromolecule. The sum of vanillyl, syringyl, and cinnamyl phenolic CuO oxidation products

(VSC) has been used as relative measure of the total lignin concentration in plants and sediments (Hedges and Mann, 1979), and in soils (Kögel, 1986). Moreover, the mass ratios of acids to aldehydes of the vanillyl (ac/al)_v and of syringyl structural units (ac/al)_s have been used to determine the degree of lignin oxidation within a sample, while a decreasing mass ratio of the syringyl to vanillyl units (S/V) has reflected selective loss of syringyl units during lignin degradation (Ertel and Hedges, 1984; Kögel, 1986). The method of CuO oxidation has been successfully used to clarify the pathways of lignin degradation in terrestrial forest ecosystems (e.g., Zech et al., 1996). This method also should be suitable for characterizing lignin dynamics in grassland soils as influenced by climate.

The objectives of this study were (i) to establish the pattern of lignin decomposition in particle-size fractions from grassland topsoils as influenced by climate, and (ii) to test whether VSC lignin could be a suitable indicator for the identification of recent and nonrecent plant residues in fine and coarse sand fractions.

MATERIALS AND METHODS

Soils

We took composite samples (0–10 cm) from each of 18 sites along mean annual temperature (MAT) and mean annual precipitation (MAP) transects across the native North American grasslands. They comprise the choice of sites from the study by Amelung et al. (1998) that have a moderate texture range (17–35% clay). The MAT ranges from 0.9 to 23.4°C, and MAP from 300 to 1308 mm (Table 1). The vegetation species at each site resembled the potential grassland vegetation of respective Land Resource Areas (USDA-SCS, 1981) with few exceptions. Buffalo grass [*Buchloe dactyloides* (Nutt.) Engelm.] and blue grama [*Bouteloua gracilis* (Kunth) Lag. ex Griffiths, nom. illeg.] were common in the drier areas in the western prairie; *Agropyron* species such as western wheatgrass [*Pascopyrum smithii* (Rydb.) Á. Löve] were typical for the northern and central plains; whereas green needlegrass [*Nassella viridula* (Trin.) Barkworth] was only found at the northern sites. Big and little bluestem (*Andropogon gerardii* Vitman, and *Schizachyrium scoparium* (Michx.) Nash, respectively) contributed to the grasses in the east central prairie, and ragweed (*Ambrosia* sp. L.) was among those at the southern sites. More details concerning site locality, sampling strategy, invasive plant species, parent material, and soil properties at the sites have been described by Amelung et al. (1998, 1999b) and USDA-SCS (1994).

Abbreviations: (ac/al)_s, mass ratio of acids to aldehydes of syringyl structural units; (ac/al)_v, mass ratio of acids to aldehydes of the vanillyl structural units; ANOVA, analysis of variance; MAP, mean annual precipitation; MAT, mean annual temperature; SOC, soil organic carbon; SOM, soil organic matter; S/V, mass ratio of the syringyl to vanillyl units; VSC, the sum of vanillyl, syringyl, and cinnamyl phenolic CuO oxidation products.

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Table 1. Soils, climate, and lignin signature of the sites under study.†

No.	Series	Locality	MAT	MAP	Bulk soil (<2000 μm)				Clay VSC	Silt VSC	Fine sand VSC	Coarse sand VSC	Classification (Soil Survey Staff, 1998)
					VSC	(ac/al) _v	(ac/al) _s	S/V					
			°C	mm	g kg ⁻¹ SOC‡				g kg ⁻¹ SOC in the fraction				
Cryic													
I	CutKnife-Naicab	Hoey, SK	0.9	456§	19.6	0.40	0.47	0.74	7.5	13.9	28.5	68.7	Fine-loamy, mixed superactive Typic Haplocryolls
II	Elstow	Aberdeen, SK	1.6	343¶	21.3	0.36	0.45	0.78	6.6	14.5	28.8	60.8	Fine-loamy, mixed superactive Ustic Haplocryolls
III	Ardill	Swift Current, SK	3.2	380#	21.5	0.36	0.39	0.83	10.6	18.4	30.7	34.6	Fine-loamy, mixed superactive Ustic Argicryolls
Frigid													
IV	Svea	Morris, MN	6.1	565	17.7	0.32	0.44	0.78	8.3	18.1	65.2	87.4	Fine-loamy, mixed, superactive frigid Pachic Hapludolls
V	Amor	Mandan, ND	5.0	419	16.7	0.28	0.43	1.03	9.5	14.8	34.8	54.6	Fine-loamy, mixed, superactive frigid Typic Haplustolls
VI	Joplin	Havre, MT	6.1	300	26.4	0.40	0.59	0.80	17.1	18.4	47.1	83.8	Fine-loamy, mixed, superactive frigid Aridic Argiustolls
Mesic													
VII	Ulm	Arvada, WY	7.2	400	17.3	0.39	0.40	0.53	7.2	14.6	22.5	61.4	Fine, smectitic, mesic Ustic Haplargids
VIII	Weld	Akron, CO	10.8	375	24.1	0.26	0.45	0.71	23.9	19.8	22.9	84.6	Fine, smectitic, mesic Aridic Argiustolls
IX	Holdrege	Holdrege, KS	11.6	666	21.0	0.39	0.39	1.45	30.7	18.9	90.3	90.3	Fine-silty, mixed, superactive mesic Typic Argiustolls
X	Armo	Hayes, KS	12.2	573	11.5	0.34	0.51	0.79	9.5	26.8	45.5	60.6	Fine-loamy, mixed, superactive mesic Entic Haplustolls
XI	Sharpsburg	Lincoln, NE	10.9	792	16.4	0.27	0.38	0.55	10.5	25.4	53.3	99.7	Fine, smectitic, mesic Typic Argiudolls
XII	Reading	Manhattan, KS	12.4	791	17.5	0.34	0.47	0.79	6.4	16.4	46.2	61.6	Fine, mixed, superactive mesic Pachic Argiudolls
XIII	Parsons	Parsons, KS	14.2	1000	18.9	0.48	0.44	0.55	6.6	11.1	28.2	68.1	Fine, mixed, superactive thermic Mollic Albaqualfs††
Thermic													
XIV	Amarillo	Big Spring, TX	17.1	466	20.5	0.21	0.58	1.32	13.8	19.7	54.9	76.8	Fine-loamy, mixed, superactive thermic Aridic Paleustalfs
XV	Hallettsville	Columbus, TX	20.0	1030	16.6	0.35	0.38	0.83	8.1	31.2	42.6	67.0	Fine, smectitic, thermic Udertic Paleustolls
XVI	Morey	Beaumont, TX	20.3	1308	23.8	0.16	0.35	0.76	17.1	20.3	72.3	77.4	Fine-silty, siliceous, superactive hyperthermic Oxyaquic Argiudolls‡‡
Hyperthermic													
XVII	Racombes	Alice, TX	22.2	700	9.1	0.34	0.32	0.76	7.1	20.6	33.2	68.5	Fine-loamy, mixed, superactive hyperthermic Pachic Argiustolls
XVIII	Moglia	Webb County, TX	23.4	440	17.2	0.25	0.42	1.08	8.3	21.1	24.7	44.5	Fine-loamy, mixed, superactive hyperthermic Ustic Haplocalcids

† MAT, mean annual temperature; MAP, mean annual precipitation directly measured at the experimental station for the last 40–60 yr, or extrapolated from the closest climate station, according to Wallis et al. (1991); VSC, sum of vanillyl (V), syringyl (S), and cinnamyl (C) units; (ac/al)_v, ratio of vanillin acids to vanillin; (ac/al)_s, ratio of syringic acid to syringaldehyde; S/V, ratio of syringyl to vanillyl structural units.

‡ SOC, soil organic carbon.

§ Snow: 155 mm.

¶ Snow: 90 mm.

Snow: 122 mm.

†† Classification is taxadjunct at mesic–thermic and thermic–hyperthermic temperature regimes, respectively.

Particle-Size Fractionation

Air-dried bulk samples screened to <2 mm were fractionated according to particle size as outlined by Amelung et al. (1998). Briefly, coarse sand-sized SOM was removed from soil by wet sieving after treating the sample ultrasonically at 60 J mL⁻¹ with a probe type sonicator (model W 185 F, Heat Systems, Long Island City, NY). After final ultrasonic dispersion at 440 J mL⁻¹, centrifugation was used to collect the clay fraction (<2 μm), and silt [2–20 μm = fine silt (USDA)] was separated from fine sand [20–250 μm = coarse silt, very fine, and fine sand (USDA)] by wet sieving. Amelung and Zech (1999) suggested that with the procedure described above, a disruption of coarse sand-sized SOM during the ultrasonic dispersion can be minimized, but that yields of clay were similar to those obtained from conventional particle-size analysis (USDA-SCS, 1994). All fractions were dried at 40°C (Christensen, 1992) and ground for chemical analysis.

Soil Analysis

The data for SOC in bulk soils and fractions were taken from Amelung et al. (1998). The pH was measured in a 0.01 M CaCl₂ solution (soil:salt solution = 1:2.5). Amount and degree of oxidative decomposition of lignin were estimated using alkaline CuO oxidation at 170°C for 2 h (modified from Hedges and Ertel, 1982). We replaced liquid–liquid extraction by a solid-phase extraction of the phenols using C-18 (Mallinckrodt Baker, Phillipsburg, NJ) as suggested by Kögel (1986). Phenolic oxidation products were dissolved and derivatized with a 1:1 mixture of pyridine and N,O-bis(trimethylsilyl)-trifluoroacetamide (Fluka Chemie, Deisenhofen, Germany), separated by capillary gas chromatography (Ultra 2 fused silica column, 25 m, Hewlett Packard, Palo Alto, CA) and detected by a flame ionization detector. Ethylvanillin was added as an internal standard to the sample prior to alkaline extraction, and phenylacetic acid was used as a second internal standard

added before derivatization in order to determine the recoveries of ethylvanillin. The recoveries increased to >80% with the addition of 50 mg of glucose to the sample, prior to alkaline CuO oxidation.

After fractionation, on average 110% of lignin-derived phenols (VSC) were recovered relative to their determination in the bulk soil samples, suggesting that size fractionation and ultrasonic treatment did not induce losses of lignin. The weighted means for other lignin indicators yielded 105% [for $(ac/al)_V$ and S/V] and 108% [for $(ac/al)_S$] relative to the values that were determined for the unfractionated bulk soils. The fractionation procedure as outlined above, in the case of lignin components of SOM, obviously does not include the risk of structural changes in SOM, as observed by Morra et al. (1991) after extended (>5 kJ) ultrasonic dispersion.

Statistical Evaluation

We used a *t* test of paired differences for the comparison of two means (Hartung, 1989). Multiple comparisons of means for the different size fractions were conducted using one-way analysis of variance (ANOVA) along with post hoc separation of means by the LSD procedure, as recommended by Huitema (1980). Multivariate statistical analysis was performed with the Windows' software package Statistica 5.0 for ANOVA (StatSoft, Tulsa, OK) and SPSS 5.0 for linear multiple regression (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Lignin in the Size Fractions

The SOM in the 20- to 50- μm and 50- to 250- μm fractions were statistically identical with respect to C/N ratios or to C and N content (Amelung et al., 1998). To test whether the SOM of these two subfractions of our fine sand had similar lignin, we determined lignin-derived phenols in selected samples of these two fractions. Similar results for VSC, $(ac/al)_{V,S}$, and S/V ratios (Table 2) suggested that both fractions comprised similar lignin pools. Conforming with Amelung et al. (1998) we continued, therefore, to work with the combined 20- to 50- μm and 50- to 250- μm fractions.

The content of lignin-derived phenols averaged $17.9 \pm 5.3 \text{ g VSC kg}^{-1} \text{ SOC}$ in the upper 10 cm of native bulk soils (<2 mm), which is similar to the lignin content in the Russian steppe (Rodionov et al., 1999). The VSC concentrations in SOM decreased with decreasing particle size in the order: coarse sand > fine sand >

Table 2. Average lignin signature of organic matter in the fine sand fraction from five selected grassland sites (III, IV, IX, XVII, XIX) of the North American prairie after subfractionation into 20 to 50- μm and 50 to 250- μm particles.†

Fraction	VSC	$(ac/al)_V$	$(ac/al)_S$	S/V
	$\text{g kg}^{-1} \text{ SOC}^\ddagger$			
20–50 μm	40.9	0.22	0.41	1.12
50–250 μm	52.7	0.20	0.38	1.01
Difference	NS§	NS	NS	NS

† VSC, sum of lignin-derived phenols; $(ac/al)_V$, ratio of vanillic acid to vanillin; $(ac/al)_S$, ratio of syringic acid to syringaldehyde; S/V, ratio of syringyl to vanillyl structural units.

‡ SOC, soil organic carbon.

§ NS, not significantly different at the $P < 0.05$ level (*t* test via paired differences; Hartung, 1989).

silt > clay (Fig. 1); thus supporting earlier evidence that particle-size separation yields different VSC pools (Guggenberger et al., 1994). In contrast, the ratios of acids to aldehydes of the vanillyl and syringyl structural units increased significantly ($P < 0.05$; Fig. 1), indicating that oxidative side-chain alteration increased as particle size decreased (Ertel and Hedges, 1984). This suggests that the decrease of VSC contents with decreasing particle size indicated a typical SOM decomposition gradient similar to those reported for forest soils in central Europe (Kögel et al., 1988). Selective losses of syringyl structural units were significantly pronounced only for the clay fraction, as indicated by lower S/V ratios ($P < 0.05$) in the clay (0.73) compared with silt (0.84) and fine-sand fractions (0.90). The S/V ratio of coarse sand-sized SOM (0.78) was lower than for fine sand-sized SOM ($P < 0.05$). This indicated a preferential decay of vanillyl relative to syringyl structures, such as was observed for the initial stages of SOM decomposition in forest soils (Kögel, 1986). The lower S/V and $(ac/al)_{V,S}$ ratios in the coarse sand compared with the fine-sand fraction confirmed, therefore, that on the average, the coarser plant residues in soil were more recent than the finer ones (Amelung et al., 1998).

It was obvious that there was no selective preservation of VSC units with increasing degree of SOM decomposition in the smaller size fractions. As intact lignin is insoluble, it must be oxidized before it can be transferred into soil solution. Here it is attracted by soil minerals (Kaiser et al., 1996). Because an oxidative degree of lignin in dissolved organic matter has been found to be similar to that of the clay fractions (Guggenberger et al., 1998), it seems reasonable to speculate that the VSC content of the clay fractions was affected by the continuous sorption of dissolved organic matter containing oxi-

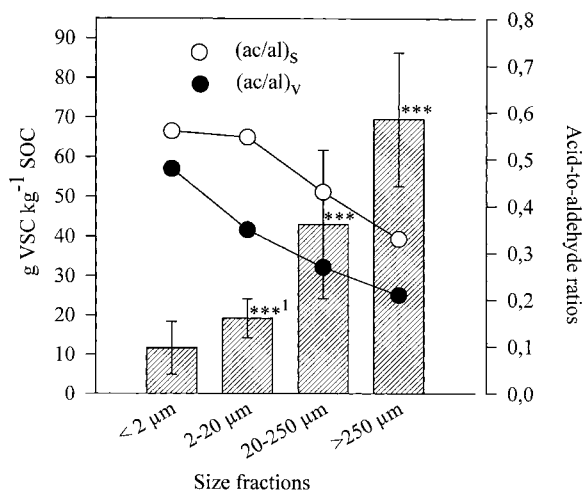


Fig. 1. Average concentration (bars) of lignin-derived phenols (VSC), as well as phenolic acid to aldehyde ratios (curves) of the syringyl $(ac/al)_S$ and vanillyl $(ac/al)_V$ structural units in particle-size fractions of 18 topsoils in the native grasslands. The symbol *** indicates that VSC is significantly higher compared to the next finer fraction at the $P < 0.001$ probability level of significance. The symbol ¹ indicates that significant difference is obtained only when ANOVA is conducted with natural log-transformed VSC values. The error bars result from variations in VSC concentrations of a respective fraction across the prairie.

dized VSC units in low concentration (Guggenberger and Zech, 1994). Real lignin contents, however, remained unknown, because only CuO reactive parts of the lignin molecule were extracted. Also, NMR measurements failed to detect a maximum of lignin-derived aromatic moieties in the clay fraction, supporting the hypothesis that there was no selective preservation of aromatic moieties in the most degraded SOM (Catroux and Schnitzer, 1987; Oades et al., 1987). In contrast, pyrolysis mass spectrometry data indicated an enrichment of degraded lignin in clay fractions (e.g., Schulten and Leinweber, 1991). In solid-state NMR research there is the risk of an underestimation of the aromatic signal (Snape et al., 1989). Furthermore, alkaline extraction of SOM for liquid-state NMR spectroscopy may render the sample poorly representative, and yields of pyrolyses may have been affected by the presence of minerals (Schulten and Leinweber, 1991, 1993). Due to these disadvantages, it still remains uncertain as to what degree bulk lignin residues accumulated in the SOM of the clay fractions.

Litter decomposition rates are positively related to the N content and negatively related to the lignin content of the litter (Aber and Melillo, 1982); i.e., they correlate negatively with the initial lignin/N ratio (Melillo et al., 1982). Parton et al. (1987) also used the lignin/N ratio for distinguishing plant residues that are difficult to degrade (*structural-C* with wide lignin/N ratio) from those that are more easily degraded (*metabolic-C* with narrow lignin/N ratio). In the samples under this study, the average ratio of VSC/N in the coarse sand-fractions (VSC/N = 15.9) exceeded that of the fine sand fractions (VSC/N = 0.29) by a factor of 55 ($P < 0.001$). Thus, the VSC/N ratio decreased with the increasing stage of decomposition from coarse to fine sand-sized SOM, and differences in the degree of decomposition were reflected more sensitively than by the C/N ratio, averaging 21 for the coarse and 14 for the fine sand fractions, respectively (Amelung et al., 1998). As the coarse sand-sized SOM was more recent than fine sand-sized SOM, it seems likely that it will decompose more quickly than fine sand-sized SOM structures that have already been altered by microbes. We suggest that in contrast to the lignin/N ratio of initial plant litter, the lignin/N ratio of SOM fractions is positively correlated with the decomposition rates.

Effects of Climate on the Dynamics of Lignin

In the bulk soil, changes of lignin-derived phenols (in g VSC kg⁻¹ soil) closely followed changes of SOC ($r = 0.90$, $P < 0.001$). In order to discuss the dynamics of lignin, we preferred to emphasize parameters of lignin alteration and the VSC concentration in SOM rather than the absolute VSC content.

There were no relationships between lignin parameters in the bulk SOM with climatic elements such as MAT or MAP, which confirms the observations of Amelung et al. (1997) for six sites. The MAT and MAP explained <20% of the variability of VSC (in g kg⁻¹ SOC), and <26% of the variations in (ac/al)_v, (ac/al)_s, and S/V of the bulk SOM.

We attribute the lack of clear relationships between the lignin characters of the bulk SOM and climatic elements to the fact that one third of the total VSC stock in these grassland soils was in coarse sand-sized SOM. The degree of side-chain oxidation, as indicated by low (ac/al)_{v,s} ratios, remained low for the coarse sand fractions (Fig. 1), and the correlations between climatic elements and VSC concentrations in the coarse sand fractions were insignificant ($r^2 < 0.1$), which suggests that SOM of the coarse sand fractions was still too little altered to be affected by climate (Amelung et al., 1998). Low coefficients of determination and standard deviations of 23% of the mean VSC concentration in the SOM of the coarse sand fractions and of ≈30% for (ac/al)_{v,s} and S/V ratios indicate that the variation in these parameters must be attributed to factors other than climate; e.g., to a qualitatively and quantitatively different lignin signature in the residues of various nonwoody vascular plant tissues (Whitehead et al., 1981).

Another picture was obtained from the characterization of lignin in fractions finer than 250 μm. The (ac/al)_s ratio of the fine sand fractions decreased significantly with increasing MAT (Fig. 2), although the other lignin parameters of this fraction did not significantly change with MAT, MAP, nor the logarithm of the MAP/MAT ratio. In the silt and clay fractions, however, both (ac/al)_s and (ac/al)_v decreased significantly with increasing MAT (Fig. 3a,b). The negative relations between the degree of lignin side-chain oxidation and MAT (Fig. 2, 3) became more pronounced the finer the particle size; i.e., the more the SOM was altered by microbes. Moreover, the r values were higher for the more reactive syringyl than for the more recalcitrant vanillyl structures. Nevertheless, it should be noted that the (ac/al)_{v,s} ratios of the clay fractions from site XIII were not included in the correlation analyses. These values were statistically extraneous according to the Grubbs test for outliers ($P < 0.001$; Hartung, 1989). Because humic substances act as electron donors for inorganic reduction processes, it seems reasonable to speculate that the high oxidative degree of lignin in the clay fraction of

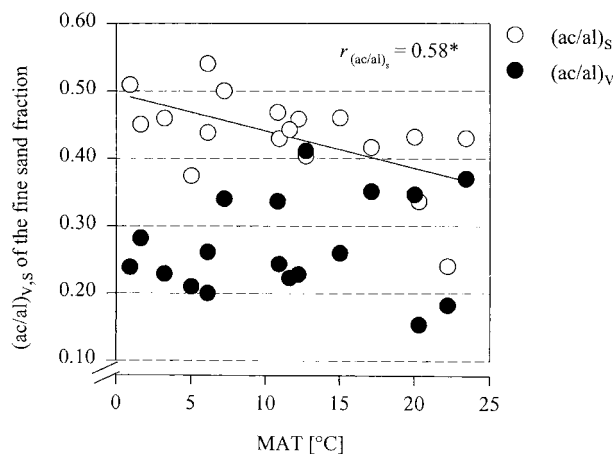


Fig. 2. Ratio of lignin-derived phenolic acids to aldehydes of the vanillyl (ac/al)_v and syringyl structural units (ac/al)_s as related to mean annual temperature (MAT, °C) for the fine sand fractions (20–250 μm).

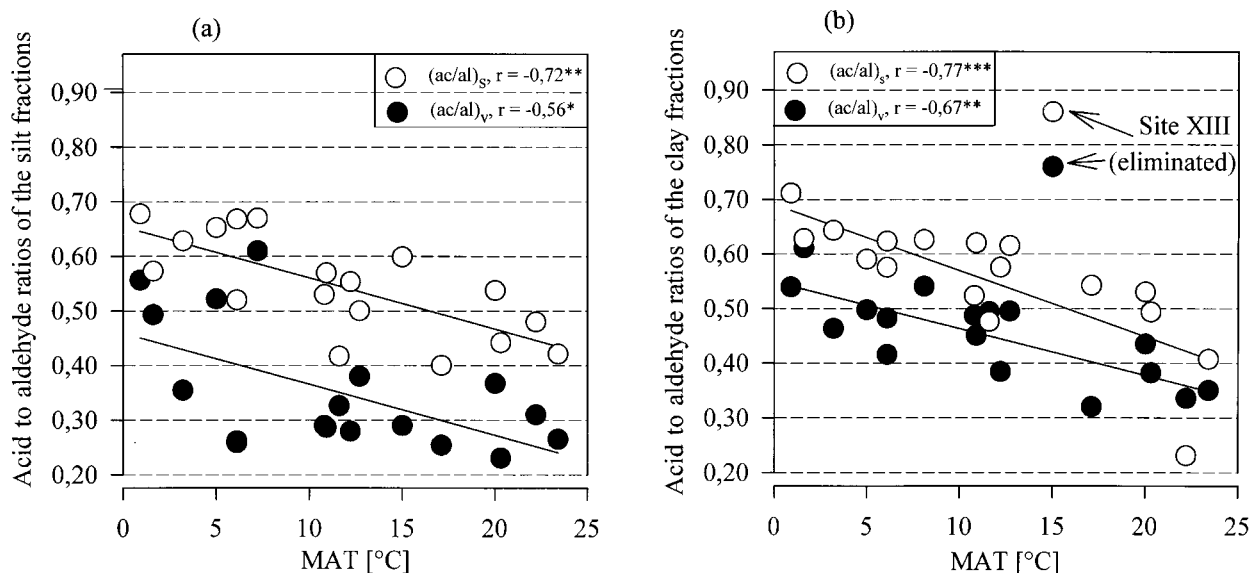


Fig. 3. Ratio of lignin-derived phenolic acids to aldehydes as related to mean annual temperature (MAT, °C) for the syringyl (ac/al)_s and vanillyl (ac/al)_v structural units in the (a) silt and (b) clay fractions (Site XIII, an Aquoll, was eliminated from the calculation of *r*).

site XIII might be the result of Mn and Fe reduction in the clay of this Aquoll (site XIII is the only site that had an aquic moisture regime).

Selective loss of the syringyl relative to the vanillyl structural units is indicated by decreasing S/V ratios (Ertel and Hedges, 1984). In the silt fractions S/V_{silt} correlated positively with MAT ($r = 0.47$, $P < 0.05$). This correlation was less clear than that for (ac/al)_s or (ac/al)_v, partly because S/V_{silt} was additionally related to MAP:

$$\begin{aligned} S/V_{\text{silt}} &= 0.84 + 0.02 \text{ MAT } (^\circ\text{C}) - 0.34 \text{ MAP (m)}, \\ R &= 0.64, P < 0.05 \end{aligned} \quad [1]$$

In the silt fractions both S/V and the acid to aldehyde ratios indicate that the alteration of the remnant lignin macromolecule was less pronounced at warmer sites than at regions of low MAT. In addition, the VSC concentrations increased with increasing MAT ($r = 0.50^*$) in the SOM of this fraction. The VSC/N_{silt} ratios also increased with MAT ($r = 0.61^{**}$). Apparently, VSC/N ratios illustrated an effect of MAT on lignin dynamics in the silt fractions more clearly than VSC concentrations did, but less clearly than the ratios of phenolic acids to aldehydes. The correlation coefficients for the VSC/N ratios were low, however, and similar trends for other fractions were insignificant.

At present we know little about the kinetics of incorporating lignin into organomineral fractions. What is more, it remains uncertain whether we can deduce higher mineralization rates of lignin from higher degrees of side-chain oxidation at cool climates (Haider, personal communication, 1996), since lignin-derived phenols comprised only the extractable part of total lignin in soil. Nevertheless, we may argue that (i) there was no correlation of lignin characteristics with MAT in the coarse sand fractions that represented the *fresh* plant residues in soil (Amelung et al., 1998); therefore, it is unlikely that plant-specific variations of lignin charac-

ters (Whitehead et al., 1981) contributed significantly to the correlations of the (ac/al)_{v,s} ratios of the <250- μm fractions with MAT, or of S/V ratios and VSC concentrations of silt with MAT. We conclude that the effects of MAT developed during the decomposition of lignin in soil. We also claim that (ii) the *r* values and significance levels for the relationship of acid to aldehyde ratios with MAT improved with decreasing particle-size diameter. This gives additional support to the hypotheses mentioned for site XIII that minerals had an effect on the increased oxidation of lignin. This does not imply, however, that interactions between lignin and mineral fractions are necessary for the correlation of lignin characters with MAT, since a significant correlation between MAT and the (ac/al)_s ratio was also found for the fine sand fraction, where little if any SOM was bound to minerals (Amelung et al., 1998).

Stott and Martin (1990) stated that lignin in soil is decomposed cometabolically. Lignin degradation depends, therefore, on additional C sources (e.g., saccharides of litter) that can be utilized by microorganisms (Kirk et al., 1976; Haider, 1992). Since saccharides of litter are decomposed faster than lignin and faster as soil temperature increases (Donnelly et al., 1990), the SOM in warm grasslands had both less litter (Amelung et al., 1998) and fewer carbohydrates than that of cool grasslands (Amelung et al., 1997). Consequently, it seems reasonable to assume that the concentration of additional C sources readily available to microorganisms is lower in warm than in cool climates. At sites of the southern grasslands (MAT > 12°C) such inadequate substrates might also have promoted the decay of microbially derived amino sugars (Amelung et al., 1999b). Lack of additional C sources could, therefore, also limit lignin oxidation rates (Kirk et al., 1976) at warm as compared with cool climates.

When considering carbohydrates as additional C source for the cometabolic decay of lignin, we have to

take into account that (i) carbohydrate concentrations also depended on MAP (Amelung et al., 1997) while $(ac/al)_{v,s}$ ratios did not, and that (ii) carbohydrate content was low in the silt fraction (Cheshire, 1979; Guggenberger et al., 1994) although silt-sized SOM did not reveal the least degraded lignin. To clarify this issue, we determined individual sugars in the fractions; the results revealed that clay-associated saccharides were mainly microbially derived and correlated closely with MAP but not with MAT (Amelung et al., 1999a). It is therefore unlikely that (i) these sugars served as an additional C source for the cometabolic, MAT-dependent decomposition of lignin, and that (ii) the carbohydrate content of a particle-size fraction controlled the degree of lignin oxidation in this specific fraction. In contrast to the saccharides of the clay fraction, sugars of the fine sand fraction correlated more closely with MAT than with MAP (Amelung et al., 1999a). Also the proportion of particulate SOM in sand fractions was primarily related to MAT, but little related to MAP (Amelung et al., 1998). We assume that only a labile portion of total soil saccharides (mainly litter carbohydrates of sand-sized SOM) served as an additional C source for cometabolic lignin oxidation.

CONCLUSIONS

Particle-size fractionation into clay, silt, fine sand, and coarse sand yielded four significantly different lignin pools; the degree of lignin alteration proceeded as particle-size diameter decreased. The small scale heterogeneity of lignin properties among particle-size fractions was at least as important as the large scale heterogeneity of lignin properties among the whole grasslands.

Because organic matter of the coarse sand fractions was too little altered to reflect climatic effects on SOM, there was no correlation for lignin characters with climatic elements when only the bulk soil material was considered. Nevertheless, climate influenced the degree of lignin oxidation in the finer particle-size fractions, especially in clay- and silt-sized separates. This was indicated by $(ac/al)_{s,v}$ ratios and, less clearly, by S/V and VSC/N ratios and VSC concentrations. Obviously, only lignin that has been altered by microbes is related to climate. Whereas S/V ratios were related to MAT as well as to MAP, side-chain oxidation depended on the temperature regime. The warmer the climate, the lower the $(ac/al)_s$ ratios of the fine sand fractions and the $(ac/al)_{v,s}$ ratios of the silt and clay fractions, irrespective of the amount of MAP these sites receive.

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