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# Retention of Tannin-C is Associated with Decreased Soluble Nitrogen and Increased Cation Exchange Capacity in a Broad Range of Soils

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Phenolic compounds, called tannins, can be retained by soil and affect soluble N but have been studied in only a few soil types. Surface samples (0–10 cm), collected from the United States and Canada, were treated with water (Control) or solutions containing procyanidin, catechin,  $\beta$ -1,2,3,4,6-pentagalloyl-O-D-glucose (PGG), tannic acid, gallic acid, or methyl gallate. Soluble C and N in treatment supernatants and after incubation (16 h, 80°C) were measured to determine retention of treatment C and effects on soluble N and cation exchange capacity (CEC). Retention varied significantly with treatment (T) and soil order (S) and was greatest for PGG > tannic acid > procyanidin > catechin > methyl gallate > gallic acid and in Alfisols, Aridisols and Mollisols compared Ultisols. However, differences among soil orders were observed only for strongly retained compounds ( $T \times S$ ,  $P < 0.001$ ). Extraction of soluble N was decreased by gallic acid and tannins, especially PGG, but unaffected by methyl gallate or catechin. All treatments decreased soluble N from Aridisols while Entisols were less affected by tannins ( $T \times S$ ,  $P < 0.01$ ). Soil CEC was significantly increased by tannins but unaffected by other compounds. However, CEC increased more in Aridisols than in Mollisols or Ultisols and treatment effects were small and unvarying in Ultisols ( $T \times S$ ,  $P < 0.001$ ). Changes to both soluble N and CEC were linearly related with retention of treatment C. Tannins produced effects associated with improved soil quality on a broad range of soils and may have a role in land management.

**Abbreviations:** CEC, cation exchange capacity; EC, electrical conductance; ODE, oven dry equivalent; PB, Prussian blue; PGG,  $\beta$ -1,2,3,4,6-pentagalloyl-O-D-glucose; S, soil order; soluble C, soluble carbon; soluble N, soluble nitrogen; T, treatment;  $\text{Trt-C}_{\text{final}}$ , final retention of treatment carbon by soil;  $\text{Trt-C}_{\text{ret}}$ , initial retention of treatment carbon by soil;  $\Delta\text{CEC}$ , net changes in cation exchange capacity attributable to treatments;  $\Delta\text{Sol-N}$ , net changes in extraction of soluble nitrogen attributable to treatments.

**T**annins are polyphenolic plant secondary compounds with molecular weights typically in the range 500 to 3000 g mol<sup>-1</sup>. Like other phenolics, tannins are redox active and form complexes with metal ions such as Fe(III) or Al(III). The defining characteristic of tannins is their ability to form cross-linked, complexes with protein (Hagerman, 2012). Lower molecular weight non-tannin phenolics may bind protein but do not form precipitable complexes. Tannins can account for a significant proportion of biomass in some terrestrial plants (Kuiters, 1990; Matthews et al., 1997), and are broadly classed as hydrolyzable or condensed (Haslam, 1981; Salminen and Karonen, 2011). Hydrolyzable tannins are galloyl esters and their derivatives attached to polyol cores, while

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condensed tannins are oligomeric and polymeric flavan-3-ols (Quideau et al., 2011).

The importance of phenolic plant secondary compounds, including tannins, to soil functions has emerged as their effects on biological, chemical, and physical processes have come to light. (Halvorson et al., 2011; Hättenschwiler and Vitousek, 2000; Horner et al., 1988; Kraus et al., 2003a; von Lützow et al., 2006). When exuded from roots or leached from living or dead plant materials, phenolic compounds can affect soil physical properties and nutrient cycling by forming complexes with mineral fractions of the soil, by stabilizing soil organic matter, proteins or other organic forms of N, and by direct or indirect stimulation or inhibition of soil microorganisms (Adamczyk et al., 2013; Cesco et al., 2012; Fabre et al., 2010; Makoi and Ndakidemi, 2007). However, their impacts may be influenced by specific tannin and soil characteristics and remain to be fully elucidated (Kraus et al., 2003b, Schmidt et al., 2012, Smolander et al., 2012). Our earlier work focused on understanding the initial effects of tannins and related non-tannin phenolics on soil processes and characteristics related to management of soil organic matter and nutrient cycling (Halvorson et al., 2012a; Halvorson and Gonzalez, 2008; Halvorson et al., 2012b; Halvorson et al., 2011; Halvorson et al., 2009; Schmidt et al., 2012). When added to soil, tannic acid affected the recovery and composition of Bradford-reactive soil protein, associated with glomalin. Retention of treatment C by soil was rapid, much greater for tannins than non-tannin subunits, and greater for hydrolyzable gallotannins than condensed tannins (proanthocyanidins). Significant amounts of retained tannin-C remained in soil even after repeated rinses with hot water and retention appeared to be favored by soil organic matter and influenced by depth, soil amendments, and soil texture. In response to increasing compound concentrations or repeated applications, tannins produced patterns of cumulative retention, consistent with saturable binding of the treatment C to soil with evidence for a maximum loading capacity. Relatively high retention of tannin-C by soil was accompanied by increases in cation exchange capacity. Solubility of soil N, particularly organic forms, was reduced by hydrolyzable tannins and related compounds and to a lesser degree by condensed tannins. The effects of treatment varied with the temperature but were little affected by soil amendments. However, reductions of soluble N were stronger in pasture than forest soil. We also found tannins and related compounds can increase the solubility of soil metals such as Ca and Mn and mitigate the toxic effects of Al on roots (Kinraide and Hagerman, 2010).

Together, these studies suggest tannins may have a role in the management of agricultural soils where the quantity and quality of soil organic matter and nutrient cycling has economic and environmental consequences (eg, Dominati et al., 2010; Robinson et al., 2013; Robinson et al., 2009). However, the initial reactions of tannins with soil, as well as subsequent biological transformations, have been evaluated for only a few soil types and land uses and thus critical assessment of their potential role in managing soil quality has lagged. More systematic, comparative studies

are needed to provide information to devise new strategies to achieve desired management goals.

The objective of this study was to document the initial interactions of tannins and closely related non-tannin phenolic compounds with a broad variety of soils. We hypothesized that the patterns we observed for pasture and forest sites from West Virginia, USA (Halvorson et al., 2012b; Halvorson et al., 2011; Halvorson et al., 2009) would be reflected consistently in samples acquired from across the United States and Canada representing a wide variety of land uses and differing management styles. We treated samples of these soils with solutions containing chemically well-defined hydrolyzable and condensed tannins, (polymers) and related non-tannin phenolic substances (monomers). We determined the retention treatment C in soil and treatment effects on soluble N and cation exchange capacity to fit within the analytical framework of our earlier studies and to relate to important soil parameters such as the accumulation or retention of organic matter or plant nutrients.

## MATERIALS AND METHODS

### Soils

Samples of surface soil (0–10 cm) representing five soil orders, a wide variety of land uses, and differing management styles were acquired from various locations distributed across 22 states and a Canadian province (Ontario) (Appendix A). Each sample ( $n = 66$ ) was comprised of multiple cores collected from an area and in such a fashion so as to accurately represent the local management conditions. Composite samples were sieved (2 mm), dried to a constant mass at 55°C, and stored at room temperature until further analysis.

Basic soil properties were determined for each composite sample (Table 1). Total soil C and N were determined on untreated soils after dry oxidation with a FlashEA 1112 NC Analyzer (CE Elantech, Lakewood, NJ) using CE Elantech NC reference soil (3.5% C and 0.37% N) and *K*-factor calibration with aspartic acid as a standard. Total soil organic C was determined similarly from samples acidified with 100  $\mu$ L 6 M HCl and oven dried (60°C for 2–3 h). Sample textures were determined by hydrometer (Midwest Labs, Omaha, NE, www.midwestlabs.com). Soil pH and electrical conductance (EC) were measured by electrode (1:1 soil/water). Cation exchange capacity (CEC) of soils was measured at the soil pH by exchange with cobalt hexamine trichloride at a concentration of 0.01667 M and expressed as centimoles of positive charge per kilogram oven dried equivalent soil ( $\text{cmol}_c \text{ kg}^{-1}$  ODE soil) (Ciesielski and Sterckeman, 1997a, 1997b, ISO 23470:2007, 2007).

### Test Compounds

Soil samples were treated with solutions containing model tannins or non-tannin phenolic compounds (organic acids and flavonoids), selected to represent a range of phenolic compounds of varying complexity present in the plant-soil continuum (Gallet and Lebreton, 1995). Our representative condensed tannin was a polymeric flavonoid-based procyanidin isolated from sorghum

**Table 1. Soil properties (0–10 cm)†.**

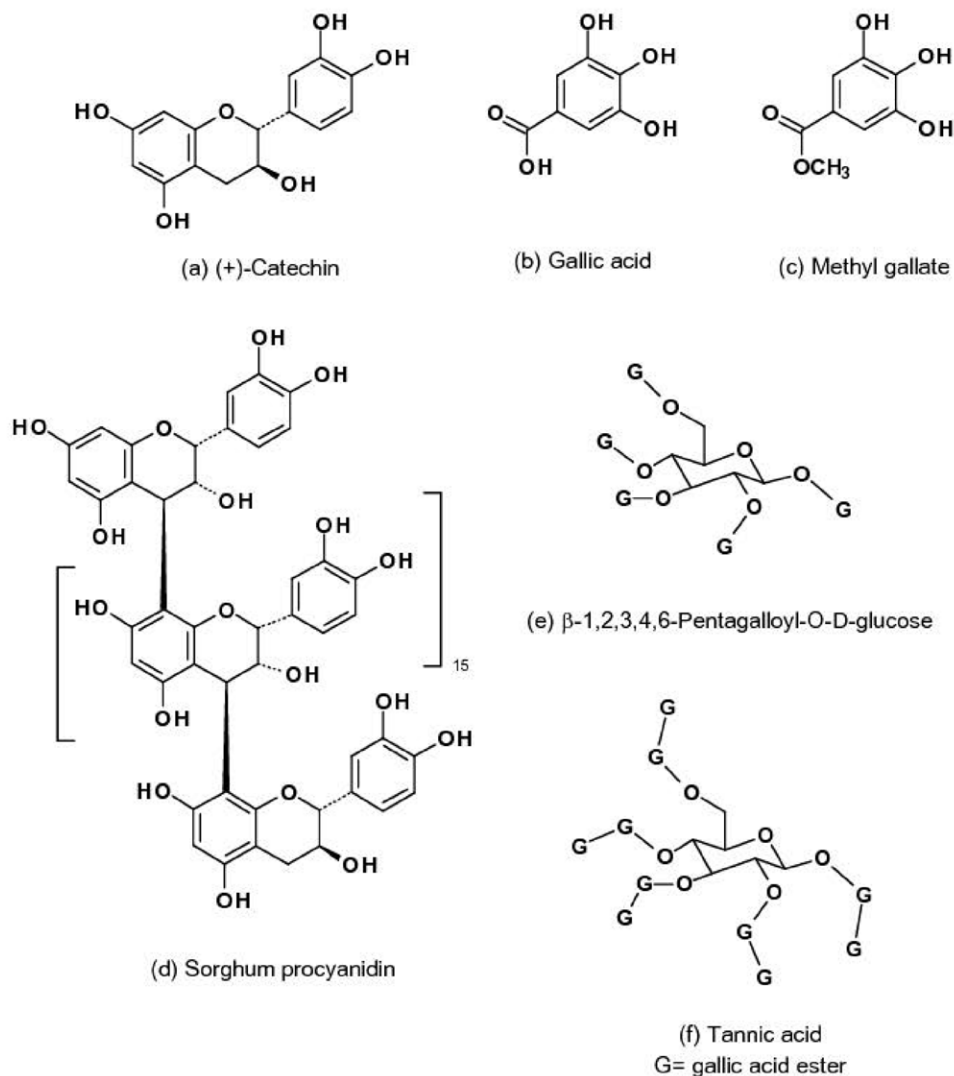
Soil type	Total soil C	Total organic soil C	Total soil N	Sand	Silt	Clay	pH	EC	CEC
Average ( $n = 66$ )	1.88±0.15 (0.35–6.46)	1.57±0.12 (0.30–4.60)	0.15±0.01 (0.04–0.40)	41.5±2.7 (8–89)	43.5±2.3 (7–76)	14.9±0.9 (2–34)	6.8±0.1 (4.3–8.7)	262 ± 22 (62–863)	12.6 ± 0.8 (1.7–26.5)
Alfisol ( $n = 10$ )	1.80±0.29 (0.35–3.74)	1.74±0.28 (0.30–3.60)	0.16±0.02 (0.04–0.23)	25.8±7.4 (8–84)	52.0±5.2 (10–66)	22.2±2.5 (6–30)	6.4±0.3 (4.3–7.2)	225 ± 58 (82–700)	11.2 ± 1.4 (5.0–18.2)
Aridisol ( $n = 5$ )	3.66±0.76 (1.85–6.46)	1.09±0.17 (0.74–1.64)	0.13±0.02 (0.09–0.18)	39.6±8.5 (18–56)	48.0±8.3 (30–68)	12.4±2.2 (8–20)	8.4±0.0 (8.3–8.4)	307 ± 77 (176–571)	14.3 ± 1.7 (10.9–18.5)
Entisol ( $n = 6$ )	0.87±0.10 (0.65–1.27)	0.68±0.07 (0.37–0.87)	0.08±0.01 (0.06–0.09)	62.2±4.4 (48–75)	26.2±4.0 (13–38)	11.7±1.0 (8–14)	8.0±0.3 (7.1–8.6)	325 ± 110 (131–863)	14.1 ± 1.7 (9.8–18.9)
Mollisol ( $n = 33$ )	2.09±0.20 (0.39–4.80)	1.93±0.19 (0.36–4.60)	0.18±0.02 (0.04–0.40)	35.0±0.3 (10–70)	49.3±3.0 (16–76)	15.7±1.1 (4–34)	6.7±0.2 (5.1–8.7)	299 ± 29 (84–854)	15.9 ± 0.9 (9.4–26.5)
Ultisol ( $n = 12$ )	1.15±0.13 (0.54–1.93)	1.08±0.13 (0.50–1.83)	0.10±0.01 (0.04–0.18)	63.0±4.8 (38–89)	27.5±4.3 (7–54)	9.5±1.6 (2–18)	6.1±0.2 (5.1–7.4)	138 ± 29 (62–419)	3.4 ± 0.4 (1.7–5.5)

† Data are arithmetic average ± standard error, (range of values). Total soil C and N were determined on untreated soils, after dry oxidation, by TCD with a FlashEA 1112 NC Analyzer. Total organic soil C was determined similarly from samples acidified with 100  $\mu\text{L}$  6 M HCl at 60°C for 2 to 3 h. Sample textures were determined by hydrometer. Soil pH and electrical conductance (EC) were measured by electrode (1:1 soil/water). Cation exchange capacity (CEC) of soils was measured at the soil pH by exchange with cobalt hexamine trichloride.

grain [*Sorghum bicolor* (L.) Moench] (Hagerman, 2002; Schofield et al., 1998). We also evaluated tannic acid, a commercially available, but imprecisely defined mixture of galloyl esters, and  $\beta$ -1,2,3,4,6-pentagalloyl-O-D-glucose (PGG), a well-defined gallotannin purified from tannic acid (Hagerman, 2002). Non-tannin phenolics included the flavonoid catechin, the phenolic acid, gallic acid, and its ester, methyl gallate (Fig. 1, Table 2).

### Retention of Treatment Carbon and Effects on Soluble Nitrogen

Sorption/desorption of test compounds and effects on soluble N were determined from patterns of soluble carbon (soluble C) and nitrogen (soluble N) in supernatants after an application of a cool (23°C) treatment solution and after a subsequent incubation in hot water (80°C). Both cool and hot water extractions were employed because they have been associated with different pools of labile C and N in soil and featured in recent work (Halvorson and Gonzalez, 2008; Halvorson et al., 2009). The C and N extracted from soil with cool water are thought to correlate to recent inputs such as fertilizer, lime, manure, or soluble plant



**Fig. 1. Chemical structures for (a) (+)-Catechin, (b) Gallic acid, (c) Methyl gallate, (d) Sorghum procyanidin, (e)  $\beta$ -1,2,3,4,6-pentagalloyl-O-D-glucose (PGG), and (f) Tannic acid. The structure shown for tannic acid is a representative molecule since it is an imprecisely defined mixture of galloyl esters.**

residues while C and N recovered after hot water incubation are positively correlated with soil microbial biomass C and N, mineralizable N and total carbohydrates. (Curtin et al., 2006; Ghani et al., 2003).

**Table 2. Properties of treatment compounds.**

Treatment	Class	Source	Compound characteristics				Treatment solution characteristics <sup>¶</sup>						
			MW†	C#	N#	C	K <sub>ow</sub> §	Soluble C	Soluble N	Phenolics#	pH		
			g mol <sup>-1</sup>	%	%	g mol <sup>-1</sup>	—	—	—	—	—	—	—
Methyl 3,4,5-trihydroxybenzoate, 98% (Methyl gallate) (MG)	Phenolic organic ester	Indofine Chemical Co., Hillsborough, NJ	184	51.7	0.047	96	6.3	510.1	0.00	4.43	4.5		
Gallic acid, certified (GA)	Phenolic organic acid	Fisher Scientific, Pittsburgh, PA	170	47.7	0.026	85	0.3	501.0	0.05	5.62	3.3		
Tannic acid, certified (TA)	Mixture of gallotannins	Fisher Scientific, Pittsburgh, PA	902	49.4	0.234	474	ND	482.2	0.22	3.38	3.5		
β-1,2,3,4,6 penta-O-galloyl-D-glucose (PGG)	Gallotannin	Purified from tannic acid (Fisher)	941	49.7	0.014	492	129	497.1	0.05	3.15	5.1		
(+)-Catechin hydrate, >98% (CAT)	Flavonoid	Sigma, St Louis, MO	290	61.6	0.076	180	2.4	605.6	0.10	3.35	5.4		
[(4β- > 8)-epicatechin] <sub>15</sub> -(4β- > 8)-catechin (Sorghum procyanidin) (PRO)	Polymeric flavonoid	[ <i>Sorghum bicolor</i> (L.) Moench]	4624	48.6	0.210	2880	0.002	471.5	0.56	1.49	5.6		

† Number average molecular weight for TA estimated by RP-HPLC by Hagerman (personal communication, 2010) and used to calculate weighted average g C mol<sup>-1</sup>.

‡ Total C and N were determined in triplicate with a FlashEA 1112 NC Analyzer (CE Elantech, Lakewood, NJ).

§ Octanol-water partition coefficients (K<sub>ow</sub>) for PGG, CAT and SOR from Hagerman et al. (1998), GA and MG from Lu et al. (2006). Low values correspond to hydrophilic compounds while higher values are indicative of hydrophobic ones.

¶ Treatment solutions contained the equivalent of 1 mg treatment compound per milliliter deionized water. Ten milliliters of solutions were applied g<sup>-1</sup> soil<sup>-1</sup>.

# Determined by the Modified Prussian Blue assay.

Seven subsamples (3 g), obtained from of each soil sample, were weighed into tared *Oak Ridge* polypropylene centrifuge tubes (50 mL, nominal). These were treated with 30 mL of a test solution to yield a final amendment of 10 mg test compound g<sup>-1</sup> soil or with 30 mL of deionized water (control). After reciprocal shaking at 200 rpm for 1 h at room temperature, samples were centrifuged for 3 min at 11,952 g and decanted. Sample tubes were weighed to account for the effects of retained solution while supernatants were analyzed for soluble C and N with a Shimadzu TOC-VCPN analyzer equipped with a TNM-1 module (Shimadzu Scientific Instruments, Columbia, MD). Sample pellets were stored for <4 h in the original tubes in a refrigerator until they could be prepared for the hot water incubation. For the hot water incubation, water (30 mL) was added to all soil samples, which were then vortexed, incubated overnight in a water bath (16 h, 80°C), and assayed for soluble C and N.

Total phenolics initially added by test solutions and remaining in the supernatants after treatment were determined with the modified Prussian blue (PB) assay for total phenols (Graham, 1992; Hagerman, 2002; Schofield et al., 1998) using gallic acid as the standard. This assay is a colorimetric determination of phenolics and other oxidizable compounds which cannot distinguish between tannins and other phenolic substances. The proportion of added PB-reactive material remaining in treatment supernatants was compared against retention of treatment C to confirm retention of phenolic compounds by the soils. Values for hot water-soluble C, and N, and PB were corrected to account for carryover from the previous treatment step and all data were adjusted to an oven dry equivalent (ODE) soil basis.

Initial retention of treatment C by soil was calculated as:

$$\text{Trt-C}_{\text{ret}} = \text{Trt-C}_{\text{added}} - (\text{Sol-C}_{\text{trt}} - \text{Sol-C}_{\text{control}}) \quad [1]$$

where Trt-C<sub>added</sub> is the soluble carbon added in treatment solutions (Table 2), and Sol-C<sub>Trt</sub> and Sol-C<sub>control</sub> are soluble carbon extracted from treated and control samples, respectively.

The final retention of treatment C after the hot water incubation was determined as

$$\text{Trt-C}_{\text{final}} = \text{Trt-C}_{\text{ret}} - (\text{HWSol-C}_{\text{trt}} - \text{HWSol-C}_{\text{control}}) \quad [2]$$

where HWSol-C<sub>Trt</sub> and HWSol-C<sub>control</sub> are soluble carbon extracted from treated and control samples with hot water. Values for initial and final retention of treatment C were determined in both absolute (mg kg<sup>-1</sup> ODE soil), and relative (percent of added treatment C) terms.

Treatment effects on the extraction of soluble N were calculated relative to the control values and used to determine if treatments decreased (net treatment < net control) or increased (net treatment > net control) extraction of soluble N from soil. The net effects of treatments on the extraction of soluble N after the initial treatment and after the hot water incubation were determined as:

$$\Delta \text{Sol-N} = \text{Sol-N}_{\text{trt}} - \text{Sol-N}_{\text{control}} \quad [3]$$



where  $\text{Sol-N}_{\text{trt}}$  and  $\text{Sol-N}_{\text{control}}$  indicate the amount of net soluble nitrogen extracted from soil samples treated with phenolic compounds or water alone, respectively.

### Measurements of Final Total Soil Carbon and Cation Exchange Capacity

The soil pellet remaining in tubes after the hot water incubation was dried (55°C), and a portion assayed for total soil-C and CEC as described above. Although we expected changes in soil-C to be the net result of the C added by the treatments balanced against losses of soluble C, we also compared  $\text{Trt-C}_{\text{final}}$  with the difference between treated and control samples (i.e., total C in treated soil–total C in control samples) to account for experimental error introduced by sample handling.

As with soluble N, treatment effects on soil cation exchange capacity (CEC) were calculated relative to the control values. The net changes in CEC attributable to treatments, ( $\Delta\text{CEC}$ ), were determined as:

$$\Delta\text{CEC} = (\text{CEC}_{\text{trt}} - \text{CEC}_{\text{control}}) [4]$$

where  $\text{CEC}_{\text{trt}}$  and  $\text{CEC}_{\text{control}}$  indicate the CEC measured in soil samples treated with phenolic compounds or water alone, respectively.

### Statistical Analyses

We analyzed initial retention of treatment C and  $\Delta\text{Sol-N}$  by ANOVA with SAS 9.2 and PROC MIXED using a model that contained both fixed (treatment) and random (sample location) effects (Littell et al., 1996; SAS, 1999). The Kenward–Roger (KR) option was used to calculate degrees of freedom while covariance structures were selected to minimize Akaike’s Information Criterion. Multiple pairwise comparisons of means were performed using the Tukey–Kramer method using a value of 5% (i.e.,  $P < 0.05$ ) as the minimum criterion for significance unless otherwise noted. Assumptions of normality were evaluated and appropriate data transformations identified with SAS/ASSIST. Bivariate relationships between treatment effects and several soil properties were characterized by Spearman correlations ( $\rho$ ), using PROC CORR. Compared to the Pearson corre-

lation, Spearman correlations do not assume bivariate normality and are less biased by outliers. Significant deviation of  $\Delta\text{Sol-N}$  and  $\Delta\text{CEC}$  from zero, indicative of a meaningful change in the extraction of soluble N or cation exchange capacity due to the treatment, was determined by the LSMEANS statement in PROC MIXED. Values indicated in text and graphs are the arithmetic mean,  $\pm$  the standard error of the mean, expressed on oven-dry soil basis.

## RESULTS AND DISCUSSION

### Retention of Treatment Carbon

The control treatment (water) extracted an average  $297 \pm 23 \text{ mg kg}^{-1}$  soluble C from soils. In contrast, differing amounts of C added in treatment solutions were retained by treated soils ( $P \leq 0.001$ ) resulting in net increases to soil C (Table 3). The greatest retention, observed for PGG, accounted for more than 70% of the C added in the treatment solution. Soils retained about 33% of procyanidin from sorghum. The lowest retentions were observed for non-tannin phenolic compounds, gallic acid, methyl gallate, and catechin, equivalent to 8, 12, and 17% of added treatment C, respectively (Table 3). Retention of all treatment compounds was positively correlated with soil organic C, total N, silt, clay, and CEC and negatively related to sand. Soil pH was negatively correlated with retention of gallic acid but positively correlated with retention of tannic acid and PGG. Soil pH, however, was unrelated to retention of methyl gallate, catechin, or procyanidin. Electrical conductivity was positively correlated with retention of catechin, procyanidin, tannic acid, and PGG but not related to retention of gallic acid or methyl gallate (Table 3).

Patterns of treatment retention observed across a broad geographical range of soil types and management were consistent with those observed earlier for West Virginia forest and pasture soils and for Oregon soils amended with various kinds of organic matter (Halvorson et al., 2012a, 2011). Retention of tannins and related non-tannin compounds in soil has been linked to compound polarity, size, and molecular weight with the greatest retention by larger, non-polar compounds (Halvorson et al., 2011;

**Table 3. Retention of treatment C† following a 1 h shake at room temperature (23°C) and Spearman correlation coefficients ( $\rho$ ) with selected soil attributes.**

Treatment	Retention of treatment C		Total organic soil C	Total soil N	Sand	Silt	Clay	pHw	EC‡	CEC
	mg kg <sup>-1</sup> soil	% of the total added								
			—Spearman correlation coefficients ( $\rho$ )—							
Gallic acid	406 (38) F	7.9 (0.7)	0.72***	0.70***	-0.58***	0.50***	0.40***	-0.42***	0.05	0.41***
Methyl gallate	639 (39) E	12.3 (0.7)	0.70***	0.72***	-0.51***	0.43***	0.39**	-0.14	0.22	0.53***
Catechin	1025 (51) D	16.6 (0.8)	0.73***	0.78***	-0.75***	0.67***	0.48***	-0.20	0.29*	0.58***
Procyanidin	1583 (71) C	32.9 (1.5)	0.64***	0.71***	-0.56***	0.41***	0.52***	-0.09	0.32**	0.41***
Tannic acid	2576 (111) B	52.4 (2.2)	0.41***	0.51***	-0.53***	0.42***	0.43***	0.32**	0.51***	0.57***
PGG	3579 (125) A	70.7 (2.4)	0.46***	0.56***	-0.53***	0.44***	0.43***	0.29*	0.53***	0.63***

\* Significant at the 0.05 level.

\*\* Significant at the 0.01 level.

\*\*\* Significant at the 0.001 level.

† Treatment solutions consisted of a water control or supplied 10 mg of gallic acid, methyl gallate, catechin, procyanidin tannin from sorghum, tannic acid or  $\beta$ -1,2,3,4,6-penta-galloyl-*O*-D-glucose (PGG) g<sup>-1</sup> soil. Data are the arithmetic averages (standard error) of all samples ( $n = 66$ ).

Treatment differences in retention of treatment C were determined by ANOVA and are delineated by letter.

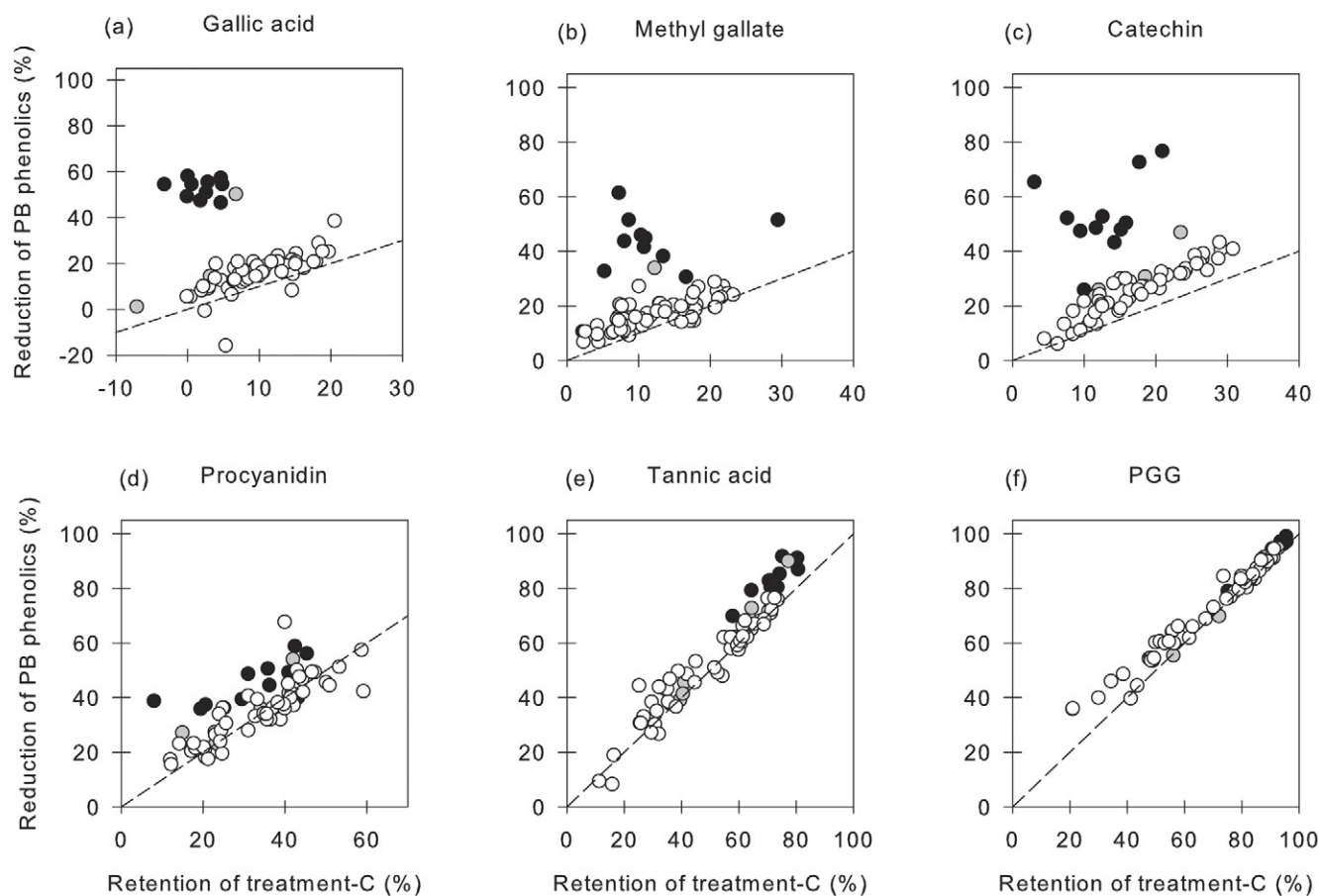
‡ EC = electrical conductance; CEC = cation exchange capacity.

Schmidt et al., 2012). Sorghum procyanidin is a polar extended molecule with high molecular mass compared to the smaller, hydrophobic and disk-like PGG (Table 2; Feldman and Smith, 1996; Hagerman et al., 1998; Kondo et al., 2006). Sorption of hydrophobic compounds is correlated with the amount of soil organic matter (e.g., Karickhoff et al., 1979; Luthy et al., 1997) but also affected by the composition of organic matter (e.g., Chen et al., 2009; Xing, 1997). Based on the importance of hydrophobic and hydrogen bonding interactions between polyphenols and biomolecules such as proteins (Hagerman, 2012) we speculate that the affinities of polyphenols for soils might involve similar interactions. Under favorable conditions of pH, ionic interactions between the weakly acidic polyphenols and soils might be important. Weak forces such as Van der Waals interactions could also contribute to soil-polyphenol interactions. The affinity of soil for the very nonpolar PGG may be dominated by hydrophobic interactions but the more polar compounds such as sorghum procyanidin might interact mainly through hydrogen bonding.

Retention has also been related to physical properties of soil such as texture. Using a small number of soils ( $n = 6$ ) Schmidt et al. (2012) found sorption of a simple phenolic, methyl gallate, was strongly related to soil organic matter but it was best modeled as a function of silt and sand composition. Conversely the sorption of a more complex polyphenol was best modeled as a

positive function of sand, silt, clay, and pH. The results of this study, however, suggest retention of treatment C is increased in the presence of silt and clay (Table 3). In comparison to sand, silt and clay fractions possess greater specific surface area and usually contain greater amounts of metal oxides (particularly iron) which are important for microaggregation and stabilization of soil organic matter and may increase the sorption of tannins via formation of tannin-metal complexes (Kaal et al., 2005; Pronk et al., 2011).

Estimates of retention of treatment C, determined with Eq. [1], generally matched the disappearance of PB reactive phenolics from treatment supernatants, especially for procyanidin, tannic acid, and PGG (Fig. 2d–2f). We also observed close agreement between retention of treatment C and disappearance of PB reactive phenolics for all treatment compounds in our earlier studies conducted on soils with pH ranging from 4.0 to 5.3 (Halvorson et al., 2012a; 2011). However, disproportionately high losses of PB phenolics together with low retention of treatment C were observed for non-tannin phenolic compounds, gallic acid, methyl gallate, and catechin in soil samples with a pH higher than 8 (Fig. 2a–2c). Phenolics are not stable at high pH, so loss of phenolics from our system could be explained by chemical decomposition. The rate and pH dependence of decomposition may vary for different polyphenolics depending on chemi-



**Fig. 2.** The relationship between retention of treatment C and reduction of total Prussian Blue (PB) phenolics in supernatants (% of added, Table 2). Open symbols indicate a starting soil pH < 7.5 while those in gray and black indicate a starting pH between 7.5 and 8.0 and >8.0, respectively. The dashed line indicates a slope of 1:1.

cal structure and position of substituents such as the phenolic hydroxyl groups. Friedman and Jürgens (2000) found that gallic acid and catechin were decomposed at high pH with different stabilities for the two compounds that may be related structural variations. The similarity between the amount of treatment C retained and disappearance of PB phenolics observed for tannins reflects the role of both pH and chemical nature of the sorptive surface in the sorption–desorption experiments. We hypothesize there is a complicated interplay between sorption and decomposition of natural phenolics in soil systems.

### Treatment Effects on Extraction of Soluble Nitrogen

The control treatment (water) extracted an average  $40.7 \pm 3.2 \text{ mg kg}^{-1}$  soluble N from soils, an amount similar to that extracted with the tannic acid or procyanidin treatments (Table 4). In contrast, the methyl gallate and, to a lesser extent, catechin treatments extracted more soluble N from samples than the control; gallic acid, and PGG extracted less. Reductions in soluble N could be attributed to formation of complexes with proteins (cf. Knicker, 2004; Kraus et al., 2003a; Rillig et al., 2007) or other N-containing compounds (Adamczyk et al., 2011, 2013) influenced by specific tannin chemistry (Coq et al., 2010; Kraus et al., 2003b; Mutabaruka et al., 2007) and the quantity and quality of soil N (Talbot and Finzi, 2008).

The  $\Delta\text{Sol-N}$  (Eq. [3]) for methyl gallate and catechin treatments was strongly positively correlated with several soil variables including retention of treatment C, total organic soil C and N, silt, and EC and negatively correlated with the amount of sand and soil pH (Table 4). In addition,  $\Delta\text{Sol-N}$  for catechin was positively correlated with clay content and CEC. Average  $\Delta\text{Sol-N}$  for gallic acid was positively correlated with silt content and negatively correlated with soil pH. The  $\Delta\text{Sol-N}$  for PGG was correlated to soil texture, positively for silt and clay and negatively with sand. Average  $\Delta\text{Sol-N}$  for the tannic acid treatment was correlated to the same soil variables observed collectively for gallic acid and PGG, significantly positive with silt and clay content but negatively with sand and pH. Tannic acid is a het-

erogeneous mixture containing gallic acid and gallates including PGG. Average  $\Delta\text{Sol-N}$  for procyanidin was negatively correlated with a single soil variable, pH.

Correlation coefficients were useful for describing the relationship between pairs of variables and provided additional insight to the apparent lack of effect of tannic acid or procyanidin on soluble N suggested by the univariate averages. The average  $\Delta\text{Sol-N}$  for tannic acid and procyanidin treatments did not differ from 0, implying that these treatments did not affect the solubility of soil N, yet the  $\Delta\text{Sol-N}$  for both of these compounds was negatively correlated with soil pH. Such a pattern indicates they extracted more N than the water control when soil pH was low and conversely, extracted less N from soil than the water control when soil pH was high. Scatter plots indicated that the pH at which this switch occurred was between 6 and 7 (data not shown). Because pH can affect the formation of tannin-protein complexes (Hagerman, 2012; Hagerman and Butler, 1981; Kraus et al., 2003a), we postulate that at low pH, tannins may form soluble tannin protein complexes. At high soil pH we speculate that tannins immobilize N by forming stable tannin-protein complexes that remain tightly soil-bound.

### Response to the Hot Water Incubation

Incubation in hot water, following the initial treatments, extracted about  $540 \text{ mg kg}^{-1}$  C from control samples (Fig. 3). Similar quantities of soluble C were extracted from samples previously treated with gallic acid, procyanidin, and methyl gallate, indicating no significant desorption of treatment C or treatment effects on soluble pools of soil organic matter. Conversely, the quantity of soluble C extracted from samples treated with catechin, tannic acid, or PGG exceeded the control by about 150 to  $180 \text{ mg of C kg}^{-1}$  soil, suggesting release of weakly retained treatment C. These releases were equivalent to 18, 7, and 4%, of retained treatment C for catechin, tannic acid, and PGG respectively (Table 3).

The hot water incubation also extracted about  $54 \text{ mg of N kg}^{-1}$  from control samples, an amount greater than

**Table 4. Treatment effects† on  $\Delta\text{Sol-N}\ddagger$  following a 1 h shake at room temperature (23°C) and Spearman correlation coefficients ( $\rho$ ) with selected soil attributes.**

Treatment	$\Delta\text{Sol-N}$ mg kg <sup>-1</sup> soil	Retention of treatment C	Total organic soil C	Total soil N	Sand	Silt	Clay	pHw	EC	CEC
		—Spearman correlation coefficients ( $\rho$ )—								
Gallic acid	-4.1 (0.6) D	0.05	0.07	0.03	-0.23	0.34**	-0.12	-0.38**	0.03	-0.12
Methyl gallate	8.5 (0.9) A	0.41***	0.68***	0.69***	-0.51***	0.49***	0.23	-0.52***	0.34**	0.17
Catechin	2.1 (0.5) B	0.35**	0.32**	0.32**	-0.52***	0.50***	0.34**	-0.35**	0.39**	0.28*
Procyanidin§	0.3 (0.4) C	-0.02	0.21	0.17	-0.17	0.14	0.14	-0.64***	0.09	-0.14
Tannic acid§	-0.8 (0.6) C	-0.19	0.21	0.18	-0.37**	0.32**	0.34**	-0.48***	0.18	0.10
PGG	-3.4 (0.6) D	-0.23	-0.06	-0.09	-0.36**	0.30*	0.36**	-0.17	0.14	0.17

\* Significant at the 0.05 level.

\*\* Significant at the 0.01 level.

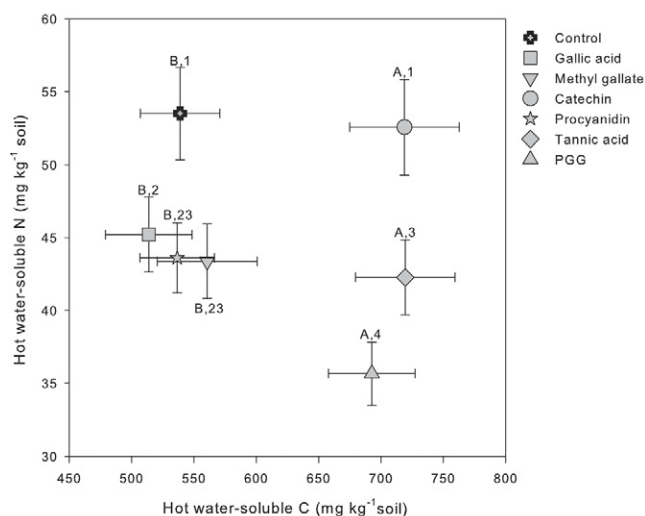
\*\*\* Significant at the 0.001 level.

† Treatment solutions consisted of a water control or supplied 10 mg of gallic acid, methyl gallate, catechin, procyanidin tannin from sorghum, tannic acid or  $\beta$ -1,2,3,4,6-penta-galloyl-O-D-glucose (PGG) per g soil. Data are the arithmetic averages (standard error) of all samples ( $n = 66$ ). Treatment differences in retention of treatment C were determined by ANOVA and are delineated by letter.

‡ As calculated with Eq. [3].

§ Not significantly different from the water Control.





**Fig. 3. Soluble C and N extracted with a 16 h hot water (80°C) incubation (Mean, SEM,  $n = 66$ ). Treatment differences for soluble C (denoted by letters) and N (denoted by number) were determined for transformed data with Tukey's HSD ( $P < 0.05$ ).**

the initial extraction with cool (23°C) water, and similar to the catechin treatment (Fig. 3). Significant reductions in hot water extractable-N were observed for all other treatments ranging from about  $-8 \text{ mg N kg}^{-1} \text{ soil}$  for gallic acid to  $-18 \text{ mg N kg}^{-1} \text{ soil}$  PGG. While the soluble N extracted with cool water or treatment solutions includes both inorganic and organic N, the N in the hot water extract is likely to be primarily composed of unspecified organic forms of N (Curtin et al., 2006; Ghani et al., 2003; Halvorson et al., 2009; Leinweber et al., 1995). Less hot-water soluble N following treatment with all of the compounds except catechin suggests they increased the ability of organic N to resist extraction by forming chemical or physical complexes with the soil matrix. We believe that unique features of the catechin structure or reactivity are responsible for its low activity in the hot water extractions.

### Analysis by Soil Order

Our initial attempts to identify a few simple explanatory relationships for treatment effects were hampered by simultaneous significant linear correlations with a number of related soil properties suggesting multicollinearity (Tables 3 and 4). We were also unable to uncover significant treatment effects using several self-defined, subjective, data stratification schemes based on management, such as cropped vs. uncropped, or on relative cultivation disturbance (more vs. less). However, a number of significant patterns emerged when treatment effects were analyzed by soil order. Soil order is a well-established taxonomic concept that provided a clearly defined and geo-referenced scheme for distinguishing among our samples based on an objective rationale. Each soil order represents a unique combination of properties that integrates parent material, vegetation, climate, soil moisture regime, diagnostic horizons, and biological chemical and physical processes (Jenny, 1941; Soil Survey Staff, 1999). We therefore determined treatment effects on  $\text{Trt-C}_{\text{final}}$ , and  $\Delta\text{Sol-N}$ , and

$\Delta\text{CEC}$  after the hot-water extraction, using an ANOVA model that included fixed effects of treatment and soil order.

### Final Retained Treatment Carbon

Final retention of treatment C after the hot water treatment ( $\text{Trt-C}_{\text{final}}$ ), varied strongly with treatment (T) and soil order (S) but also exhibited a  $T \times S$  interaction (all  $P \leq 0.001$ , Table 5). Treatment effects were arranged similarly within each soil order with values for PGG > tannic acid > procyanidin > catechin > methyl gallate > gallic acid. Among soil orders, retention was greatest in Alfisols, Aridisols, and Mollisols, the groups that contained relatively large amounts of soil C, N, and silt and was lowest in poorly developed (Entisols) or degraded (Ultisols), soils characterized by relatively low amounts of soil organic material or largely comprised of sand (Table 1). The  $T \times S$  interaction showed that retention of treatment C varied among the different soil orders only for strongly retained compounds, especially tannins (Table 5). Significant differences in  $\text{Trt-C}_{\text{final}}$  among soil orders could be detected only for PGG, tannic acid, procyanidin, and catechin. Alfisols, Aridisols, and Mollisols retained 76, 84, and 71% of the PGG treatment, compared to only 49% by Ultisols. Tannic acid displayed a similar pattern. Sorghum procyanidin was retained more in Alfisols than Entisols, while more catechin was retained in Alfisols than Ultisols. Conversely, soil characteristics had little influence on poorly retained compounds. Only small amounts of the applied gallic acid or methyl gallate remained in soil, about 8 and 12%, respectively, and did not vary among the soil orders.

The main effects of treatment on  $\text{Trt-C}_{\text{final}}$  were confirmed by direct measurements of total soil C (Fig. 4a). Based on previous studies (Halvorson et al., 2012a) we expected changes in soil C to be the net result of the C added by the treatments balanced against losses of soluble C. However the actual measured change of soil C in control samples, a loss of  $2418 \pm 425 \text{ mg kg}^{-1} \text{ soil}$ , was greater than could be attributed to solely to extraction of water-soluble C,  $836 \pm 51 \text{ mg kg}^{-1} \text{ soil}$  (Table 5) and a similar disparity between actual and expected soil C was also observed for the other treatments. The average disparity between actual and expected soil C observed for all treatments as well as the control ( $1779 \pm 160 \text{ mg kg}^{-1} \text{ soil}$ ,  $n = 460$ ) suggests a common source for unintentional losses of soil C associated with sample handling, perhaps during the decanting step. Assuming similar C losses from all samples due to handling, we accounted for these errors by subtracting the total C in control samples from the amount in treated samples and found we found good agreement with  $\text{Trt-C}_{\text{final}}$ , confirming meaningful amounts of each compound remained on soil C (Fig. 4b, Table 5; also Fig. 5b in Halvorson et al., 2011).

### Final Effects on Soluble Nitrogen

Net change in soluble N after treatments and hot water incubation,  $\Delta\text{Sol-N}$ , varied with treatment ( $P < 0.001$ ) but also exhibited a significant  $T \times S$  interaction ( $P < 0.01$ ) (Table 5). Main effects indicated that extraction of soluble N was not sig-

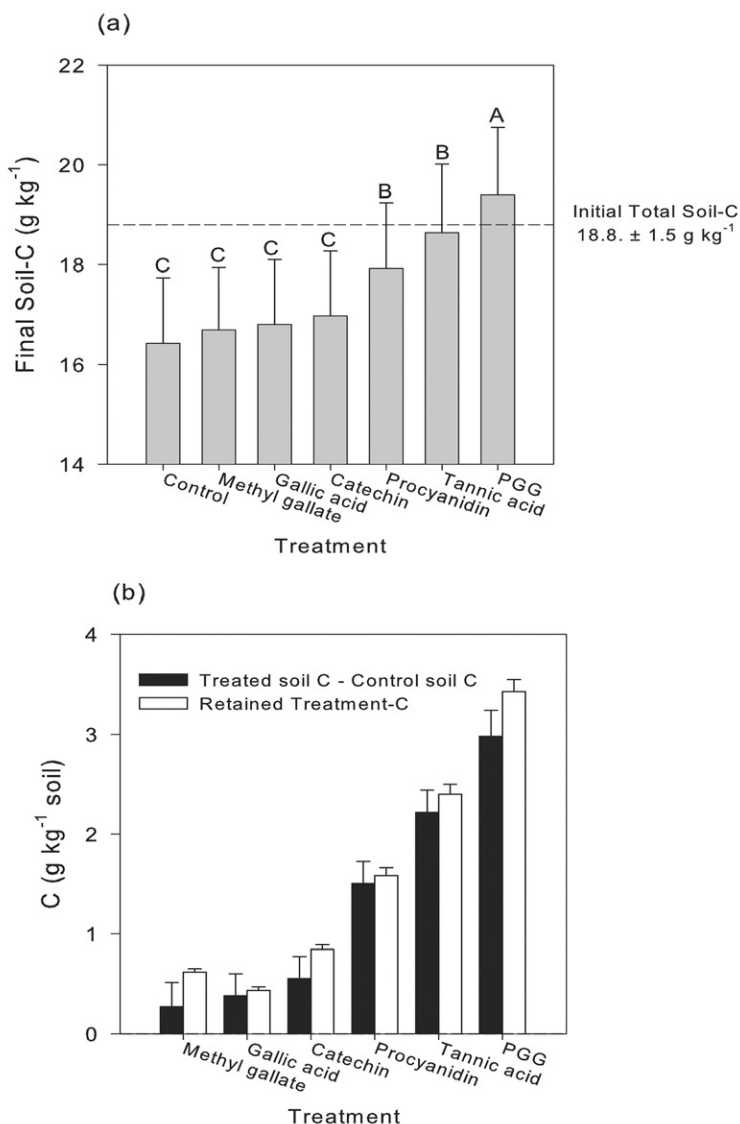
**Table 5. Final response to treatment solutions† following a 16 h water incubation at 80°C.**

Soil order	Retained treatment C§												ΔSol-N§												ΔCEC§					
	Control Soluble C				Mean Control Soluble N				Mean Control Soluble N				Mean Control Soluble N				Control CEC				Mean (nx6)				Mean (nx6)					
	GA	MG	CAT	PRO	TA	PGG	TA	PGG	GA	MG	CAT	PRO	TA	PGG	TA	PGG	CEC	CEC	GA	MG	CAT	PRO	TA	PGG	TA	PGG	TA	PGG		
Alfisols (n = 10)	692 (105) a5	778 (91) a5	1280 (143) a4	2131 (219) a3	2840 (263) a2	3872 (374) a1	1932 (173) X	96.8 (15.4)	-13.2 (5.0) a2	3.27 (2.71) a1	0.53 (2.29) a1	-10.2 (3.8) a2	-10.4 (4.3) a2	-21.2 (5.2) a2	-8.53 (1.91)	11.1 (1.5)	11.1 (1.5)	-0.40 (0.34) a2	-0.34 (0.71) a2	0.14 (0.38) a12	0.84 (0.29) a12	1.10 (0.33) a12	1.97 (0.51) ab1	1.10 (0.33) a12	1.97 (0.51) ab1	0.56 (0.21)				
Aridisols (n = 5)	242 (97) a4	808 (206) a3	907 (138) ab3	2045 (108) ab2	3258 (103) a1	4255 (271) XY	1919 (19.5)	72.3 (19.5)	-15.6 (3.9) a12	-14.5 (2.8) a12	-12.1 (3.2) a1	-22.1 (3.5) a12	-20.1 (2.7) a2	-31.3 (5.8) a2	-19.3 (1.8)	14.3 (1.7)	14.3 (1.7)	-0.40 (0.43) a3	1.75 (0.43) a23	-0.22 (0.57) a3	0.81 (0.89) a3	4.09 (1.03) a12	6.26 (0.73) a1	4.09 (1.03) a12	6.26 (0.73) a1	2.05 (0.52)				
Entisols (n = 6)	287 (93) a3	431 (59) a3	582 (106) ab23	1025 (178) b2	2185 (304) ab1	2901 (184) YZ	1235 (8.1)	57.2 (8.1)	-11.2 (2.8) a1	-4.68 (5.20) a1	-4.56 (5.53) a1	-6.92 (4.38) a1	-12.4 (5.8) a1	-15.8 (2.03)	-9.27 (2.03)	13.7 (1.9)	13.7 (1.9)	-1.04 (0.93) a2	0.47 (0.88) a12	-0.07 (0.99) a12	0.99 (0.80) a12	1.46 (0.63) a12	2.75 (0.69) ab1	1.46 (0.63) a12	2.75 (0.69) ab1	0.76 (0.37)				
Mollisols (n = 33)	472 (48) a5	669 (41) a4	857 (55) ab4	1581 (92) ab3	2473 (129) a2	3617 (146) a1	1611 (88) XY	105.7 (8.6)	-12.7 (1.8) a2	-0.78 (1.52) a1	4.40 (1.25) a2	-9.64 (2.04) a2	-10.9 (1.9) a2	-21.0 (2.1) a3	-8.43 (0.93)	15.4 (1.0)	15.4 (1.0)	-0.02 (0.57) a2	0.84 (0.54) a12	0.01 (0.46) a2	1.36 (0.45) a1	0.77 (0.44) a12	1.68 (0.51) b1	0.77 (0.44) a12	1.68 (0.51) b1	0.77 (0.20)				
Ultisols (n = 12)	253 (62) a4	356 (40) a34	557 (60) b3	1232 (135) ab2	1557 (166) b2	2445 (105) Z	1067 (9.5)	87.9 (9.5)	-10.0 (3.7) a23	-1.06 (2.14) a12	1.13 (2.11) a1	-5.21 (1.11) a123	-12.9 (2.1) a34	-20.6 (2.5) a4	-8.10 (1.29)	3.2 (0.4)	3.2 (0.4)	-0.65 (0.16) a1	-0.17 (0.12) a1	-0.02 (0.10) a1	0.11 (0.11) a1	-0.08 (0.15) a1	0.58 (0.15) b1	-0.08 (0.15) a1	0.58 (0.15) b1	-0.04 (0.07)				
Mean (n = 66)	431 (37) F	617 (35) E	845 (47) D	1585 (75) C	2396 (102) B	3426 (123) A	94.2 (5.6)	94.2 (5.6)	-12.4 (1.4) B	-1.61 (1.17) A	1.16 (1.11) A	-9.61 (1.34) B	-12.0 (1.3) B	-21.2 (1.5) C	-8.10 (1.29)	12.3 (0.8)	12.3 (0.8)	-0.31 (0.30) E	0.51 (0.31) CD	-0.00 (0.25) DE	0.98 (0.25) BC	0.98 (0.27) B	1.97 (0.32) A	0.98 (0.27) B	1.97 (0.32) A	0.76 (0.37)				

**ANOVA**

Source of variation	df	Retention of treatment C¶	ΔSol-N#	ΔCEC ††
Treatment (T)	5	***	***	***
Soil order (S)	4	***	NS †	NS
T × S	20	***	**	***

\*\*\* Significant at the 0.01 level.  
 \*\* Significant at the 0.001 level.  
 † NS = not significant.  
 ‡ Treatment solutions consisted of a water control or supplied 10 mg of gallic acid (GA), methyl gallate (MG), catechin (CAT), procyanidin tannin from sorghum (PRO), tannic acid (TA) or β-1,2,3,4,6-penta-galloyl-D-glucose (PGG) per g soil. Data are arithmetic averages (standard error).  
 § CEC = cation exchange capacity; Main effects of treatment (column averages, n = 66) or soil order (row averages, n = 66) are denoted by capital letters. For interactions, differences among soil orders within each treatment are denoted by lowercase letters while differences among treatments are denoted by numbers (Tukeys HSD, P ≤ 0.05).  
 ¶ Calculated with Eq. [2]. Data were square root-transformed before ANOVA.  
 # Calculated with Eq. [3]. Data were power-transformed (1.5) before ANOVA.  
 †† Calculated with Eq. [4].



**Fig. 4.** (a) Main treatment effects on total soil C. Differences among treatments are denoted by letters (Mean, SEM,  $n = 66$ ,  $P \leq 0.05$ , Tukey's HSD) and (b) control corrected changes in total soil C (treated sample soil C minus control sample soil C) compared to final sorption from Table 5 (Mean, SEM,  $n = 66$ ).

nificantly affected by the methyl gallate or catechin treatments but was decreased by the other treatments and especially by PGG. Treatment with PGG decreased soluble N by more than 20% in Mollisols to about 45% in Aridisols, equivalent to about 30 to 40 kg of soluble N ha<sup>-1</sup> in the 0- to 10-cm depth, assuming a soil bulk density of 1.3 Mg m<sup>-3</sup>. Main treatment effects on values of  $\Delta\text{Sol-N}$  reflect the net sum of soluble N in treatment supernatants (Table 4) and extracted by the hot water incubation (Fig. 3). In the case of catechin, large differences from the water control did not result after either cool treatment step or subsequent hot extraction; consequently we conclude it does not interact with soil N. However, as observed in earlier work (Halvorson et al., 2012a, 2012b), methyl gallate increased extraction of soluble N in treatment supernatants but subsequently decreased hot water-extractable soluble N, a pattern that implies it increases the efficacy of the initial extraction process. At this time, we do not have sufficient data to identify the mechanisms responsible for this increase of ef-

ficacy but the combined net effect was not different from water. Alternatively, neither procyanidin or tannic acid initially affected soluble N (Table 4) but both compounds decreased soluble N extracted by hot water (Fig. 3) resulting in significantly less final soluble N. Finally, soluble N was decreased by gallic acid and PGG in both treatment supernatants and the hot water extracts. For most treatments, average values of  $\Delta\text{Sol-N}$  were negatively and linearly related to retention of treatment C (Fig. 5a) suggesting a common mechanism for both. However, despite having the lowest retention by soil, gallic acid decreased soluble N to levels similar to tannic acid or PGG. We postulate that the decrease seen with gallic acid could be due, in part, to redox reactions with metals, especially Mn, in the soil (Halvorson et al., 2012b; Hem, 1965; Pohlman and McColl, 1989). Insoluble Mn(IV) could be reduced to the soluble Mn(II) as gallic acid oxidized to quinones and semi-quinones that formed "humic-like" polymers with amino-containing compounds and were retained in the soil matrix. However, this explanation cannot explain the lack of effect seen for catechin since it also reacts with soil metals and forms polymers (Chen et al., 2010).

The  $T \times S$  interaction further revealed that unlike the other soil orders, soluble N was significantly decreased in Aridisols by all of the treatment compounds, including methyl gallate and gallic acid. In addition, significant differences among treatments were observed for most soil orders, especially in Mollisols and Ultisols. Formation of these soils typically results in strong patterns of horizonation. However, treatment effects on  $\Delta\text{Sol-N}$  were relatively unvarying in Entisols, the soil order distinguished by a lack of profile development. This pattern suggests the effects of phenolic compounds on soluble N will vary with the age or composition of soil organic matter, or changes in chemical characteristics and physical properties with depth. Decreasing  $\Delta\text{Sol-N}$  was also linearly related with increasing retention of treatment C for each individual soil order and appeared steepest for Ultisols, intermediate and similar for Alfisols and Mollisols and relatively shallow and similar for Entisols and Aridisols (Fig. 5b). These differences suggest that in addition to the amounts of treatment C retained, reductions in soluble N were influenced by soil properties such as pH, EC, and/or CEC (Table 1). Although Ultisols contained intermediate amounts of organic matter compared to Entisols and Aridisols, they had significantly lower pH, electrical conductivity, and CEC (Table 1). In addition, differences observed among soil orders could reflect variations in the chemical composition of soil organic matter associated with land use or pedogenic lineage. Halvorson et al. (2012b) found phenolic treatments reduced net soluble N in pasture soil significantly more than in soil from adjacent woodlands.

### Changes to Cation Exchange Capacity

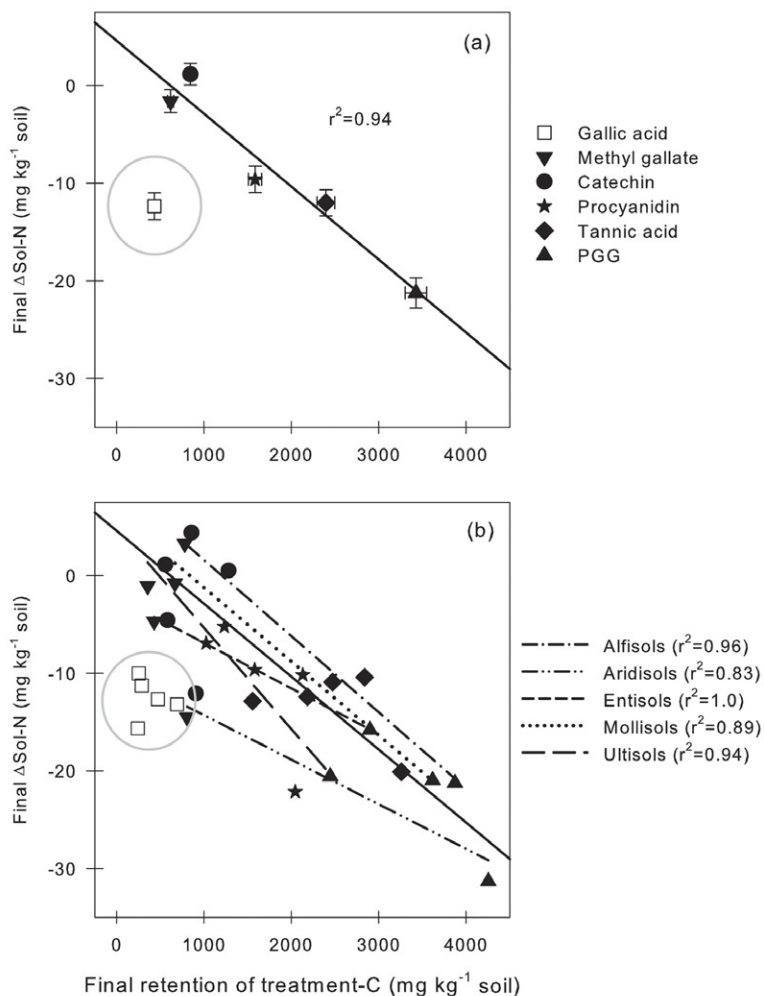
Treatment with water alone had little effect on soil CEC. The average CEC of control samples decreased by about

0.3 cmol<sub>c</sub> kg<sup>-1</sup> soil or 4%, compared to the initial value, 12.6 ± 0.8 cmol<sub>c</sub> kg<sup>-1</sup> soil (Tables 1 and 5). Net treatment effects (ΔCEC), determined with Eq. [4], varied strongly with treatment and as a T × S interaction (*P* ≤ 0.001). The main effects of treatment showed non-tannin phenolic compounds had no impact on soil CEC beyond that seen for the control. The Least Squares Means for ΔCEC, calculated for the gallic acid, methyl gallate, and catechin treatments, were not different from 0 (Table 5). Alternatively, significant net increases in soil CEC were observed for the tannin treatments, averaging about 1 cmol<sub>c</sub> kg<sup>-1</sup> soil for both procyanidin and tannic acid, and about 2 cmol<sub>c</sub> kg<sup>-1</sup> soil for PGG-treated samples or by about 8 and 16%, respectively. Treatment effects on CEC and especially the differences between procyanidin and PGG have been hypothesized to relate to polarity, molecular shape, and additional substituents that contribute in specific ways unique to each compound (Halvorson et al., 2011). Because it is a nonpolar compound, PGG is thought to interact with the soil via hydrophobic interactions, while sorghum procyanidin is a polar compound and probably interacts by hydrogen-bonding mechanisms (Hagerman et al., 1998). Different mechanisms of interaction with the soil may mask existing sites or present new sites for CEC depending on the surface chemistry of the soil and the amount of phenolic compound retained (Halvorson et al., 2011). The positive linear relationship between retention of treatment C and ΔCEC (Fig. 6a) supports a hypothesis that additional exchange sites are formed.

The T × S interaction showed treatment effects on CEC were small and did not vary in Ultisols, whereas PGG increased ΔCEC in all other soil orders. Though the effects of the other treatments were consistent among soil orders, PGG increased CEC more in Aridisols (44%) than in Mollisols (11%) or Ultisols (18%). No treatment differences, observed in Ultisols, may relate to their weathered state, for example, clay mineralogy and pH, and corresponded to a weak relationship between ΔCEC and retention of treatment C. The strongest linear increases in ΔCEC with retained treatment C were observed for Aridisols and Entisols (Fig. 6b).

## CONCLUSIONS

This study corroborated and expanded the results of our earlier limited work and we now generally conclude tannins entering soil are retained to a much greater degree than related phenolic compounds and significantly reduce the solubility of N. Retention may be important on a landscape scale and be linked to patterns of soil organic matter or texture while tannin effects related to nutrient cycling such as increased CEC, immobilization of soil N, or interactions with soil toxicants (Kinraide and Hagerman, 2010) may be of greatest significance in the vicinity of plant roots. Retention of plant phenolics, added by leaching or from plant residues, represents a relatively rapid incorporation of



**Fig. 5. (a) Main effects of ΔSol-N plotted against retained treatment C (Mean, SEM, n = 66) and (b) ΔSol-N plotted against retained treatment C for individual soil orders. Data for gallic acid (circled) were omitted from the linear regressions.**

organic matter to soil. However, little is known about the consequences of microbiological degradation or the chemical transformations that occur once they enter soil, or interactions with other forms of soil organic matter (cf. Kögel-Knabner, 2002; Rovira and Vallejo, 2002). Immobilization of organic soil N by tannins, presumably through the formation of complexes, has not been well studied in agricultural soils and could be a useful means for managing soil fertility providing nutrient uptake by crops or root functions are not inhibited.

That no single soil attribute explained the patterns of tannin retention or decreased N solubility suggests both are influenced by compound specific characteristics such as molecular size, polarity, and configuration and involve several mechanisms. The differences observed among soil orders suggest that the magnitude and consequences of tannin effects will covary across the landscape together with patterns of attributes that reflect soil-forming processes including management. Besides a central conceptual theme, our use of soil orders provides a geo-referenced context with which to develop future research by identifying the distribution of soil types or suites of soil characteristics that would be likely to respond to tannins. In this study, tannin effects were especially evident in



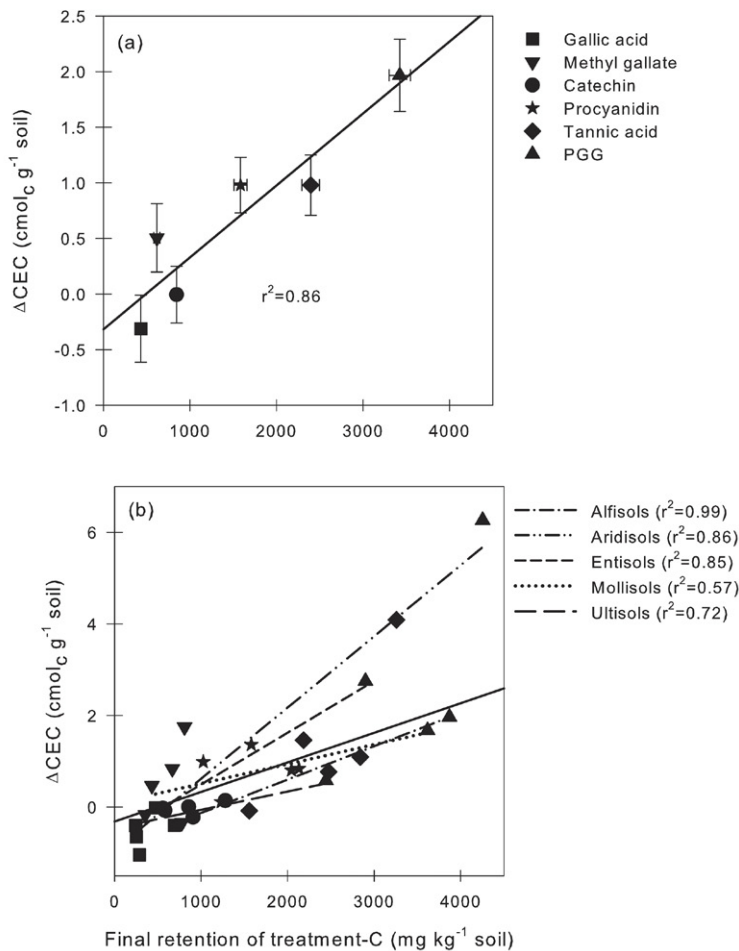


Fig. 6. (a) Main effects of  $\Delta\text{CEC}$  plotted against retained treatment C (Mean, SEM,  $n = 66$ ) and (b)  $\Delta\text{CEC}$  plotted against retained treatment C for individual soil orders.

#### Appendix A. Selected soil properties for individual samples.

Contact†	State/ Province	Lat. (North)/ Long. (West)	Soil order	Management‡	Sand	Silt	Clay	pH	Total	Organic	Total	EC§	CEC
					— % —	— g kg <sup>-1</sup> —	soil C		soil C	soil N			
Acosta-Martinez	TX	33.68/101.77	Mollisol	CT, cotton-sorghum	50	32	18	7.57	4.54	4.55	0.44	169	11.8
Acosta-Martinez	TX	33.68/101.77	Mollisol	CT, continuous cotton	54	28	18	7.38	3.90	3.60	0.43	181	10.5
Acosta-Martinez	TX	33.68/101.77	Mollisol	NT, cotton-sorghum	48	32	20	7.81	5.17	5.15	0.53	192	12.0
Arriaga	AL	32.43/85.89	Ultisol	CT, cotton-corn	75	19	6	6.37	5.44	5.03	0.45	125	1.9
Arriaga	AL	32.43/85.89	Ultisol	CsT., cotton-corn	75	23	2	6.54	7.78	7.27	0.60	75.1	2.2
Arriaga	AL	32.42/85.90	Ultisol	CT, cotton-corn	48	34	18	5.79	8.16	8.03	0.85	171	4.4
Arriaga	AL	32.42/85.90	Ultisol	CT + M, cotton-corn	48	34	18	5.69	12.98	11.89	1.24	419	5.1
Baker.	MN	44.75/93.07	Mollisol	CT, corn-soybean	20	62	18	6.07	22.22	21.52	1.87	319	17.3
Baker.	MN	44.75/93.07	Mollisol	ST, corn-soybean	22	62	16	6.09	27.17	25.29	2.25	351	19.1
Busscher	SC	34.29/79.75	Ultisol	CsT, corn-wheat	66	28	6	7.35	12.43	10.13	1.04	72.8	4.9
Busscher	SC	34.29/79.75	Ultisol	CT, corn-wheat	70	22	8	6.22	9.89	9.28	0.79	68.5	2.7
Emmerich	AZ	31.74/109.94	Entisol	UC, G, grass under trees	75	13	12	8.13	6.54	6.04	0.74	131	9.8
Emmerich	AZ	31.74/109.94	Entisol	UC, G, grass	72	16	12	8.43	12.68	7.80	0.82	203	16.7
Emmerich	AZ	31.74/110.05	Aridisol	UC, G, grass, high CaCO <sub>3</sub>	50	30	20	8.35	64.64	12.94	1.55	203	13.0
Follett	CO	40.32/103.15	Mollisol	NT, wheat-fallow	22	60	18	5.73	11.22	9.88	1.26	407	11.6
Follett	CO	40.32/103.15	Mollisol	CT, wheat-fallow	24	60	16	6.47	7.46	7.06	0.89	281	12.8
Follett	CO	40.32/103.15	Mollisol	CT, continuous corn	24	58	18	5.67	10.73	10.22	1.20	368	11.6
Follett	ID	43.52/111.48	Mollisol	NT, continuous wheat	10	76	14	7.35	19.24	18.60	1.94	571	18.1
Follett	ID	43.52/111.48	Mollisol	CT, continuous wheat	10	76	14	7.98	11.71	10.56	1.25	305	16.8

Continued on next page

Aridisols, the soil order with highest average pH and inorganic C but were lowest in Ultisols characterized by relatively low pH, high amounts of sand and low CEC (Table 1).

Further efforts are needed to extend the results of our studies to supplement the limited information about tannin-soil interactions and to determine their utility at the field scale for achieving specific soil management goals. This will be aided by new methods that can determine the quantity or composition of phenolic plant secondary compounds in soil as well as identification of useful functional classes of tannins and related phenolic compounds. Tannins may be part of management strategies that intensify productivity of sustainable systems (Herrero et al., 2010; Tilman et al., 2011), play a role during transition from one management to another, or as part of efforts to improve or remediate soil ecosystem services (Robinson et al., 2013). However, additional work is needed to devise and evaluate cost effective strategies to incorporate desirable phenolic compounds into agroecosystems. One option might be through selection of crop rotations or pasture forage mixtures able to add useful polyphenolic compounds to the soil directly with residues or indirectly by through livestock feeding. The livestock-soil linkage may be especially attractive if the benefits that dietary tannins may confer to ruminant physiology, such an improvement of N use efficiency (McSweeney et al., 2001; Min et al., 2003), and reduction of greenhouse gas formation (e.g., Eckard et al., 2010; Goel and Makkar, 2012; McAllister et al., 2011) can be married to the emerging research that show how tannins affect the

Appendix A (continued).

Contact†	State/ Province	Lat. (North)/ Long. (West)	Soil order	Management‡	Sand	Silt	Clay	pH	Total soil C	Organic soil C	Total soil N	EC§ $\mu\text{S m}^{-1}$	CEC $\text{cmolc kg}^{-1}$
					— % —	— $\text{g kg}^{-1}$ —							
Follett	ID	43.52/111.48	Mollisol	UC, UG	22	70	8	7.70	37.76	32.73	2.86	488	20.5
Follett	VA	37.52/76.85	Ultisol	CT, corn–sorghum	79	13	8	6.20	6.70	6.02	0.64	78.3	1.7
Follett	VA	37.62/77.11	Ultisol	NT, corn–sorghum	89	7	4	5.91	9.06	8.27	0.80	140	2.3
Follett	NE	41.22/102.98	Mollisol	CT, wheat–fallow	30	54	16	6.99	14.15	14.04	1.45	854	17.7
Follett	NE	41.22/102.98	Mollisol	NT, wheat–fallow	22	58	20	6.70	23.05	22.71	2.17	459	24.1
Franzluebbbers	GA	33.87/83.42	Ultisol	UC, G, inorganic fertilizer	62	28	10	5.95	12.67	11.46	1.12	112	2.9
Franzluebbbers	GA	33.87/83.42	Ultisol	UC, G, broiler litter fert.	68	14	18	5.71	19.34	18.30	1.83	232	4.0
Gollany	OR	45.72/118.63	Mollisol	CT, M, wheat–fallow	26	62	12	7.84	14.07	14.06	1.30	172	16.8
Gollany	OR	45.72/118.63	Mollisol	CT, wheat–fallow	24	64	12	6.65	10.51	10.45	0.82	93.8	12.7
Gollany	OR	45.72/118.63	Mollisol	CT, B, wheat–fallow	28	60	12	6.31	9.57	9.52	0.70	84.3	13.0
Gregorich	ON	45.37/75.73	Mollisol	NT, continuous corn	60	32	8	7.30	18.51	18.09	1.57	339	10.9
Gregorich	ON	45.37/75.73	Mollisol	CT, continuous corn	64	32	4	6.35	19.60	18.02	1.85	462	9.4
Halvorson.	WV	37.83/80.72	Alfisol	UC, G, pasture	40	48	12	5.71	24.44	24.09	2.32	91	6.9
Halvorson.	WV	37.83/80.72	Alfisol	UC, UG, oak trees	36	44	20	4.35	37.42	36.04	2.15	82.4	5.7
Herrick	NM	32.57/106.6	Aridisol	UC, UG	54	38	8	8.40	31.60	7.39	0.94	176	11.1
Herrick	NM	32.57/106.6	Aridisol	UC, UG	56	36	8	8.38	35.53	9.48	1.11	187	10.9
Koehn	ID	42.52/114.38	Aridisol	CT, corn rotation	18	68	14	8.43	18.51	8.26	1.06	396	18.5
Koehn	ID	42.52/114.38	Aridisol	as above w/compost	20	68	12	8.44	32.66	16.35	1.83	571	18.2
Laird	IA	42.02/93.76	Mollisol	CT, corn–soybean	40	40	20	6.34	25.23	23.38	2.05	271	22.8
Laird	IA	42.02/93.76	Mollisol	NT, corn–soybean	46	36	18	6.19	21.15	19.49	1.78	184	17.0
Lerch	MO	39.23/92.12	Alfisol	CsT, corn–soybean	10	62	28	6.96	13.14	12.56	1.32	257	16.7
Lerch	MO	39.23/92.12	Alfisol	NT, corn–soybean	12	58	30	6.53	13.27	13.02	1.45	297	18.2
Lerch.	MO	39.23/92.12	Alfisol	UC, UG	14	66	20	7.21	20.32	17.90	1.79	250	14.0
Liebig	ND	46.77/100.92	Mollisol	G, crested wheatgrass	36	52	12	5.50	39.04	37.15	3.69	203	17.5
Liebig	ND	46.77/100.92	Mollisol	G, native, heavily grazed	40	52	8	6.68	47.99	45.96	3.95	182	23.1
Liebig	ND	46.77/100.92	Mollisol	G, native, mod, grazed	34	58	8	6.31	43.74	42.19	3.65	156	20.6
McLain	AZ	31.81/110.89	Entisol	UC, UG	62	28	10	8.64	6.54	3.74	0.57	863	10.5
McLain	AZ	31.81/110.89	Entisol	UC, G	64	28	8	8.51	8.94	7.55	0.77	300	10.4
Morgan	WY	40.82/104.77	Mollisol	UC, G	70	18	12	6.41	12.93	11.58	1.13	120	10.0
Morgan	WY	40.82/104.77	Mollisol	UC, UG	66	24	10	6.29	26.57	23.74	2.03	142	10.7
Pote	AR	35.07/93.99	Ultisol	UC, G, grass	38	54	8	5.95	18.40	16.54	1.74	96.7	5.5
Pote	AR	35.07/93.99	Ultisol	UC, G, loblolly pine	38	54	8	5.10	15.49	16.83	1.36	62	3.8
Sanderson, M.	PA	40.72/77.94	Alfisol	CT, corn rotation	20	54	26	6.59	15.79	15.97	1.53	178	9.7
Sanderson, M.	PA	40.72/77.94	Alfisol	UC, big bluestem	24	50	26	6.08	23.46	23.07	1.90	128	9.8
Sistani	KY	36.56/86.28	Alfisol	CT, continuous corn	8	64	28	7.15	11.34	11.45	1.24	142	11.9
Sistani	KY	36.56/86.28	Alfisol	as above w/poultry litter	10	64	26	6.78	17.78	17.11	2.31	700	14.1
Smith, D	IN	40.30/86.90	Mollisol	CT, continuous corn	20	58	22	6.97	21.00	20.41	1.88	319	15.2
Smith, D	IN	40.30/86.90	Mollisol	NT, sorghum sudangrass	54	36	10	6.88	27.44	26.02	2.25	84	10.0
Smith, D	IN	40.50/87.00	Mollisol	CT, continuous corn	22	52	26	6.07	31.03	28.59	2.15	205	25.0
Smith, D	IN	40.50/87.00	Mollisol	CT, M, continuous corn	22	50	28	6.49	28.75	27.38	2.41	553	26.5
Smith, J	WA	46.76/117.19	Mollisol	CT, continuous wheat	20	70	10	5.42	32.19	31.35	2.56	471	13.1
Smith, J	WA	46.76/117.19	Mollisol	NT, continuous wheat	22	64	14	5.06	28.21	26.82	2.19	328	11.8
Steenwerth	CA	38.53/121.79	Entisol	CsT, vineyard	48	38	14	7.08	9.83	8.70	0.94	199	18.9
Steenwerth	CA	38.53/121.79	Entisol	CsT, vineyard berm	52	34	14	7.28	7.54	6.74	0.86	256	18.0
Zibilski	TX	26.43/97.95	Alfisol	CT, vegetables	84	10	6	7.02	3.53	3.00	0.36	124	5.0
Zibilski	TX	26.22/97.99	Mollisol	CT, vegetables	54	22	24	8.67	14.47	7.54	0.72	315	14.6
Zibilski.	TX	26.14/97.96	Mollisol	CT, grapefruit	50	16	34	8.56	19.16	8.78	0.96	245	20.7

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‡ CT, conventional tillage leaving <30% residue cover; CsT, conservation tillage leaving >30% residue cover; NT, no-tillage; M, manure added; ST, strip tillage; UC, uncropped; UG, ungrazed; B, residue burned off.

§ EC = electrical conductance; CEC = cation exchange capacity.

composition and characteristics of animal waste (Powell et al., 2011; Powell and Broderick, 2011).

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