



Plant residue biochemistry regulates soil carbon cycling and carbon sequestration

Dean A. Martens*

USDA-ARS National Soil Tilth Laboratory, 2150 Pammel Road, Ames, IA 50011, USA

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Abstract

Substrate composition is one of the most important factors influencing the decomposition of plant residues in soils. The interaction of organic residue biochemistry with residue decomposition rates, soil aggregation and soil humus composition was determined in a laboratory experiment. Addition of seven different organic residues (2% w/w alfalfa, oat, canola, clover, soybean, corn and prairie grasses) to a Webster soil resulted in a rapid, transient increase in aggregate mean weight diameters (MWD) when incubated for 9 d with residues with low phenolic acid content (alfalfa, canola and clover) and was inversely correlated with soil carbohydrate content ($r = -0.63$). More pronounced improvement in aggregate size was noted upon increased incubation to 84 d with organic residues higher in phenolic acid content (corn, prairie grasses, oat and soybean) and was related to soil phenolic acid ($r = 0.65$) and soil carbohydrate content ($r = 0.70$). Total plant residue phenolic acid content was related to MWD measured after incubation for 84 d by a quadratic response and plateau function ($r = 0.96$) and the MWD quadratically increased with an increase in vanillin-vanillic acid concentrations in the plant residues ($r = 0.997$). Soil organic C after 84 d was related to the MWD ($r = 0.82$) and the residue's vanillin-vanillic acid content ($r = 0.86$), suggesting that C remaining in the soil following decomposition may be related to the specific phenolic acid content. The results suggest that transient aggregate stability initiated by microbial decomposition of the carbohydrate and amino acid content of the residue, is then strengthened by the interaction with phenolic acids such as vanillin or vanillic acid released by microbial decomposition from residues structural components. Published by Elsevier Science Ltd.

Keywords: Phenolic acids; Vanillin; Vanillic acid; Carbon cycling; Carbon sequestration; Aggregate stability; Soil respiration

1. Introduction

Variation in environmental factors including temperature, moisture, composition of the decomposer community and differences in residue composition have been shown to influence plant residue decomposition (Parr and Papendick, 1978; Swift et al., 1979; Stott et al., 1986). Plants in general contain the same classes of organic compounds such as cellulose, hemicellulose, starches, proteins, lipids and polyphenols, but the proportions of each, which depend on the species and maturity, may influence the degree and

rate of decomposition (Kononova, 1966). Holding environmental conditions constant, residue composition has been shown to influence the rate and extent of residue decomposition. Carbon and N concentrations have been extensively used to measure residue quality (Christensen, 1986; Jawson and Elliot, 1986; Taylor et al., 1989). Additional research has shown that element ratios of added substrates, usually C to N ratios are often used to explain different turnover rates for early residue decomposition (Aber and Melillo, 1980; Hendrickson, 1985; Oades, 1988; Cheshire and Chapman, 1996). The decomposition of more recalcitrant organic residues is considered to be controlled by the lignin content (Fogel and Cromack, 1977) or lignin-to-N ratios (Melillo et al., 1982; Tian et al., 1992).

Soil aggregates are the basic units of soil structure

* Tel.: +1-515-294-8412; fax: +1-515-294-8125.

E-mail address: martens@nssl.gov (D.A. Martens).

(Lynch and Bragg, 1985) and organic residues applied to soil has been shown to improve structure by increasing soil aggregate stability (Waksman, 1936; Kononova, 1961). Stabilization of soil aggregates is a function of the physical forming forces present in soils to form aggregates and the release of aggregating agents by soil microorganisms upon organic residue decomposition (Allison, 1968). Soil structure improvement is not solely dependent on the total amount of organic C present, but is a function of several factors including the composition of the organic matter (Strickling, 1950; Martin, 1971; Dormaar, 1983). Research has shown that the longevity of the changes in soil properties measured with organic residue addition is related to the rate of residue decomposition. In general, materials which are quickly decomposed exert a rapid stabilization effect, which is transient (Griffiths and Burns, 1972). Materials which are slower to decompose require a longer time for maximum aggregation to occur, but aggregation effects are effective for a longer period (Martin and Waksman, 1941; Martin, 1942).

Several theories on how organic matter stabilizes soil aggregates have been proposed. Various theories of aggregate stabilization may reflect recent findings that different mechanisms of aggregate stabilization may be operating at different size scales for microaggregates < 250 μm dia and macroaggregates > 250 μm dia, (Tisdall and Oades, 1982; Dormaar and Foster, 1991; Monreal et al., 1995). One theory implies that soil polysaccharides from plant and microbial sources play a key role in the stabilization of soil microaggregates (Martin, 1971; Cheshire, 1979). In theory, the binding activity is related to the length and linear structure of polysaccharides that allow them to bridge the space between soil particles (Martin, 1971).

A second theory of microaggregate stabilization is thought to involve soil humic-like compounds. During the decomposition stages, biotic and abiotic reactions lead from the decomposition of plant residues to a complex mixture of aromatic compounds of plant and microbial origin that compose the bulk of stable organic matter (Haider et al., 1975). Readily available constituents, such as cellulose, peptides and most simple organics will rapidly be mineralized for energy and the synthesis of microbial biomass. More resistant compounds, especially lignins and other plant phenolic compounds are decomposed more slowly and combine with the products of microbial synthesis to constitute the main component of humus. Humus formation has been reported to promote long-term soil aggregation (Fortun et al., 1989a, 1989b; Piccolo and Mbagwu, 1989). Chaney and Swift (1984, 1986) reported addition of humic acids resulted in long-term stabilized soil aggregates under conditions where additions of extracellular polysaccharides were ineffective. Martens

(in press) reported that aggregate stability in a native prairie soil and a neighboring corn-soybean rotation comparison was highly correlated to soil phenolic acid content and to alkaline extractable humic acids. The amount of humic acids in soil decreased with increased tillage and with decomposition of soybean residues due to the low concentration of phenolic acids (precursor of humic substances) in the soybean residue when compared to a corn residue (Martens, in press).

Macroaggregates (> 250 μm dia) may have organic cores and encrustation of plant fragments, plant roots and fungal hyphae by mineral particles has been suggested as a mechanism in aggregate stabilization (Oades and Waters, 1991). The formation of organo-mineral phases is also thought to protect the residue from further degradation and to play an important role in carbon sequestration (Sagar et al., 1996).

All of the proposed theories for soil aggregation involve the sequestration of C by macro and microaggregates. If the stabilization of soil microaggregates by organic residue is regulated by the rate of residue decomposition and the rate of decomposition is determined by the residue's chemical composition, then improvement in aggregate stability and C sequestration should be directly related to the chemical composition of the plant residue. For this study, I have investigated the biochemistry of different organic residues, especially phenolic acid content, on the rate of residue decomposition and promotion of aggregation substances in a laboratory experiment.

2. Methods and materials

2.1. Soil and plant samples

The properties of the Webster silty-clay loam soil (Typic Haplaquoll) soil used were: pH, 7.28; organic C content, 28.82 g kg^{-1} ; inorganic C 1.44 g kg^{-1} ; total N, 1.86 g kg^{-1} ; sand, 409 g kg^{-1} ; clay, 195 g kg^{-1} soil. The soil was collected in April 1997 and stored moist at 4°C until use. The pH was measured in 10 mM CaCl_2 (2.5 to 1 ratio) and texture by a pipette method of Gee and Bauder (1986). Total C, organic C (total C after acid neutralization) and total N were determined by dry combustion with a Perkin Elmer 2400 C/H/N analyzer (Perkin Elmer, Inc.) and inorganic C was determined by the difference between total C and organic C. The properties of the corn (*Zea mays*), soybean (*Glycine max*), prairie vegetation, alfalfa (*Medicago sativa*), oat (*Avena sativa*), clover (*Trifolium pratense*) and canola (*Brassica napus*), harvested from field sites (sampled April 1997) or from glasshouse pots, are listed in Table 1. The plant samples analyzed were a mix of both leaf and stem portions (ground

through a 1 mm sieve) and prairie grass species sampled were not identified.

2.2. Analyses

Total carbohydrate content was measured by acid extraction and ion chromatography with pulsed amperometric detection of individual monosaccharides (Martens and Frankenberger, 1990). Briefly, 500 mg soil or 100 mg plant residue were refluxed with 2 N H₂SO₄ at 80°C for 16 h, neutralized to pH 4 to 5 with 5 M KOH, centrifuged to remove precipitate and an aliquot was diluted for analysis.

Total protein content was measured by acid extraction and ion chromatography with pulsed amperometric analysis of the individual amino acids and sugars (Martens and Frankenberger, 1992a). Briefly, 500 mg soil or 100 mg plant residue were autoclaved for 16 h with 4 N methanesulfonic acid (2 mg tryptamine ml⁻¹), neutralized to pH 5 with 5 M KOH, centrifuged to remove precipitate and an aliquot diluted for analysis. The monosaccharides and amino acids were separated on a Dionex DX-500 (Dionex Corp. Sunnyvale, CA) ion chromatograph equipped with a CarboPac PA10 (2 mm i.d.) for monosaccharide analysis and an AminoPac PA10 (2 mm i.d.) for amino acid analysis. Separation was achieved with a NaOH gradient (5 to 80 mM) for monosaccharides and a NaOH–Na acetate gradient (30 to 80 mM NaOH; 0 to 500 mM Na acetate) for amino acids.

Plant (50 mg) and soil (2 g) phenolic acids were extracted with 5 ml 1 M NaOH for 16 h in a reciprocal shaker at 30°C as described by Provan et al. (1994). After extraction, the sample was centrifuged and the supernatant was placed in a glass disposable test tube and heated at 90°C for 2 h to release the conjugated phenolic acids (Whitehead et al., 1983). The heated extract was then cooled, titrated with 4 M HCl to <pH 2.0, diluted to 10 ml, centrifuged to remove the formed precipitate and an aliquot was passed through a Varian (Varian Assoc. Harbor City, CA) Bond Elut[™] PPL solid phase extraction tube. The

tubes were dried under a stream of air and the phenolic compounds were eluted with 1 ml of ethyl acetate into gas chromatography autosampler vials. The phenolic compounds (1 µl, 10:1 split) were then analyzed for composition with a Hewlett-Packard 6890 gas chromatograph equipped with a HP-5 (5% crosslinked phenylmethyl siloxane) capillary column (30 m length, 0.32 mm column i.d., 0.25 µm film thickness) and detected with a flame ionization detector. The following conditions were employed for phenolic acid separation: injector temperature, 230°C; temperature ramp, 70°C for 2 min then ramped to 250°C at 10°C min⁻¹; detector temperature, 250°C. This extraction procedure releases both possible free and conjugated phenolic acids, although analysis of the nonheated extracts determined very low levels of free phenolics in plant or soil samples.

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). All soils were passed field moist through an 8 mm sieve, wet sieved in the moist condition (180 to 250 g water kg⁻¹ soil) and corrected for sand content by dispersion in sodium metaphosphate. The mean weight diameter (MWD) was calculated with the following equation (Haynes and Beare, 1997).

$$\text{MWD} = \left(\sum X_i S_i \right) / W$$

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

2.3. Residue mineralization experiment

To determine the effect of the plant residue biochemistry on the rate of residue decomposition and resulting soil aggregate stability, 30 g (dry weight) moist samples of the soil were mixed with 2% (0.6 g

Table 1
Properties of plant leaf and stem residues (dry weight) used in this study

Vegetation	Organic C (g kg ⁻¹ residue)	Total N (g kg ⁻¹ residue)	C to N ratio	Total carbohydrates (g kg ⁻¹ residue)	Total protein (g kg ⁻¹ residue)	Total phenolic acid (g kg ⁻¹ residue)
Corn	445	4.3	103	106.5	2.3	37.7
Oat	433	17.0	25	152.8	3.4	14.3
Prairie ^a	453	3.7	122	110.2	3.0	11.1
Alfalfa	350	36.0	10	54.0	8.7	3.1
Soybean	397	10.7	37	73.2	4.6	2.4
Clover	464	44.9	10	47.0	10.9	0.8
Canola	410	12.8	32	173.9	3.7	0.7

^a Unidentified native grass species.

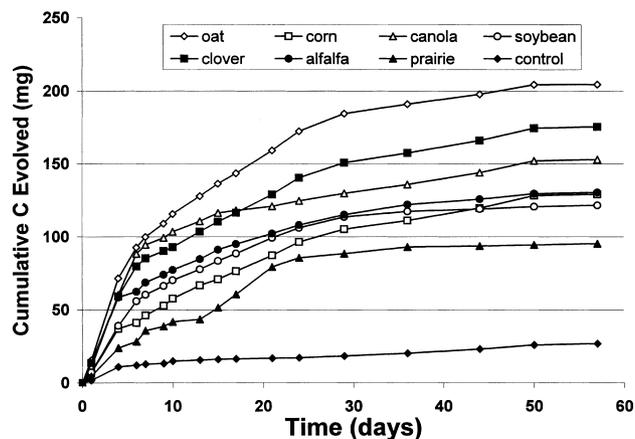


Fig. 1. Cumulative C evolution from the Webster soil treated with or without 2% (w/w) of the seven organic residues for 57 d at 22.0°C.

DW) residue and incubated at 34 kPa moisture tension at 22°C. Samples were weighed at weekly intervals and the soil moisture corrected for the drying effects induced during incubation. At 9, 29 and 84 d, dupli-

cate samples from each treatment were removed from an experimental setting that passed CO₂-free compressed air over each sample and collected the CO₂ evolved in a solution of 1 M NaOH. The CO₂ evolved was determined by back titration (0.25 M HCl) of the NaOH trap treated with excess BaCl₂ (Hassink, 1994). The soil samples were divided into two portions. One half of moist soil was used to determine soil aggregate stability and the second half was air-dried, sieved (1 mm) and analyzed for organic C, total N, carbohydrate, amino acids and phenolic acid content as described above. The plant residues were chosen for this study based on the total phenolic acid content and phenolic acid composition.

Humic acids were extracted from selected residue treatments after incubation for the specified times by a method described by Schnitzer (1982). The method utilizes a 0.2 M NaOH overnight extraction (excluding O₂) and acid precipitation of the humic acids (pH < 2.0). The humic acids were purified by repeated dissolution in 0.2 M NaOH with acid precipitation, centrifu-

Table 2

Total carbohydrate, protein and phenolic acid content (\pm S.D.; $n = 2$) determined for the Webster soil treated with different plant residues (2%) and incubated for up to 84 d^a

Residue	Time (d)	Total carbohydrates (g kg ⁻¹)	Total protein (g kg ⁻¹)	Total phenolic acids (mg kg ⁻¹)
Soil ^b	Initial	1.33	3.62	47.00
Corn	0	6.16	4.48	796.26
	9	3.17 \pm 0.11	5.12 \pm 0.04	480.34 \pm 5.53
	29	3.68 \pm 0.30	3.72 \pm 0.20	393.74 \pm 56.90
	84	4.42 \pm 0.38	5.11 \pm 0.07	263.91 \pm 45.34
Oat	0	6.74	4.85	365.81
	9	3.32 \pm 0.88	5.08 \pm 0.34	180.95 \pm 11.54
	29	3.49 \pm 0.10	3.65 \pm 0.20	129.30 \pm 0.20
	84	4.81 \pm 0.41	5.87 \pm 0.46	87.17 \pm 7.69
Prairie ^c	0	2.57	4.74	217.00
	9	3.40 \pm 0.36	4.87 \pm 0.09	199.36 \pm 6.50
	29	3.73 \pm 0.06	3.90 \pm 0.06	145.85 \pm 19.68
	84	5.02 \pm 0.30	5.05 \pm 0.12	124.73 \pm 8.67
Alfalfa	0	3.82	5.78	185.43
	9	2.43 \pm 0.50	5.95 \pm 0.02	64.40 \pm 1.11
	29	3.14 \pm 0.01	4.28 \pm 0.51	52.14 \pm 10.38
	84	3.84 \pm 1.14	5.48 \pm 0.03	42.67 \pm 0.49
Soybean	0	5.10	5.12	119.02
	9	2.49 \pm 0.56	4.84 \pm 0.31	67.86 \pm 24.03
	29	3.75 \pm 0.23	4.07 \pm 0.73	69.97 \pm 14.76
	84	4.27 \pm 0.48	5.76 \pm 0.01	71.40 \pm 5.66
Clover	0	4.77	6.49	79.45
	9	2.26 \pm 0.32	5.93 \pm 0.25	58.41 \pm 3.41
	29	2.76 \pm 0.32	4.77 \pm 1.03	48.02 \pm 2.12
	84	4.30 \pm 0.46	5.73 \pm 0.39	45.73 \pm 6.04
Canola	0	5.86	5.18	73.07
	9	2.12 \pm 1.21	4.87 \pm 0.23	66.35 \pm 4.46
	29	3.60 \pm 0.35	3.88 \pm 0.39	51.57 \pm 3.42
	84	3.33 \pm 0.01	5.21 \pm 0.37	40.86 \pm 0.36

^a The carbohydrate, protein and phenolic acid content for d 0 was calculated by mixing the amount of residue (2%) with the Webster soil, extraction and conducting the respective carbohydrate, amino acid and phenolic acid analysis.

^b The value for initial soil is the biochemical analysis of soil alone (no residue added).

^c Unidentified native grass species.

gation and then the humic acids were washed free of Cl^- and freeze dried.

3. Results and discussion

The amount and phenolic acid composition (polyphenol content) of the organic residues have been suggested to control the rate of organic matter decomposition (Herman et al., 1977; Neely et al., 1991). Residues (Table 1) with a wide range in phenolic acid content (0.7 to $37.7 \text{ g kg}^{-1} \text{ DW}$), carbohydrate content (47 to $173.9 \text{ g kg}^{-1} \text{ DW}$) and total protein content (2.3 to $10.9 \text{ g kg}^{-1} \text{ DW}$) were incubated (2.0% w/w) with the soil at ambient temperatures ($22 \pm 1.0^\circ\text{C}$) for up to 84 d for evaluation of this hypothesis. Simple regression analysis found that mg CO_2 evolved from the treatments at $\text{d } 57$ (Fig. 1) with or without residue was related to soil carbohydrate content ($r = 0.93$, $P = 0.01$) and soil amino acid content ($r = 0.67$, $P = 0.05$) and not significantly correlated with soil phenolic acid content ($r = 0.21$) at time 0 (Table 2) suggesting that the majority of C evolved during the initial stages of decomposition resulted from mineralization of the added carbohydrates and amino acids. Stevenson (1986) discussed the microbial decomposition rates of the organic residues with carbohydrates and amino acids respired to CO_2 very quickly in soils in times as short as hours or a few days and structural macromolecules required a much longer time for decomposition to simpler units before respiration.

Studies have shown that different organic residues have different CO_2 evolution rates. Ajwa and Tabatabai (1994) reported the CO_2 evolution from different organic residues in a 30 d incubation ranged from

Table 3

Mean weight diameter (MWD) of the Webster soil ($\pm \text{S.D.}$; $n = 2$) with and without treatment with different plant residues (2%) and incubation for 84 d

Residue	MWD (mm)			> 1.0 mm ^a (%)		
	9 d	29 d	84 d	9 d	29 d	84 d
Corn	0.76 ± 0.02	2.02 ± 0.01	1.41 ± 0.07	50.0	75.8	72.6
Oat	0.85 ± 0.11	0.98 ± 0.06	1.39 ± 0.01	56.0	75.3	69.0
Prairie ^b	0.68 ± 0.09	1.08 ± 0.19	1.45 ± 0.25	49.5	67.7	71.4
Alfalfa	1.17 ± 0.01	1.07 ± 0.08	1.18 ± 0.12	63.0	63.0	65.8
Soybean	0.80 ± 0.15	1.86 ± 0.06	1.22 ± 0.00	53.5	79.2	72.0
Canola	1.11 ± 0.12	1.22 ± 0.09	1.00 ± 0.38	61.4	70.5	62.8
Clover	0.80 ± 0.03	0.97 ± 0.05	0.90 ± 0.09	55.0	63.5	51.2
Control	0.37 ± 0.21	0.42 ± 0.10	0.66 ± 0.35	28.6	35.6	43.6

^a Percentages are aggregates (by weight) remaining on the sieves > 1.0 mm size.

^b Unidentified native grass species.

27% of C added for corn residue to 58% of C added for alfalfa residue and $> 50\%$ of the total CO_2 produced was evolved in the first 6 d . Their data reinforces the evidence that residue chemical composition influences the soil decomposition rates. Herman et al. (1977) reported that decomposition rates of plant residues in a soilless medium could not be predicted from properties of the original materials studied such as C to N ratio, lignin or carbohydrate content when evaluated individually, but these properties when combined, could accurately estimate the decomposition rates.

Soil total carbohydrate content, total protein content and total phenolic acid content in the soil treated with the seven residues at time 0, 9, 29 and 84 d are given in Table 2. The total protein content only

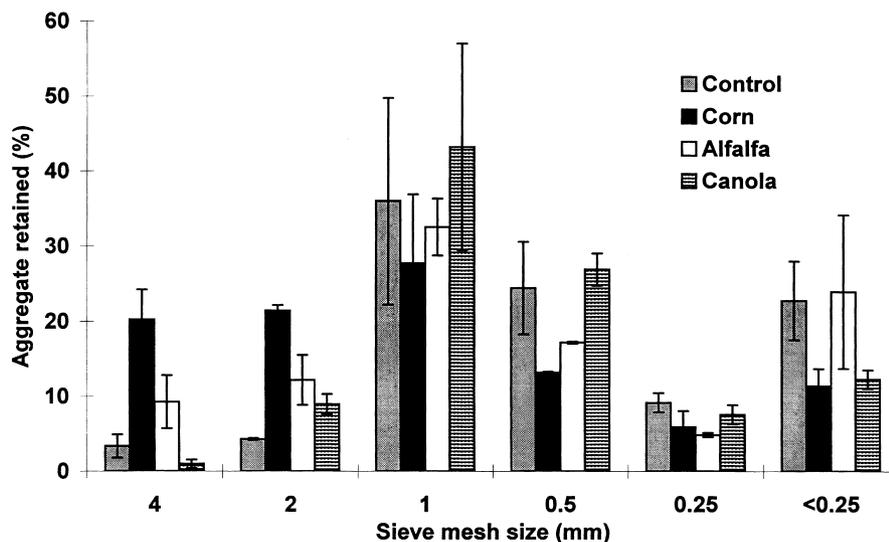


Fig. 2. Size distribution of aggregates separated by wet sieving from the Webster soil treated with corn, alfalfa, canola or no residue and incubated for 84 d .

Table 4

Organic C content of Webster soil (\pm S.D.; $n = 2$) after treatment with different residues and incubation for 84 d

Residue	Organic C (g C kg ⁻¹ soil)			
	0 d ^a	9 d	29 d	84 d
Corn	39.58	39.29 \pm 2.67	39.70 \pm 3.73	39.50 \pm 1.84
Oat	40.24	37.25 \pm 2.78	36.59 \pm 4.53	36.27 \pm 0.65
Prairie ^b	41.06	41.60 \pm 2.17	36.68 \pm 1.68	36.87 \pm 2.02
Alfalfa	39.85	36.48 \pm 0.89	35.76 \pm 0.73	34.50 \pm 0.81
Soybean	39.64	39.00 \pm 1.03	37.06 \pm 0.04	35.73 \pm 0.12
Clover	40.95	35.91 \pm 1.35	35.91 \pm 0.48	35.50 \pm 0.51
Canola	39.70	32.83 \pm 2.85	34.69 \pm 1.39	33.25 \pm 0.94
LSD _{0.05}		3.25	3.05	2.83

^a The organic C value for Day 0 was calculated by mixing the amount of residue (2%) with the Webster soil and the analysis was done by dry combustion. All values are corrected for inorganic C content.

^b Unidentified native grass species.

showed slight changes with time, but the carbohydrate content initially decreased rapidly in response to microbial decomposition and then carbohydrate content increased during the 84 d incubation. In contrast, the total phenolic acid content decreased with time for all of the amendments, with decomposition ranging from 16% of the soybean phenolics to 69% of the oat phenolics in 84 d.

Addition of the seven organic residues increased MWD for the soil at all incubation times when compared to the control (no residue added) (Table 3). The residues resulted in a rapid increase in MWD after 9 d of incubation, but the alfalfa, clover and canola residues resulted in nearly identical MWDs after incubation for 29 or 84 d. Residues with higher phenolic acids such as the corn, oat, prairie grasses increased MWD with increased incubation time and were the most effective residues for increasing MWD after 84 d. The mechanism for stabilization of soil particles by plant residues depends on the size of the stabilized aggregate. Products of microbial metabolism may be involved with stabilization of microaggregates < 20 μ m dia, where plant debris play a major role in the stability of microaggregate between 100–200 μ m dia (Dorbeer and Foster, 1991), with plant roots and fungi stabilizing soil particles and organic matter in macroaggregates (Monreal et al., 1995).

In the absence of plant roots and lack of visible fungal activity, the binding of soil particles into macroaggregates in my study appears to be a function of decomposing organic residue becoming encrusted with soil particles. A size-distribution comparison of the soil aggregates resulting from the decomposition of the corn, the alfalfa and the canola residue for 84 d is presented in Fig. 2. It is evident that the soil with the greatest amount of organic C (Table 4) remaining (corn residue, highest phenolic acid content, Table 1)

Table 5

Correlation coefficients (r values) between plant chemical composition and soil organic C content (mg or g kg⁻¹) and soil and plant chemical composition (mg or g kg⁻¹) and MWD (mm) following decomposition of added organic residues to the Webster soil. *0.05 > P > 0.05; **0.01 > P > 0.001; *** P < 0.001

Chemical composition	d 9	d 29	d 84
<i>Soil organic C content</i>			
Organic C	0.28	0.32	0.52
Total N	-0.48	-0.47	-0.44
C to N ratio	0.74*	0.58	0.67*
Carbohydrates	-0.27	-0.13	-0.10
Protein	-0.37	-0.44	-0.42
Phenolic acids	0.47	0.91**	0.90**
<i>MWD; incubated soil content</i>			
Organic C	-0.46	0.81*	0.79*
Total N	ND	ND	ND
C to N ratio	ND	ND	ND
Carbohydrates	-0.63*	0.56	0.70*
Protein	0.31	-0.33	0.28
Phenolic acids	-0.47	0.63*	0.65*
<i>MWD; initial plant content</i>			
Organic C	-0.78*	0.04	-0.04
Total N	0.39	-0.55	-0.63*
C to N ratio	-0.63*	0.40	0.84*
Carbohydrates	0.10	0.06	0.24
Protein	0.31	-0.46	-0.72*
Phenolic acids	-0.43	0.55	0.96****

^a The value represents a quadratic response and plateau function.

has the greatest amount of soil aggregates remaining in the 2 and 4 mm sieve when compared to the alfalfa (low phenolic acid content) and canola (lowest phenolic acid content tested) amendments. The canola residue had the highest carbohydrate content of the seven residues tested and resulted in a slight improvement in the 0.5 mm and 1.0 mm aggregates size fractions compared with the control. Degens and Sparling (1996) reported that ¹⁴C-glucose added to a soil (31 mg OC g⁻¹ soil) resulted in a short-lived increase in the MWD of the 0.25 and 0.50 mm aggregate classes, but had no statistical effect on the measured aggregate classes after 56 d of incubation. Carbohydrates such as glucose stimulate microbial activity measured as respiration and can result in transient soil aggregation. Martens and Frankenberger (1992b) reported that a single pulse of glucose (1 mg glucose-C g⁻¹ soil) was detectable by ion chromatographic analysis for up to 5 d, but after 7 d incubation, the acid extractable monosaccharide content of the glucose-treated soil was identical to the control soil (no glucose added). The results reported by Martens and Frankenberger (1992a, 1992b) suggest that simple carbohydrates stimulate microbial activity, but not the synthesis of polysaccharide binding agents. Microbial products resulting from the residue decomposition interacting with weekly wet-dry cycle increased the MWD distribution, but the specific material stabilizing the soil particles could not be

identified by trends in total carbohydrate, protein or phenolic acid content (Table 2).

The soil organic C content measured during the 84 d incubation also reflected greater loss of C from residues with lower amounts of phenolic acids and less C loss from residues higher in phenolic acids (Tables 1 and 4). The organic C remaining in the treated soils (Table 4) was not significantly correlated with the initial residue organic C, total N, carbohydrate content, protein content, or phenolic acid content at d 9, but was significantly correlated with phenolic acid content at d 29 and phenolic acid content and C to N ratio at d 84 (Table 5), indicating that residues with higher amounts of phenolic acids result in more C remaining in the soil following short-term incubation. The organic C remaining after incubation for 29 and 84 d was also significantly correlated with MWD measured at d 29 and 84 (Table 5). The evidence suggests that understanding the plant biochemistry will provide evidence for subsequent soil decomposition rates and serve as a means for possibly predicting C sequestration.

Statistical analysis showed total soil carbohydrate content (Table 2) was significantly negatively correlated with MWD (Table 4) at 9 d, not significant at 29 d and positively correlated at 84 d incubation; total soil protein content was not significantly correlated with MWD at 9 d, at 29 d and at 84 d incubation; total soil phenolic acid content was not correlated to MWD at 9 d, but was found significant at the 29 d and 84 d incubation (Table 5). The change in the correlation coefficients noted with the soil biochemistry composition and aggregate size suggests that several mechanisms are functioning for stabilization of soil particles (Table 5). The initial mechanism of stabilization involves the most labile of the residue biochemistry, stimulation of microbial activity by mineralization of carbohydrates and amino acids and may play a major role in the early stages of aggregate stabilization. Residues with higher amounts of carbohydrates and amino acids decompose faster (Fig. 1) and later microbial release of phenolic compounds, if present, reinforces the stabilization initiated by the microbial mineralization of the labile carbon pool. This mechanism is very similar to the results of an experiment conducted by Griffiths and Burns (1972) in which short-term stable aggregates (<6 weeks) were produced with incubation of synthetic soil aggregates with polysaccharide alone or mixed together with tannic acid (phenolic acid source), but adding the tannic acid after the synthetic soil aggregate had been incubated with the polysaccharide and dried resulted in aggregate stability that endured with no sign of diminished aggregation for up to 6 months.

Soil humic acids have also been proposed to be formed from phenolic acids released from plant residue

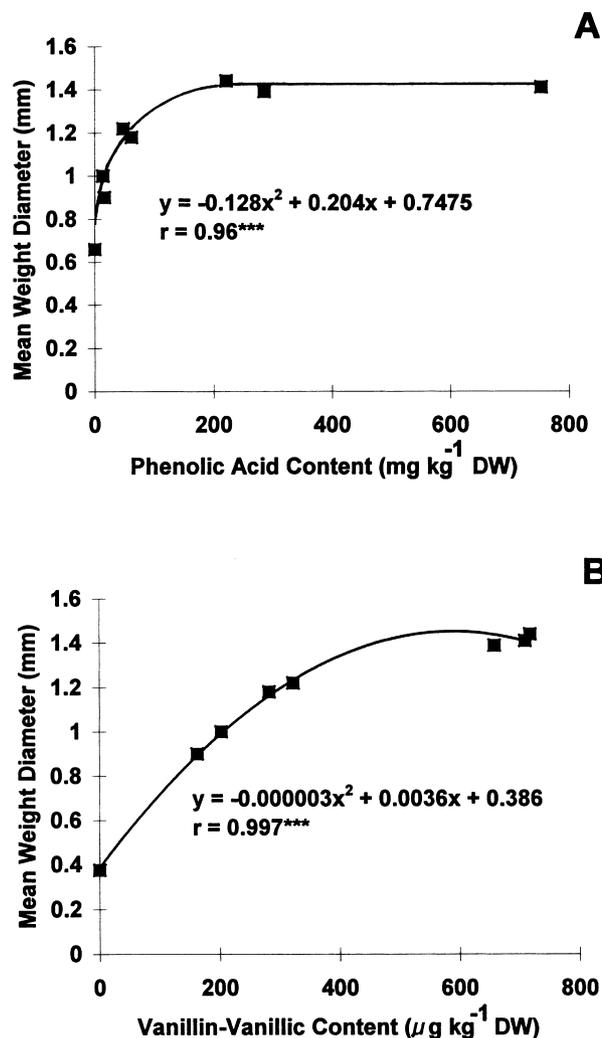


Fig. 3. Mean weight diameter of the Webster soil treated with or without 2% (w/w) of the seven organic residues for 84 d and (a) total residue phenolic acid content and (b) the sum of the residue vanillin and vanillic acid content.

or synthesized by decomposing soil microorganisms and are important in soil stabilization. Martens (in press) reported that the quantities of humic acids extracted from a prairie and a nearby producer corn-soybean rotation were directly related to the soil MWD. In the work presented here, alkaline extraction of humic acids from the control soil, the corn (high phenolic acids content) and canola (low phenolic acid content) amended soil after incubation for 29 and 84 d found the extractable humic acids in the nonamended soil maintained 2.8 g kg^{-1} soil during incubation, while in the corn amended soil, humics increased to 3.3 g kg^{-1} soil (d 29) and 3.6 g kg^{-1} soil (d 84). Incubation of the canola residue resulted in 2.8 g kg^{-1} soil (d 29), but a decrease of humic acids to 2.0 g kg^{-1} soil after 84 d incubation suggesting that the canola residue may increase decomposition of native soil organic matter. Stevenson (1994) reported

that polyphenols from plant sources or microbial synthesis were important precursors of soil humic substances and the formation of humic substances with incubation of the corn residue and the decrease in humic substances with incubation of the canola residue supports the importance of plant polyphenols as precursors of soil humic acids. My results indicate that biochemical analysis of plant tissue can provide information on decomposition rates, but this information is dependent on accurate measurement of the biochemical composition of plant residue and soils as the first step to understanding the effects of crop management systems on soil processes.

Most of the literature concerning the composition of organic residues on decomposition rates and soil structure has measured residue quality by use of a proximate analysis initially described by Goering and Van Soest (1970). This proximate method, originally used to measure forage digestibility, gravimetrically determines soluble cell constituents (cold H₂SO₄), cellulose (heated H₂SO₄ for several hours) and lignin (heated H₂SO₄, 24 h) by solubility differences in concentrated H₂SO₄. Clearly, more specific methodology must be employed to accurately determine the relationships between the biochemical composition of plant residue and soil decomposition. Replacing gravimetric measurements with precise extraction and detection techniques as employed in my study determined that plant residue carbohydrate, protein and phenolic acid contents (time 0 values, Table 2) were not significantly correlated with MWD at 9 d, or 29 d, but after 84 d incubation, the protein content was significantly correlated and a significant quadratic response and plateau relationship ($r = 0.96$, $P > 0.001$) was determined for the phenolic acid content (Fig. 3a). The critical concentration of phenolic acids needed to result in the maximum increase in MWD under the conditions employed here is in the range of 200 to 220 mg kg⁻¹ soil. Statistical analysis of the residue's phenolic acid composition determined that the concentration of vanillin-vanillic acid in the plant residues was found to be significantly related with MWD with a quadratic response function ($r = 0.997$, $P > 0.001$) after 84 d incubation (Fig. 3b). The results indicate that the phenolic acid content of the plant residues appeared to be important in the aggregate stability mechanisms and for maintaining organic C and that determination of the organic residue's phenolic composition may help predict C sequestration potential when incorporated into biofilters, conservation reserve acreage or biofuel production.

My study showed that C accumulation in soil improved soil aggregate stability. The aggregate stability improved by addition of organic residue was a result of increased microbial activity due to metabolism of carbohydrates and the interaction of plant

phenolic acids released during decomposition of residue structural components. Investigating the residue biochemistry determined a significant relationship between the sum of vanillin and vanillic content and MWD suggesting that measuring phenolic acid content and composition would be beneficial for prediction of soil structure improvement.

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