Relationship Between Plant Phenolic Acids Released during Soil Mineralization and Aggregate Stabilization

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ABSTRACT

Phenolic acids (PAs) from plant and microbial sources have been implicated as important components in a variety of soil processes, but little information is available on the decomposition rates of plant-derived PAs, synthesis of soil microbial PAs, incorporation of PAs into humic substances and stabilization of soil aggregate fractions. To obtain this information, a Webster soil (fine-loamy, mixed, superactive, mesic Typic Endoaquoll) was amended with seven plant residues (2% w/w) and incubated at 22 $\pm$ 2°C. Duplicate samples were extracted after incubation for 9, 29, and 84 d and analyzed for PA composition. The plant residues contained large amounts of ferulic [3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid] and coumaric acids [3-(4-hydroxyphenyl)-2-propenoic acid] (both C_6–C_3, phenylpropyl type), which decomposed according to pseudo first-order kinetics. Most of the remaining PAs showed little change in concentration after 9 d and only microbial synthesis of 4-hydroxybenzoic acid was noted during the study. Analysis of six purified soil microbial polymers isolated from pure culture confirmed that the tested microbial extracellular polymers contained benzoic and benzaldehyde PAs (C_6–C_1, phenylmethyl type). Evaluation of humic acids isolated from different residue-amended soil showed the majority of humic-PAs were C_6–C_3 PAs of plant origin. Increased mean weight diameter of soil aggregates was closely related to the increase in soil PA concentration and organic C content in the amended soil during incubation. The results suggest that plant PAs are extremely important in the soil C cycle, soil aggregation and in the formation of stable C, and measurement of soil ester-linked PA composition can provide an index of plant-derived C in soil.

FORMATION AND COMPOSITION of stable soil organic matter (SOM) is not completely understood, but stable SOM is thought to be derived from microbial conversion of plant derived PAs complexed with amino acids, polysaccharides, and other organic constituents (Haider and Martin, 1975; Verma et al., 1975; Haider et al., 1975). Phenolic acids from plant or microbial sources are important precursors of soil humic substances (Stevenson, 1994), but the importance of each source is not known (Martin and Haider, 1976). The greatest source of PAs in nature is from primary plant cell walls and structural lignins (Carpita and McCann, 2000). Phenolic acids constitute the second most abundant organic constituent cycled in soil, next to cellulose (Freudenberg, 1968; Harkin, 1973) and can account for 20 to 30% of the biological C cycling in the biosphere (Croteau et al., 2000). Phenolic acids from the primary cell wall of plants and lignin polymers of vascular plants have a relatively high chemical stability and the conversion of PAs back to CO_2 during mineralization has been reported to present the rate-limiting step in recycling biological C (Croteau et al., 2000). Phenolic acids found in cell wall and lignins have a unique chemical structure of C_6–C_3 (phenylpropanoid type) that is essentially absent from other living organisms (Sarakanen and Ludwig, 1971). By contrast, PAs determined to be of microbial origin (Moorman et al., 1992) are of the form C_6–C_1 (phenylmethyl type). Although polymeric PAs are relatively slow to degrade compared with the plant carbohydrate fractions, the polymers are decomposed under natural conditions and certain fungi are especially active in the decomposition process (Haider et al., 1977).

Little is known about the decomposition rates of plant-derived PA polymers in nature, although the fate of PAs in soil has generally been determined by adding known amounts of radiolabeled monomeric PAs and simple PA polymers (Martin and Haider, 1976; Haider et al., 1977) or adding nonradiolabeled monomeric PAs and then attempting to extract the added PA without extracting the native, background levels of PA (Dalton et al., 1987; Dalton et al., 1989; Blum et al., 1994; Blum, 1997, 1998). Other studies have extracted PAs from soils with NaOH to determine an estimate of soil PAs present at a point in time (Hartley and Buchan, 1979; Whitehead et al., 1981, 1982, 1983; Kelley et al., 1994; Martens, 2000a, 2002).

Since the quantity and composition of PAs in plant biomass is uniquely different (Martens, 2000a, 2002), characterizing the composition of plant-derived PAs has been proposed as a method to determine the contribution of specific vascular plant sources to soil C (Hedges and Mann, 1979). Cell walls of monocot plants such as grasses can accumulate extensive interconnecting networks of PAs in the form of ferulic and coumaric acids that link cellulose and protein structures to the core lignins (Carpita and McCann, 2000). Provan et al. (1994) noted that the ferulic and coumaric acids from the cell walls were predominantly found as ester-linked compounds that were extracted with cold 1 M NaOH. The PAs extracted by this method were not of lignin origin, as a release of PAs from lignin requires much stronger oxidizing conditions (cupric oxide-NaOH, 175°C) (Chen, 1992). Martens (2002) reported that the quantity and composition of ester-linked PA in soil extracted with NaOH may provide an estimate of the level of identifiable plant residues remaining in soil and thus could provide a rapid chemical analysis for determining the level of plant biomass-derived C present in soil under different management systems.

The objectives of this study were to follow the decomposition of plant-derived ester-linked PAs from plant biomass with contrasting PA composition in soil, to determine the contribution of the PAs to development of

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Abbreviations: MWD, mean weight diameter; PA, phenolic acid; SOM, soil organic matter.
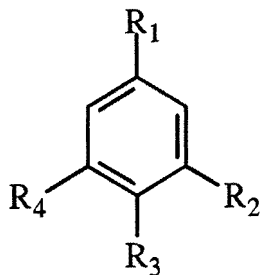
Fig. 1. Model chemical structure for the phenolic acids (PAs) studied here. The identities of the R_1–R_4 groups for the various PAs are listed in Table 1.

soil aggregate stability and the role of plant and microbial PAs in formation of stable soil aggregate stability.

MATERIALS AND METHODS

Soil and Plant Samples

All soil collection sites are located within the same soil map unit delineation (Finnley and Lucassen, 1985) and three samples of each soil were collected from within a 1-m² grid and combined into one composite sample. The soil was collected in April 1997, stored moist at 4°C, and a full description of soil properties is reported in Martens (2000a, 2002). The properties of the corn (*Zea mays*), soybean (*Glycine max*), an unidentified grass species collected from a native prairie site, alfalfa (*Medicago sativa*), oat (*Avena sativa*), clover (*Trifolium pratense*) and canola (*Brassica napus*), harvested from field sites (sampled April 1997) or from glasshouse pots at physiological maturity, are given in Martens (2000a). The plant samples analyzed included leaves and stems ground to pass through a 1-mm sieve.

Analyses

A detailed description of the PAs identified, and the soil PA extraction, purification, separation and detection procedure was reported by Martens (2002). Briefly, the soil and humic acid PA composition was determined by extraction with 1 *M* (ambient temperature) or 4 *M* NaOH [autoclave (15 min at 121°C; 104 kPa)] followed by purification using Varian PPL solid-phase extraction tubes (Varian Assoc., Harbor City, CA) with separation and detection with a Hewlett-Packard 6890 (Hewlett-Packard, Palo Alto, CA) gas chromatograph equipped

with a flame ionization (FID) or a mass spectral detector. The structures and identification key of the PAs reported in this study are given in Fig. 1 and Table 1, respectively.

Residue Mineralization Experiment

To determine the effect of the plant PA composition on the soil phenolics recovered following residue decomposition, 30 g (dry weight) moist samples of the soil were treated with 2% (0.6 g dry weight) residue and incubated at −34 kPa moisture tension at 22°C. The properties of the Webster silty-clay loam soil (Typic Haplaquoll) soil used were: pH, 7.28; organic C content, 28.8 g kg⁻¹; inorganic C 1.44 g kg⁻¹; total N, 1.86 g kg⁻¹; sand, 409 g kg⁻¹; and clay, 195 g kg⁻¹ soil and had been planted to soybeans the previous year. Samples were weighed at weekly intervals and the soil moisture corrected for the drying effects induced during incubation. After 9, 29, and 84 d, duplicate samples were removed from a closed chamber that passed humidified CO_2–free compressed air over each sample. The soil was air-dried, passed through a 1-mm sieve and analyzed for ester-linked phenolic acid content as described by Martens (2002). At each sampling date, the PA values for the control samples (no amendment added) were subtracted from the PA values obtained from the amended soil resulting in a value for plant or microbial PAs remaining in the soil. The plant residues were chosen for this study based on their total PA content and composition.

Soil aggregate stability was determined with a nested sieve arrangement (4, 2, 1, 0.5, and 0.25 mm), wet sieved in degassed, distilled water for 5 min as described by Kemper and Rosenau (1986). Approximately 15 g of the 30 g soil sample were wet sieved in the moist condition (180–250 g water kg⁻¹ soil) and corrected for sand content by dispersion in sodium hexametaphosphate. The mean weight diameter (MWD) was calculated with the following equation (Haynes and Beare, 1997).

$$MWD = (\Sigma X_i S_i)/W$$

Where X_i equals sand corrected weight of soil remaining on sieve size, S_i, and W is the weight of soil (minus sand) used for the analysis. The potential upper and lower limits of the MWD in this study were 4 and 0.25 mm, respectively.

Isolated microbial polymers were extracted with 1 *M* NaOH and PA purification and compositional analysis was completed as described by Martens (2002). The extracellular polymers were extracted from the following soil organisms; *Arthrobacter viscosus* (ATCC 19584, Cadmus et al. 1963), *Azotobacter indicus* (ATCC 9037, Martin et al., 1965), *Bacillus subtilis* (ATCC

Table 1. Names and structures of phenolic compounds investigated.

ID number	Compound names		Structure†			
	Common	**Chemical**	**R_1**	**R_2**	**R_3**	**R_4**
1	**Phenol**	**Phenyl hydroxide**	**H**	**H**	**OH**	**H**
2	**Acetophenone**	**1-Phenylethanone**	**COCH₃**	**H**	**H**	**H**
3	**Cinnamadehyde**	**3-Phenyl-2-propenal**	**(CH)₂CO₂H**	**H**	**H**	**H**
4	***p*-Hydroxybenzaldehyde**	**4-Hydroxy-benzaldehyde**	**COH**	**H**	**OH**	**H**
5	**Vanillin**	**4-Hydroxyl-3-methoxybenzaldehyde**	**COH**	**OCH₃**	**OH**	**H**
6	***p*-Hydroxyacetophenone**	**1-(4-Hydroxyphenyl)ethanone**	**COCH₃**	**H**	**OH**	**H**
7	**Acetovanillone**	**1-(4-Hydroxy-3-methoxyphenyl)ethanone**	**COCH₃**	**OCH₃**	**OH**	**H**
8	***p*-Hydroxybenzoic acid**	**4-Hydroxybenzoic acid**	**COOH**	**H**	**OH**	**H**
9	**Vanillic acid**	**4-Hydroxy-3-methoxybenzoic acid**	**COOH**	**OCH₃**	**OH**	**H**
10	**Syringaldehyde**	**3,5-Dimethoxy-4-hydroxybenzaldehyde**	**COH**	**OCH₃**	**OH**	**OCH₃**
11	***c*-Coumaric acid**	**3-(4-Hydroxyphenyl)-2-propenoic acid**	**(CH)₂CO₂H**	**H**	**OH**	**H**
12	**Acetosyringone**	**1-(3,5-Dimethoxy-4-hydroxyphenyl) ethanone**	**COCH₃**	**OCH₃**	**OH**	**OCH₃**
13	***t*-Coumaric acid**	**3-(4-Hydroxyphenyl)-2-propenoic acid**	**(CH)₂CO₂H**	**H**	**OH**	**H**
14	**Syringic acid**	**3,5-Dimethoxy-4-hydroxybenzoic acid**	**COOH**	**OCH₃**	**OH**	**OCH₃**
15	**Ferulic acid**	**3-(4-Hydroxy-3-methoxyphenyl)-2-propenoic acid**	**(CH)₂CO₂H**	**OCH₃**	**OH**	**H**
16	**Sinapyl aldehyde**	**3-(3,5-Dimethoxy-4-hydroxyphenyl)-2-propenal**	**(CH)₂COH**	**OCH₃**	**OH**	**OCH₃**
17	**Sinapinic acid**	**3-(3,5-Dimethoxy-4-hydroxyphenyl)-2-propenoic acid**	**(CH)₂CO₂H**	**OCH₃**	**OH**	**OCH₃**

† The structure of each compound is shown in Fig. 1 (model phenolic acid [PA]) with the appropriate groups for R_1, R_2, R_3 and R_4.

15192, Martin, 1946), *Chromobacterium violaceum* (ATCC 9544, Martin and Richards, 1963), *Cryptococcus laurentii* (ATCC 10668, Cadmus et al., 1962), and *Hansenula holstii* (ATCC 2448, Anderson et al., 1960) cultured under optimum growth conditions outlined.

Humic acids were extracted from selected residue treatments after incubation for the specified times using a method described by Schnitzer (1982). The method utilizes a 0.2 *M* NaOH overnight extraction (excluding O_2) and acid precipitation of the humic acids (pH < 2.0). The humic acids were purified by repeated dissolution in 0.2 *M* NaOH followed by acid precipitation, and centrifugation. The humic acids were washed free of Cl^- and freeze dried. The humic acid-PAs were then extracted with 4 *M* NaOH and quantified as described by Martens (2002).

RESULTS AND DISCUSSION

Research on the role of the polymeric PAs in soil stabilization and organic C cycling involves the need to quantify the composition of the PAs in plant residue and PAs in the soil. The method described by Martens (2002) quantifies the monomeric ester-linked PA composition extracted from plants and soils and was used to investigate the impact of plant PAs on plant C cycling and development of soil aggregates. Examples of the methodology are shown in the chromatograms representing ester-linked PAs extracted from the Webster soil amended with alfalfa and the control (no amendment added) Webster soil after 9-d incubations (Fig. 2). Analysis of ester-linked plant C_6–C_3 PAs in soil that are essentially unique to vascular plants (Sarakanen and Ludwig, 1971) has potential for determining the amount of identifiable plant C accumulating in soil (Martens, 2002).

The quantity and composition of ester- and ether-linked PAs extracted from the plant residues added to amend the Webster soil in the study described here were previously presented by Martens (2002). After soil amendment, concentrations of all ester-linked plant PAs decreased when incubated for 9 d (Table 2). The plant-derived coumaric and ferulic acids continued to decrease at pseudo first-order rates when the residue-amended soil was incubated for 84 d with the exception of the canola residue, which initially contained very low levels of coumaric acid. The PA, *cis*-coumaric acid
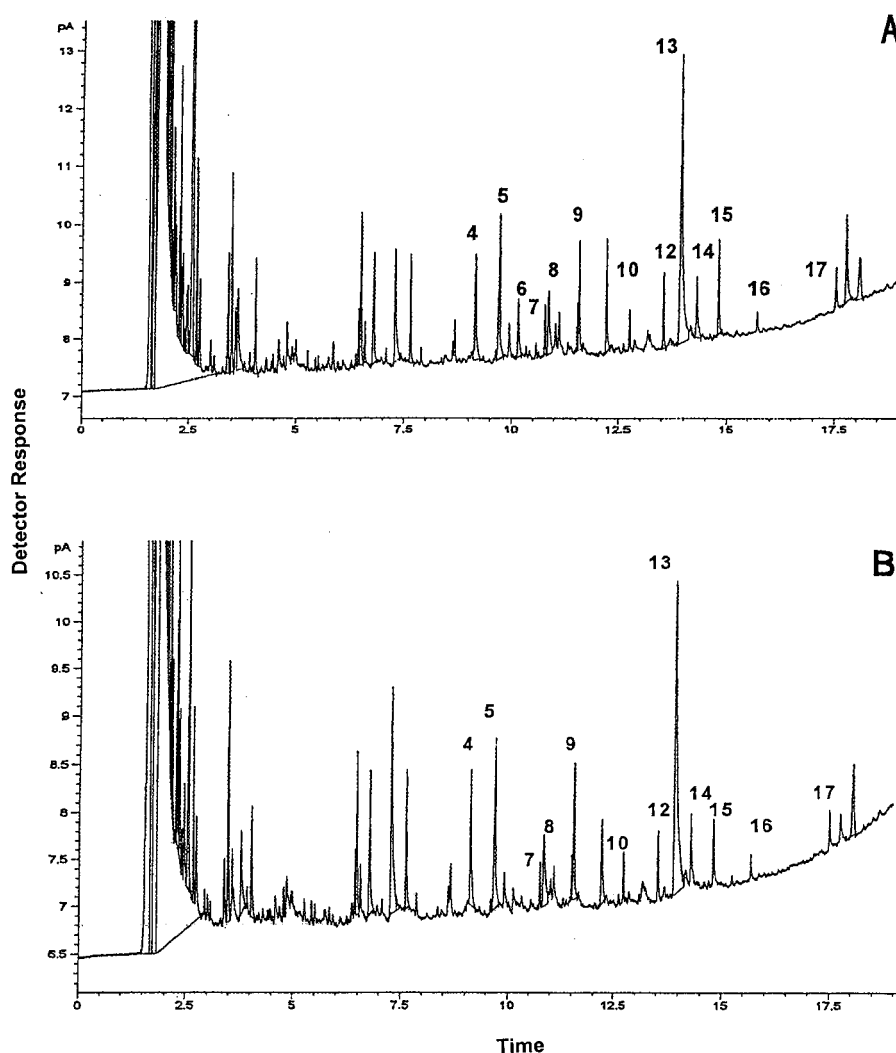

Fig. 2. Chromatographic trace of phenolic acids (PAs) isolated with alkaline extraction of the (a) Webster soil (unamended control) and (b) Webster soil incubated for 9 d with alfalfa residue. The identities of the PA compounds are given in Table 1.

SOIL SCI. SOC. AM. J., VOL. 66, NOVEMBER–DECEMBER 2002

Table 2. Composition of ester-linked phenolic acids in Webster soil incubated with plant residues for up to 84 d.†

Plant species	Day	4	5	6	7	8	9	10	12	13	14	15	Total
								g kg^{-1}					
Corn	0	9.32	10.5	ND	0.23	3.69	1.48	9.98	14.1	587	2.84	89.5	729 ± 35
	9	5.49	4.81	ND	0.60	ND	1.08	4.58	5.72	360	5.74	35.2	433 ± 40
	29	5.08	4.00	ND	ND	6.60	0.85	4.23	5.99	289	5.69	25.7	345 ± 45
	84	2.87	2.64	1.47	ND	6.63	1.00	3.73	5.26	178	5.14	12.9	220 ± 46
Oat	0	3.93	11.0	10.1	1.29	2.79	2.71	6.08	11.4	146	4.61	94.3	294 ± 45
	9	2.37	3.02	0.22	0.46	ND	0.97	2.73	2.66	57.6	2.85	22.4	131 ± 32
	29	2.18	2.30	1.63	0.78	ND	1.23	2.49	3.61	38.7	2.46	13.2	68.6 ± 26
	84	1.22	1.20	ND	0.58	ND	0.95	1.03	2.36	13.0	1.77	5.63	27.7 ± 5.2
Prairie	0	4.16	9.28	ND	1.59	2.11	3.25	4.19	8.41	102	5.14	54.3	195 ± 35
	9	3.13	6.47	ND	ND	ND	2.23	2.84	2.65	88.6	3.69	37.7	147 ± 40
	29	2.70	4.09	ND	ND	6.56	2.08	2.62	3.34	70.9	2.39	10.9	106 ± 18
	84	1.96	0.71	0.93	1.24	4.93	0.97	2.33	4.26	19.4	2.70	11.6	51.1 ± 5.6
Alfalfa	0	2.63	4.57	9.00	7.19	3.70	2.68	1.77	15.4	25.1	2.46	46.8	121 ± 10
	9	0.56	0.98	0.4	ND	1.54	0.82	ND	1.14	5.32	1.66	1.72	14.2 ± 3.2
	29	0.55	0.89	1.94	ND	1.10	0.73	ND	1.69	4.41	1.16	1.54	14.0 ± 1.2
	84	0.12	0.28	1.37	0.91	ND	0.50	ND	0.99	6.26	0.74	0.55	11.7 ± 3.6
Soybean	0	1.51	5.40	ND	ND	ND	1.41	3.75	2.29	46.9	1.89	4.77	68.0 ± 10
	9	1.19	2.89	ND	0.33	ND	0.80	2.20	0.84	18.8	1.67	1.48	30.2 ± 3.2
	29	0.98	2.00	0.70	ND	2.82	0.86	1.62	1.29	18.8	1.47	1.62	32.2 ± 3.3
	84	1.06	2.58	1.67	ND	6.10	1.40	2.57	2.22	10.6	1.67	1.30	31.1 ± 3.8
Clover	0	ND	1.23	2.40	1.44	2.08	1.28	ND	ND	6.43	ND	4.96	19.8 ± 1.2
	9	0.96	0.55	0.25	1.42	0.71	0.68	ND	ND	3.44	1.58	0.28	9.8 ± 2.2
	29	0.96	0.49	1.40	1.11	3.37	0.46	ND	ND	2.89	0.08	0.44	11.2 ± 0.9
	84	ND	0.31	1.40	0.24	2.07	ND	ND	ND	ND	0.20	0.20	4.4 ± 0.8
Canola	0	0.42	1.50	ND	ND	ND	0.80	2.57	ND	1.21	ND	4.96	11.4 ± 0.9
	9	0.42	0.65	ND	1.19	0.28	0.58	0.92	0.43	5.76	1.16	0.93	12.2 ± 0.6
	29	0.65	0.98	ND	0.77	2.63	0.40	ND	0.37	4.82	ND	0.65	11.3 ± 0.7
	84	ND	ND	ND	0.64	ND	ND	ND	ND	3.05	0.24	0.50	4.4 ± 0.6
Control	0	2.37	1.09	ND	ND	ND	2.35	ND	ND	16.8	ND	1.85	24.4 ± 2.4
	9	2.17	0.85	ND	ND	ND	2.01	ND	ND	15.6	ND	1.65	22.2 ± 1.9
	29	1.98	0.80	ND	ND	ND	2.05	ND	ND	16.1	ND	1.33	22.2 ± 0.8
	84	2.03	0.85	ND	ND	ND	2.32	ND	ND	15.3	ND	1.45	21.9 ± 1.1

† The plant-derived ester-linked phenolic acids (PAs) were determined by subtracting the PA content of the unamended soil from the PA content of the amended soil. Compounds 11, 16 and 17 were not detected in the amended soils.
‡ Identity of PA compounds given in Table 1. ND, not detected.

(Compound 11), was found in several of the plant residues (Martens, 2002), but was not identified in the amended soil. Recovery of plant PAs from soil amended with corn residue for up to 84 d are shown in Fig. 3. Calculated half-lives ($t_{1/2}$) for the most abundant PA in the plant residue, coumaric acid, ranged from 27 d in the oat residue, 30 d (alfalfa), 34 d (prairie and clover), 50 d (soybean), and 56 d (corn) suggesting that not only the total quantity of PA in the residue is important, but the composition of the PA determines resistance to residue decomposition. Similar half-lives were calculated for ferulic acid decomposition. Coumaric and ferulic acid bridge both structural carbohydrates and proteins in monocot plants to the lignin structure (monolignols) of the plant (Hartley and Jones, 1977), but are not involved in the polymeric lignin structures (Chen, 1992; Carpita and McCann, 2000). The percentage of coumaric acid lost from the soil amended with the seven residues was correlated ($r = 0.70*$) with total CO_2 respired (Martens 2000a). Martens (2000a) reported that total CO_2 respired for this experiment was correlated with carbohydrate ($r = 0.93**$) and amino acid content ($r = 0.64*$) of the amended soil at time 0. In general, the remaining plant PAs did not show changes in concentration after 9 d (Table 2), except for 4-hydroxybenzoic acid, which increased in concentration in five of the seven residue amended soils due possibly to microbial synthesis as previously reported by Moorman et al. (1992). The slow decomposition rates for the majority of the PAs identi-

fied here were similar to the results reported by Stott and Martin (1990). They reported small losses of $^{14}CO_2$ from wheat lignin ($<20\%$) added to soil after 1-yr incubation and that $>99\%$ of the remaining ^{14}C was found in the humus fraction.

Tisdall and Oades (1982) classified organic binding agents involved in soil aggregate stability as short-term (mainly polysaccharides or simple carbohydrates), temporary (roots and fungal hyphae) and persistent (resistant aromatic compounds). After 84 d, the aggregate MWD (mm) for the amended and unamended soils were 1.41 + 0.07 (corn), 1.39 + 0.01 (oat), 1.45 + 0.25 (unidentified prairie grass), 1.18 + 0.08 (alfalfa), 1.22 + 0.00 (soybean), 1.00 + 0.09 (canola), 0.90 + 0.05 (clover), and 0.42 + 0.10 (unamended control soil). Organic C content and MWD for Day 9 and 29 incubations are reported in Martens (2000a). Total plant PAs added and certain monomeric PAs were correlated with aggregate MWD and also with organic C content during the 84-d incubation (Table 3). Research has shown that soil stabilization increased in the amended soil, and the processes were related to the biochemical composition of the different plant residue additions (Martens 2000a). Martens (2000a) reported that residues with a higher carbohydrate and amino acid content decomposed quickly and resulted in a short-term aggregate stability (<29 d). The majority of the soil PA concentrations at 9 d were negatively correlated (Table 3) with aggregate stability (9 of the 12 PAs detected) and organic C con-
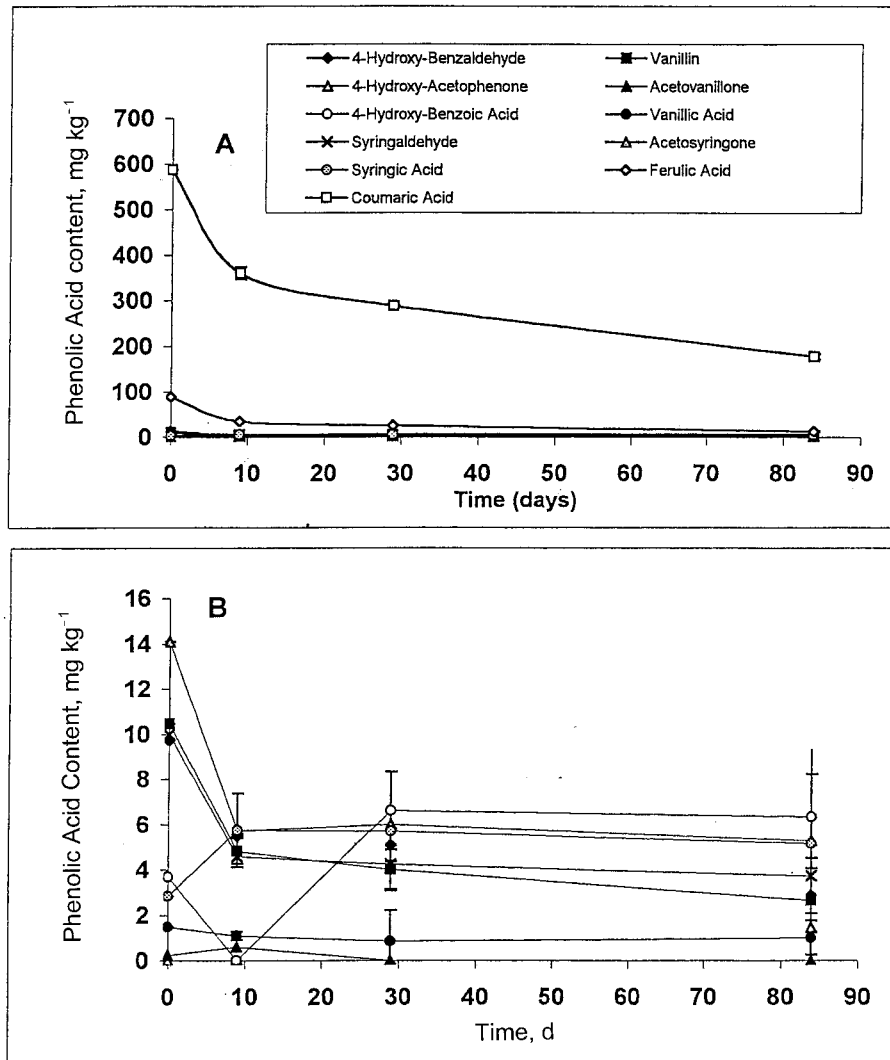
Fig. 3. Recovery of (a) the isolated phenolic acids (PAs) and (b) the isolated PAs except for coumaric and ferulic acid after incubation of corn residue for 9, 29, 84 d. The identities of the PA compounds are given in Table 1. Points without standard error bars have standard errors less than the marker size (n = 2).

tent (10 of the 12 PAs detected) indicating that plant PAs were not related with short-term soil stabilization (Table 3). Nearly all of the PAs were positively correlated with MWD (10 out of 12 PAs detected) and organic C content (11 out of 12 PAs detected) after 84 d incubation. In support of the proposed classification of organic binding agents, Martens and Frankenberger (1992) reported that a single pulse of glucose (1 mg glucose-C g^{-1} soil) resulted in short-term aggregation (<21 d), but after 7 d, the carbohydrate composition of the glucose-treated soil was identical to the control soil (no glucose added) and they concluded that simple carbohydrates stimulate microbial activity, but not the synthesis of persistent extracellular binding agents. The results reported here support the hypothesis discussed by Tisdall and Oades (1982) that residues with slower decomposition rates resulted in persistent soil aggregation that was related to increased soil organic C content in the soil aggregates.

Oades and Waters (1991) found that soil aggregates destroyed by ultrasonic treatment released plant debris that under scanning electron microscopy showed vascular and structural components of plants, which are enriched in PAs (Capita and McCann, 2000). Figures 4 and 5 show the relationships of the total soil PA content and selected soil PAs with aggregate stability and organic C content, respectively. The results show that the y intercepts of the regression and quadratic lines approximate the aggregate MWD (Fig. 4) and the organic C content (Fig. 5) of the soil before addition of the organic amendment suggesting that the PA quantity and composition are important for increasing the parameters. The aggregate stability relationship measured at Day 84 approximates a linear response and plateau suggesting that critical levels of PAs are necessary for maximum aggregate stability in this Webster soil. Martens (2000a) reported that the critical amount of total PAs added as plant residue at time 0 for aggregation during this study was 200 to 220 mg kg^{-1} soil. From the relationship in Figure 4b, it appears that a critical concentration of soil PAs for aggregation in the Webster soil tested was 90 to 95 mg kg^{-1} soil after 84 d or an apparent efficiency

Table 3. Correlation coefficients (*r* values) between plant phenolic acids (PAs) (mg kg^{-1}) added at Day 0, extracted soil PAs (mg kg^{-1}) and mean weight diameter (MWD) (mm) and soil organic C content (g kg^{-1}) following decomposition of added organic residues to the Webster soil at the designated time (*n* = 7).

Day	Compound† 4	5	6	7	8	9	10	12	13	14	15	Total
						r values						
Plant PAs Day 0 vs. MWD at Day:												
9	−0.38	−0.52	0.41	0.55	−0.04	−0.18	−0.40	0.06	−0.40	−0.43	−0.25	−0.37
29	0.57	0.30	−0.50	−0.40	−0.05	−0.37	0.64*	0.13	0.67*	−0.02	0.11	0.56
84	0.78*	0.96**	0.10	−0.04	0.42	0.66*	0.79*	0.72*	0.60	0.92**	0.82*	0.70*
Extracted Soil PAs vs. MWD at Day:												
9	−0.62	−0.72*	0.68*	−0.06	0.55	−0.60	−0.54	−0.44	−0.43	−0.59	−0.63*	−0.47
29	0.55	0.47	−0.45	−0.25	−0.47	−0.11	0.59	0.48	0.66*	0.62	0.48	0.65*
84	0.83*	0.77*	−0.06	−0.13	0.50	0.73*	0.74*	0.82*	0.53	0.74*	0.83*	0.63*
Plant PAs Day 0 vs. Organic C Content at Day:												
9	0.56	0.71*	−0.20	−0.07	0.30	0.59	0.47	0.41	0.42	0.75*	0.44	0.46
29	0.65*	0.13	−0.41	−0.05	0.29	−0.35	0.55	0.35	0.74*	−0.15	0.19	0.65*
84	0.57	0.08	−0.61	−0.23	0.05	−0.43	0.53	0.17	0.67*	−0.19	0.06	0.56
Extracted Soil PAs vs. Organic C Content at Day:												
9	0.65*	0.89**	−0.73*	−0.54	−0.41	0.80	0.52	0.57	0.44	0.65*	0.73*	0.50
29	0.90**	0.74*	−0.31	−0.26	0.57	0.25	0.89**	0.85**	0.66*	0.96***	0.86**	0.92**
84	0.97***	0.72*	0.36	−0.28	0.79*	0.69*	0.79*	0.93**	0.85**	0.97***	0.86**	0.89**

† Identity of PA compounds given in Table 1.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
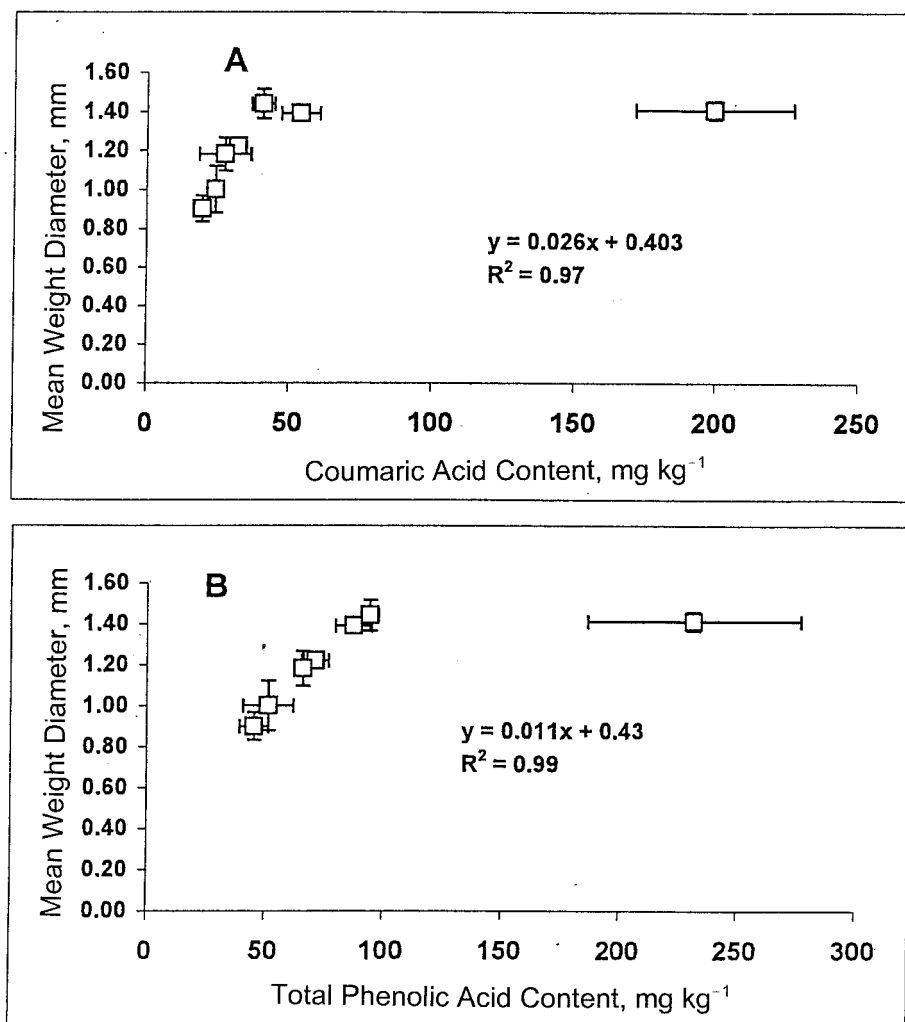*** Significant at the 0.001 probability level.

Fig. 4. Linear response and plateau regression relationship between aggregate mean weight diameter (MWD) (mm) measured at d 84 and (a) soil coumaric acid content and (b) total soil ester-linked phenolic acids (PAs) content at Day 84.
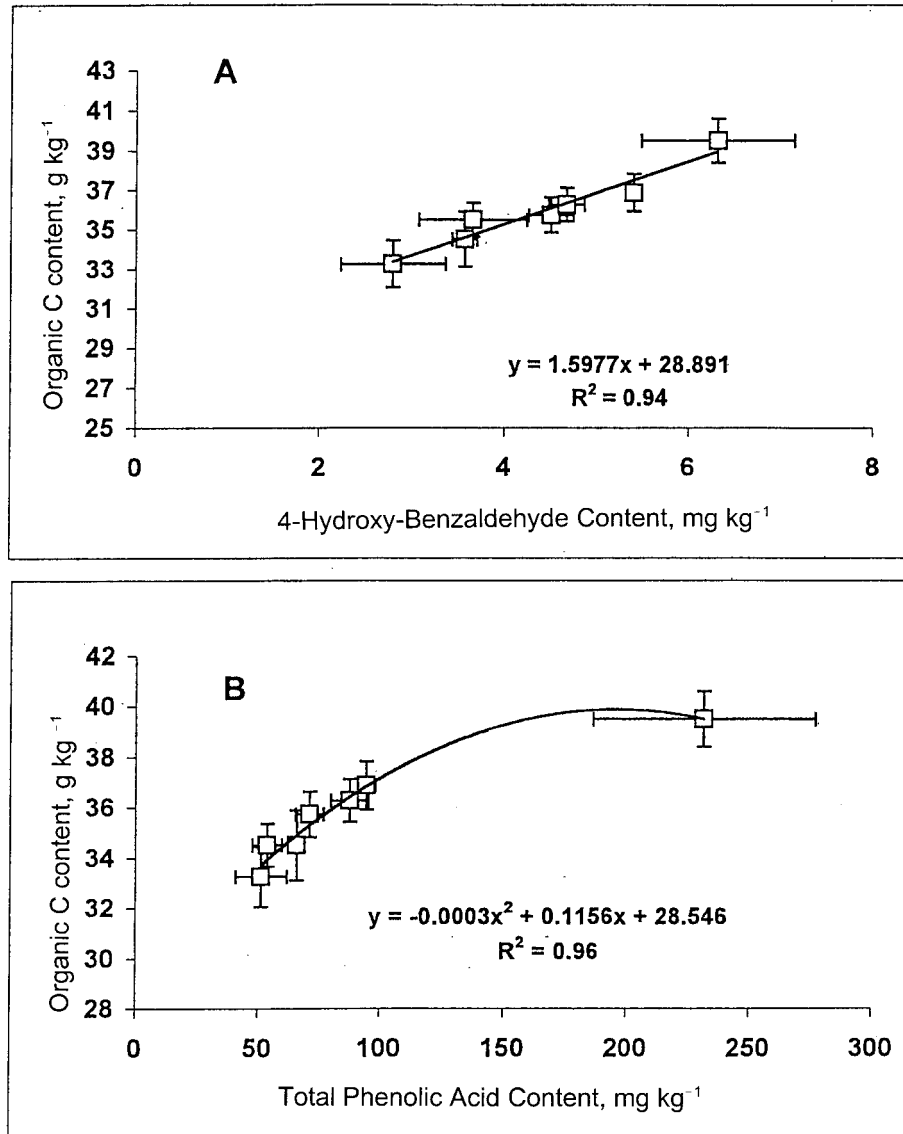
Fig. 5. Relationship between organic C content at d 84 and (a) soil 4-hydroxy-benzaldehyde content and (b) total soil ester-linked phenolic acid (PA) content at d 84.

rate for aggregate stability of approximately 50% for residue PAs added. In contrast, when a quadratic relationship was fitted to the data for total soil PAs and organic C content remaining in the Webster soil incubated for 84 d, the y intercept (28.5 g organic C kg^{-1} soil) was identical to the organic C content for the unamended soil (control) at the end of the experiment (Fig. 5b). This relationship between total PA content and organic C content provides additional evidence that PA content of the residue is important for C cycling and deserves additional research as an index of plant biomass C in soil.

Organic materials organize and stabilize soil aggregates (Tisdall and Oades, 1982; Oades and Waters, 1991) and changes in water-stable aggregates are dependent on the type (biochemical composition) of decomposing organic residue (Tisdall and Oades, 1982). The aggregate MWD calculated from Eq. [1] is based on the sum-

mation of the weight of the different aggregate-size fractions compared with the total soil weight used. The MWD value gives an overall result for soil aggregation, but provides little detail of the impact of the residue in question on the different aggregate-size distribution. The interaction of the plant residues and the percentage distribution of the aggregate fractions at 9 and 84 d are reported in Table 4. All seven amendments reduced the percentage of soil particles in the <0.25-, 0.25-, and 0.5-mm aggregate-size fractions compared with the control (no amendment added) incubated for the same time period suggesting that differences in the biochemical composition of the residues was not evident for the smallest aggregate sizes measured. At 9 d, the amendments increased the sum of the 4-, 2-, and 1-mm size fraction percentages compared with the control values in the following order of increasing effectiveness, prairie > corn > oat > clover > soybean > alfalfa = canola

Table 4. Aggregate–size distribution (%) and percentage change from the incubated Webster control soil (no amendment added) incubated for 9 and 84 d after addition of different plant residues.

Amendment	Day	Aggregate–Size Distribution, mm†					
		4.00	2.00	1.00	0.50	0.25	<0.25
		%					
Corn	9	3.8 ± 1.5 (3.8)	8.3 ± 2.2 (6.0)	33 ± 0.5 (15)	16 ± 1.9 (−3.8)	4.1 ± 0.5 (−10)	34 ± 2.2 (−11)
	84	15 ± 3.6 (13)	19 ± 0.5 (15)	31 ± 8.9 (−1.9)	11 ± 0.3 (−10)	2.9 ± 1.5 (−3.8)	20 ± 3.1 (−11)
Oat	9	4.5 ± 0.5 (4.5)	12 ± 4.6 (10)	30 ± 4.0 (12)	15 ± 3.9 (−4.9)	3.9 ± 0.8 (−10)	34 ± 6.0 (−11)
	84	7.3 ± 4.0 (4.4)	8.1 ± 2.1 (4.2)	31 ± 8.9 (−2.0)	18 ± 4.1 (−3.7)	6.4 ± 2.0 (−0.3)	29 ± 13 (−2.7)
Prairie	9	2.3 ± 1.0 (2.3)	6.5 ± 1.9 (4.2)	34 ± 2.1 (17)	17 ± 1.6 (−3.3)	4.5 ± 1.1 (−9.7)	36 ± 2.3 (−10)
	84	20 ± 7.4 (17)	11 ± 1.9 (7.0)	34 ± 8.8 (0.8)	16 ± 3.9 (−5.8)	2.9 ± 0.5 (−3.8)	16 ± 3.9 (−15)
Alfalfa	9	13 ± 2.6 (13)	14 ± 6.6 (11)	28 ± 1.1 (10)	12 ± 2.2 (−8.2)	4.0 ± 0.1 (−10)	30 ± 0.7 (−16)
	84	8.8 ± 1.1 (6.0)	12 ± 1.4 (8.0)	32 ± 3.0 (−1.0)	16 ± 1.7 (−5.3)	3.9 ± 0.4 (−2.8)	27 ± 7.6 (−4.8)
Soybean	9	3.5 ± 5.3 (3.4)	10 ± 1.8 (7.9)	35 ± 9.1 (17)	15 ± 4.6 (−5.5)	3.9 ± 1.6 (−10)	33 ± 7.7 (−13)
	84	15 ± 4.5 (12)	11 ± 0.1 (7.0)	31 ± 3.8 (−1.9)	13 ± 4.0 (−8.4)	2.3 ± 0.4 (−4.4)	27 ± 2.8 (−4.2)
Clover	9	2.9 ± 0.4 (2.9)	13 ± 1.2 (11)	31 ± 1.6 (14)	14 ± 0.1 (−6.6)	4.4 ± 0.5 (−10)	30 ± 0.7 (−11)
	84	16 ± 1.0 (13)	12 ± 1.4 (8.0)	31 ± 2.1 (−2.4)	13 ± 2.0 (−9.0)	3.9 ± 0.4 (−2.3)	19 ± 4.1 (−12)
Canola	9	13 ± 2.8 (13)	13 ± 4.3 (10)	29 ± 0.2 (11)	12 ± 4.4 (−8.0)	3.8 ± 1.9 (−10)	30 ± 1.1 (−16)
	84	6.4 ± 7.9 (3.6)	13 ± 6.5 (8.6)	38 ± 3.0 (4.5)	18 ± 7.2 (−3.1)	4.7 ± 1.0 (−2.0)	20 ± 3.1 (−12)

† Value in parentheses indicates the percentage increase or decrease in the aggregate size fraction compared with the control (no amendment added) size fraction incubated for the same time period.

(Table 4). Martens (2000a) reported that the alfalfa, canola, soybean, and oat amendments had the highest carbohydrate content of the residues tested and they mineralized faster than the corn and prairie residues. After 84 d, the amendments increased the sum of the 4-, 2-, and 1-mm aggregate-size fraction percentages in order of increasing effectiveness, oat > alfalfa > canola > soybean > clover > prairie > corn (Table 4). The results from the 9- and 84-d incubations clearly separate the residues that mineralize quickly and result in short-term aggregation (canola, oat, alfalfa, and soybean) from the residues that are slower to mineralize such as corn and prairie residues that were enriched in PA compared with the other residues tested (Martens 2000a, 2002). Short-term soil aggregation in this study appeared to be stimulated by plant constituents that mineralized rapidly (carbohydrates and amino acids), while longer-term aggregation was noted from residues with slower decomposition rates and higher concentrations of PAs. Griffiths and Burns (1972) reported a similar pattern of stabilization because of production of short-term stable aggregates (<6 wk) upon incubation of soil with an extracellular microbial polysaccharide (*Lipomyces starkeyi*), but persistent aggregation was noted following addition of tannic acid (a rich PA source) after initiation of polysaccharide-induced soil aggregation. Importance of PAs in stable soil aggregates has also been shown by Monreal et al. (1995), who reported that percentages of water-stable soil aggregates were highly correlated with the soil concentration of PA molecules ($r = 0.98$)

determined by pyrolysis field ionization mass spectrometry.

The relationships between the quantity and composition of plant PAs added to the soil suggest that plant PAs are important to soil aggregation and organic C cycling. The data show that since approximately the same amount of organic C was added in this experiment for each of the seven residues, the improvement in soil aggregation from plant residue treatments was possibly because of the concentration of plant PAs introduced. While the importance of the plant-derived PAs is evident, a persistent argument in soil science has centered on the relative importance of products of microbial metabolism such as microbial PA synthesis from nonlignin sources (hemicellulose and cellulose fractions) vs. plant PAs to the development of stable SOM or humus (Martin and Haider, 1976; Stevenson 1994).

To investigate the role of microbial PAs in MWD development, four purified bacterial and two deuteromycete extracellular polymers were analyzed for PAs and were found to have a range in total quantity and composition of the PAs (Table 5). The microbial polymers tested have been reported to be effective for promotion of soil aggregation that was not significantly correlated with polymer carbohydrate content (Martens and Frankenberger, 1992). Use of 4 M NaOH to determine total ester- and ether-linked PA content showed that >95% of the microbial PAs were recovered in the 1 M NaOH analysis (data not reported here). These microbial polymers may play a significant role in soils

Table 5. Composition of phenolic acids (PAs) extracted from isolated extracellular polymers of soil microorganisms.†

Microorganisms	Compound‡								Total
	3	4	5	6	7	8	9	10	
	mg kg^{-1}								
Arthrobacter viscosus	438	402	ND	ND	263	1540	ND	ND	3048 ± 97
Azotobacter indicus	100	111.4	144	196	94.2	ND	86.5	ND	838.2 ± 75
Bacillus subtilis	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chromobacterium violaceum	ND	355	291	ND	123	225	554	ND	1548 ± 82
Cryptococcus laurentii	ND	ND	ND	ND	ND	46.7	121	40.4	290.2 ± 18
Hansenula holstii	ND	27.0	ND	ND	ND	46.3	266	ND	423.6 ± 23

† Total PA content also includes small amounts of catechol (1,2-benzenediol), except for the *Bacillus subtilis* and *Chromobacterium violaceum* polymers.
‡ Compounds 11 through 17 were not detected in the microbial polymers tested. Identity of PA compounds given in Table 1. ND, not detected.
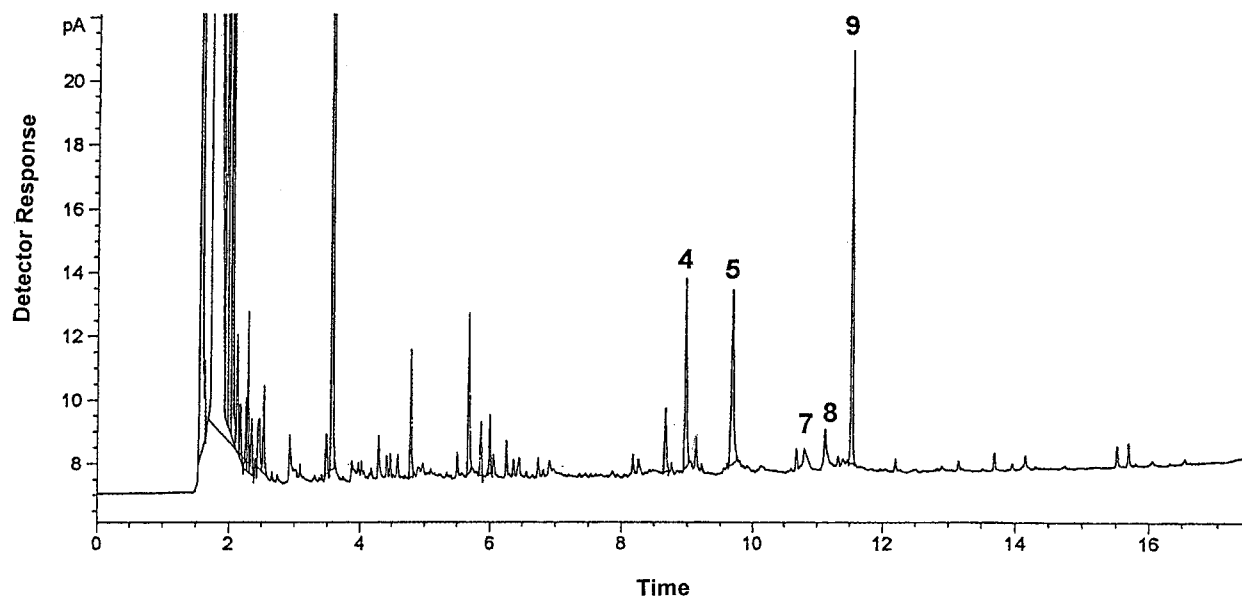
Fig. 6. Chromatographic trace of phenolic acids (PAs) following alkaline extraction of the extracellular polymer produced by *Chromobacterium violaceum*. The identities of the PA compounds are given in Table 1.

since the microbial species studied can account for a majority of the total colonies isolated from certain soils (Alexander, 1977). Quite noticeable is the total lack of PAs in the *Bacillus* polymer and also a lack of the phenylpropene (C_6–C_3) compounds in the other polymers tested that are so prominent in the plant PAs (Martens, 2002). Phenolic compounds isolated from the *Chromobacterium* violaceum polymer were identified as phenylmethyl-type PAs (C_6–C_1) (Fig. 6). This supports the previous finding of Sarakanen and Ludwig (1971) that PAs of the phenylpropene type are unique to vascular plants and not synthesized by soil microbes tested here. The results also suggest that the phenyl-methyl PAs in soil may be from a plant source or from microbial synthesis, but the phenylpropenes are confirmation of plant PAs remaining in soil. In Table 3, PA compounds extracted at Day 84 that were highly correlated with organic C content were nearly all identified as being of plant origin (Compounds 10–15). The results appear to support the conclusion that plant derived PAs are important in soil aggregate stability and in contribution to the regulation of C cycling.

The results show that the bulk of the plant PAs (cinnamic acids) were metabolized in soil (Table 2), but the soil PAs (plant and microbial sources) remaining were closely related to the organic C content following a period of decomposition (Table 3). Martens (2000a) reported that the weight of alkali-extractable humic acids from the unamended Webster soil did not change during the 84-d incubation, but the amount of humic acids extracted from the canola-amended soil incubated for 84-d decreased 29% while the humic acids extracted from the corn-amended soil increased 22% during the same time period. This evidence suggests that soil humic acids are subject to change reflecting the PA content or lack of PA content of the plant residue introduced to the soil.

To investigate the role of management and plant residues on the formation of soil humic substances in an agricultural setting, the Webster soil studied here was sampled in the field following soybean, corn, or native prairie growth and the humic acids were extracted and analyzed for PA content. A description of the soils and properties was given in Martens (2000b). The PA composition of the humic acids from soils sampled in the spring and fall is shown in Table 6. The amount of humic acid isolated from the sites decreased in the order prairie > corn > soybean as shown by Martens (2000b) and

Table 6. Composition of the phenolic acids (PAs) extracted from humic acids isolated from Webster soil under different management at a spring and a fall sampling.†

Soil	Compound‡												Total
	4	5	6	7	8	9	10	12	13	14	15	17	
	mg kg^{-1} soil												
Prairie spring	4.87	5.74	1.23	5.04	4.41	5.61	8.21	12.41	6.51	6.89	7.48	10.2	81.6 + 4.6A
Prairie fall	4.28	6.09	1.65	5.63	5.94	6.61	8.64	14.87	6.09	7.59	8.49	10.1	86.0 + 6.2A
Corn spring	1.66	2.14	0.66	1.72	1.29	2.08	5.33	9.06	4.55	3.88	3.24	4.97	40.4 + 2.1B
Corn fall	1.74	2.13	0.94	1.75	1.36	2.11	3.63	6.50	3.74	3.24	2.77	4.35	34.3 + 1.9B
Soybean spring	0.80	0.94	ND	0.80	0.88	0.94	2.44	3.62	2.42	1.58	1.26	3.52	19.2 + 2.1C
Soybean fall	ND	0.35	ND	ND	ND	0.38	1.19	1.57	1.10	0.64	0.53	2.49	8.2 + 0.9D

† The values represent milligrams of extracted humic acid-PAs per kilogram of soil.
‡ Identity of PA compounds given in Table 1. The letter following total PA value indicates statistical differences based on LSD of 10.2. Values with the same letter indicate no statistical difference.
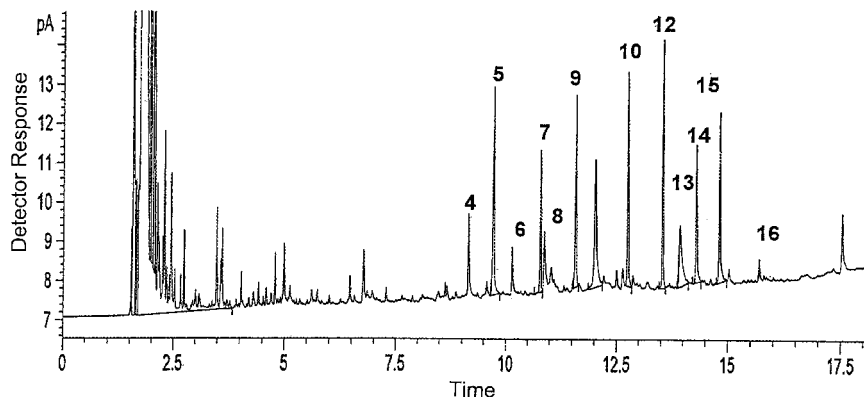
Fig. 7. Chromatographic trace of phenolic acids (PAs) following alkaline extraction of the humic acid isolated from the Webster soil under native prairie vegetation. The identities of the PA compounds are given in Table 1.

the concentration of PAs extracted also decreased in that order (Table 6). A chromatogram of the PAs extracted from the prairie soil humic acids is given in Fig. 7. Identification of the PAs of both plant and microbial origin (Compounds 4–10) and the PAs of predominantly plant origin (Compounds 12–17) suggest that the majority of the PAs isolated were from plant sources. If we assume that half of the concentration of Compounds 4 to 10 was from microbial synthesis, then >70% of the PAs isolated in the humic acids were of plant origin. Burges et al. (1964) also discussed on the importance of the plant PAs to the formation of soil humic acids. Additional evidence that the type of plant residue contributes greatly to the formation or decomposition of soil humic acids is presented in the total extractable humic PA content (Table 6), which decreased slightly in the year following corn production (15%), but decreased 57% the year following soybean production. Little change was noted in the humic acid amount isolated from the prairie soil. This suggests that the decomposing soybean residue with a marked decrease in PA content compared with the corn residue (Martens, 2002) was not as effective for maintaining humic acid levels as was the corn residue with the higher PA content. A possible explanation for the decrease in extractable-humic acids from the soybean soil was given by Mwale and Walters (1994). They reported that the reduced dependence of corn on fertilizer N following soybean production was in part due to the stimulated mineralization of native soil N by the presence of the soybean residue. The evidence again suggests that formation of soil humic material is dependent on the chemistry of the plant residue inputs (Stevenson, 1994) and may fluctuate because of management of plant residues (Martens 2000b).

Since approximately the same amount of organic C was added for each of the seven residues in the incubation experiment, the noted improvement in long-term soil aggregation was related to the concentration and composition (quality) of plant PAs introduced, not to the total C loading (quantity). Added plant PAs were metabolized in soil, but at a much slower rate than reported for the plant carbohydrates and amino acids (Martens, 2000a). Phenolic acid analysis indicated that C_6–C_3 compounds, dominant in plant biomass and humic

acids were not found in the microbial extracellular polymers tested indicating that soil humic acids extracted from the Webster soil strongly reflect the chemistry of the added plant biomass. The results of this study supports the hypothesis that soil analysis for PA composition has merit for estimating the amount of plant-derived C remaining in soil.

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