

Article

Sorption of Tannin and Related Phenolic Compounds and Effects on Extraction of Soluble-N in Soil Amended with Several Carbon Sources

Jonathan J. Halvorson ^{1,*}, Hero T. Gollany ², Ann C. Kennedy ³, Ann E. Hagerman ⁴,
Javier M. Gonzalez ¹ and Stewart B. Wuest ²

¹ USDA-ARS, Appalachian Farming Systems Research Center, 1224 Airport Road, Beaver, WV 25813-9423, USA; E-Mail: Javier.Gonzalez@ars.usda.gov

² USDA-ARS, Pendleton, OR 97810, USA; E-Mails: Hero.Gollany@ars.usda.gov (H.T.G.); Stewart.Wuest@ars.usda.gov (S.B.W.)

³ USDA-ARS, Pullman, WA 99164, USA; E-Mail: Ann.Kennedy@ars.usda.gov

⁴ Miami University, Oxford, OH 45056, USA; E-Mail: hagermae@muohio.edu

* Author to whom correspondence should be addressed; E-Mail: Jonathan.Halvorson@ars.usda.gov; Tel.: +304-256-2858; Fax: +304-256-2921.

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Abstract: Some tannins sorb to soil and reduce soluble-N. However, we know little about how they interact with organic amendments in soil. Soil (0–5 cm) from plots, which were amended annually with various carbon substances, was treated with water (control) or solutions containing tannins or related phenolic subunits. Treatments included a proanthocyanidin, catechin, tannic acid, β -1,2,3,4,6-penta-*O*-galloyl-D-glucose (PGG), gallic acid, and methyl gallate. We applied solutions of each of these materials to soil and measured soluble-C and -N in supernatants after application and following extraction with hot water (16 h, 80 °C). Sorption was low for non-tannin phenolics, methyl gallate, gallic acid, and catechin, and unaffected by amendment. Sorption of tannins, proanthocyanidin, tannic acid, and PGG, was higher and greater in plots amended with biosolids or manure. Extraction of soluble-N was not affected by amendment or by catechin, proanthocyanidin, or methyl gallate, but was reduced with PGG, tannic acid and gallic acid. Soil cation exchange capacity increased following treatment with PGG but decreased with gallic acid, irrespective of amendment. Tannins entering soil may thus influence soil organic matter

dynamics and nutrient cycling but their impact may be influenced by the composition of soil organic matter.

Keywords: tannins; C-sorption; soil organic matter; amendments; soluble-N; CEC

1. Introduction

Tannins are a common, but highly diverse, class of plant secondary phenolic compounds that undergo complex abiotic and biotic reactions and transformations in soil. These interactions include the formation of complexes, chelation, and oxidation/ reduction reactions together with their direct and indirect influences on soil biota (e.g., [1–3]). Tannins, and related phenolics, may thus couple primary productivity to biogeochemical cycles and regulate soil ecosystem processes such as formation of soil organic matter and nutrient cycles.

Some tannins and related phenolic compounds can rapidly form complexes with the mineral or organic fractions of soil (e.g., [4–7]) resulting in net gains of phenolic-C in the soil matrix and reductions in the extractable organic-N [8,9]. Tannins and other phenolics can also affect the activity or composition of extracellular soil enzymes [10,11] or glycoproteins [12]. Phenolic compounds are also known to rapidly mobilize or solubilize soil metals such as Ca, Al, and Fe probably through chelation and oxidation/reduction reactions, which have important environmental and agricultural implications [13]. Interactions between metals and phenolic compounds may variously affect plant growth. For example, tannic acid has been reported to reduce the rate of root growth by itself but has also been shown to mitigate the toxic effects of some metals [14].

Beyond initial chemical reactions, tannins affect decomposition and nutrient cycling by their direct and indirect effects on numbers, diversity, and functioning of soil biota that reduce mineralization by increasing immobilization in microbial biomass [8,15,16]. Interactions between soil microorganisms and tannins appear complex and vary among different taxonomic groups [17–19], which can be influenced by the characteristics of the tannins [20–23], availability of alternative substrates [24], or even plant genetics [25–29].

Despite this knowledge, more information is needed about the mechanisms of tannin-soil interactions that could be used to manage the quantity and quality of soil organic matter (SOM) and nutrient cycling in agricultural systems. Our previous work established that some tannins could react quickly with soil proteins [30], and water-soluble soil organic matter [12]. Once sorbed to soil tannin-C was not extracted by hot water [8]. Soils treated with several applications of representative phenolic compounds showed a high affinity and a fixed capacity for both hydrolyzable and condensed tannins but lower capacity for small phenolic compounds and reduced extractions of soluble-N [9,31].

The specific objectives of this study were to evaluate sorption and effects on soluble-N of tannins and related non-tannin phenolic compounds in soils amended with different kinds of organic matter. We applied solutions of chemically defined hydrolyzable and condensed tannins (polymers) and related non-tannin phenolic substances (monomers) to soil samples and measured soluble-C and -N in supernatants after application and following extraction with hot water to quantitate sorption or effects on extractable of soil-N as a function of amendment and phenolic treatment.

2. Materials and Methods

2.1. Field site and Sampling

A field study was initiated in 2002 in spring wheat (*Triticum aestivum* L.) stubble, at the Columbia Plateau Conservation Research Center, Pendleton, OR in northeastern Oregon (45°43'N, 118°38'W with 454-m elevation) [32]. The climate is semiarid with an annual mean temperature of 10.2 °C and precipitation of 420 mm, 70% of which occurs mainly as winter rain (November to April) [33]. Soil is a Walla Walla silt loam (coarse-silty, mixed, superactive, mesic Typic Haploxeroll) with a uniform slope of less than 2%.

The main plots (a continuous winter wheat and a continuous fallow treatment) were subdivided (split blocks) into twelve carbon source subplots and arranged in a randomized complete block design with four replications ($2 \times 12 \times 4 = 96$ total plots). Each plot was approximately 6 m long by 1.5 m wide. Several carbon substances (amendments) having specific biological and chemical properties were applied annually for five years (2002–2007) to the surface soil of individual plots. Carbon substances were chosen to mimic components of crop residues (cellulose, lignin, soluble carbohydrate, amino acids). In addition, certain amendments and residues of local interest were included for comparison. Plot treatments included no amendment (as a control), manure, municipal biosolids, wood pellets, sucrose, cotton fibers, alfalfa (*Medicago sativa* L.) pellets, winter wheat straw, composted wheat straw, winter brassica (*Brassica rapa*) crops, winter brassica residue, and tall fescue grass (*Festuca arundinacea* L.).

Amendments were added at an equivalent of 250 g C m⁻² every fall. After application, a no-till drill was used to seed the plots with winter wheat. The same drill was used in the fallow plots to give the same slight soil disturbance and surface roughness in the top 2–7 cm of soil, which also reduced movement of the residues by wind. Weeds such as downy brome (*Bromus tectorum* L.) and volunteer spring wheat were controlled on all treated plots with herbicides or hand weeding. Winter wheat and brassica plots were fertilized with nitrogen at rates sufficient to meet yield goals.

For this study, composited surface soil samples (10 cores per plot) were taken from each plot on 30 August 2007, after harvest, from 0–5 cm soil depth. Air-dried, sieved (2.0 mm) samples were stored at room temperature until analysis. Soil properties were determined for each composited sample (Table 1). Soil pH was measured by with an electrode (1:1 soil: water). Total soil-C and -N content was determined for by dry combustion [34] with a FlashEA 1112 NC Analyzer (CE Elantech, Lakewood, NJ). Cation exchange capacity (CEC) was measured at the soil pH by exchange with cobalt hexamine trichloride [35–37].

Table 1. Final soil properties[†] from a field study in which amendments were added to agricultural soil at the Columbia Plateau Conservation Research Center, Pendleton, OR, USA in 2002 through 2007.

Amendment	pH _{water} (1:1)	CEC (cmolc·kg ⁻¹)	Total-C (%)	Total- N (%)	C:N
Cotton fiber	5.23 (0.02)	14.7 (0.2)	1.65 (0.08)	0.16 (0.01)	10.4 (0.3)
Wheat residue	5.22 (0.03)	15.2 (0.3)	1.65 (0.06)	0.16 (0.01)	10.4 (0.3)
No amendment	5.21 (0.03)	14.4 (0.6)	1.71 (0.20)	0.16 (0.02)	10.7 (0.5)
Brassica crop	5.22 (0.03)	15.0 (0.3)	1.88 (0.08)	0.18 (0.01)	10.3 (0.3)
Brassica residue	5.19 (0.03)	16.3 (0.3)	1.90 (0.11)	0.18 (0.01)	10.4 (0.3)
Sucrose	5.20 (0.03)	14.6 (0.4)	1.97 (0.20)	0.16 (0.01)	11.8 (0.7)
Grass	5.22 (0.04)	15.6 (0.2)	2.01 (0.10)	0.18 (0.01)	10.9 (0.2)
Compost	5.14 (0.05)	15.9 (0.6)	2.23 (0.27)	0.18 (0.01)	12.2 (1.2)
Alfalfa	5.20 (0.04)	15.6 (0.4)	2.26 (0.13)	0.22 (0.01)	10.6 (0.5)
Manure	5.21 (0.05)	17.5 (0.4)	2.50 (0.28)	0.24 (0.02)	10.4 (0.3)
Wood	5.20 (0.05)	15.2 (0.4)	2.75 (0.17)	0.18 (0.01)	15.4 (1.1)
Biosolids	5.18 (0.04)	15.7 (0.6)	2.90 (0.23)	0.30 (0.02)	9.6 (0.2)

[†] Values are the arithmetic average and (SE), n = 8.

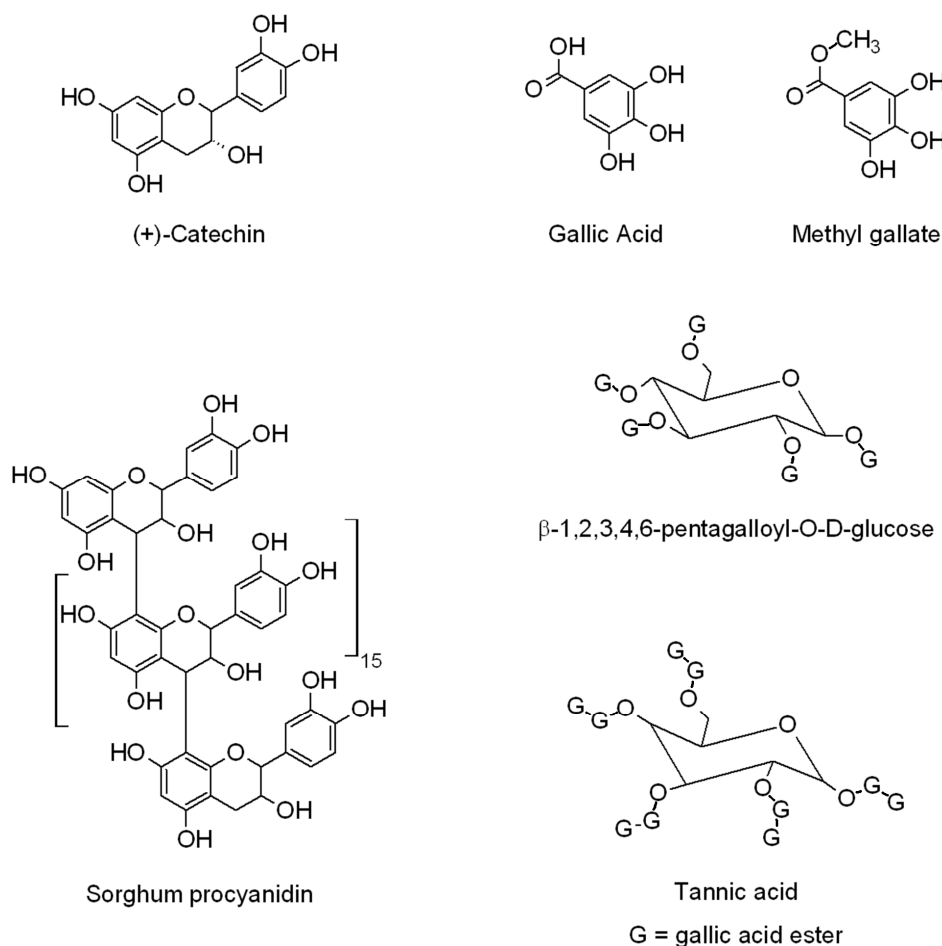
2.2. Sorption of Test Compounds

Sorption/desorption of test compounds was determined from the amount of soluble carbon (soluble-C) and nitrogen (soluble-N) in supernatants after an application of a cool (23 °C) aqueous solution and subsequent hot water (80 °C) incubation as described below. Both cool and hot water extractions were used to determine the different pools of labile C and N in soil [8,12,38,39].

2.3. Test Compounds

Soil samples were treated with deionized water (control) or with solutions containing model tannins or non-tannin phenolic compounds selected to represent a range of phenolic compounds of varying complexity present in the plant-soil continuum [40]. Our representative condensed tannin was a polymeric flavonoid-based proanthocyanidin isolated from sorghum grain (SOR) [41,42]. We also evaluated tannic acid (TA), a commercially available, but imprecisely defined mixture of galloyl esters, and β -1,2,3,4,6-penta-*O*-galloyl-D-glucose (PGG), a well-defined gallotannin purified from the tannic acid. Non-tannin phenolics included the flavan-3-ol catechin (CAT), the phenolic acid gallic acid (GA), and its ester, methyl gallate, (MG) (Figure 1 and Table 2).

Figure 1. Chemical structures for (+)-catechin (CAT), gallic acid (GA), methyl gallate (MG), penta-galloyl-glucose (PGG), sorghum proanthocyanidin (SOR), and tannic acid (TA). The structure shown for tannic acid is a representative molecule for tannic acid, an imprecisely defined mixture of hydrolysable tannins.



2.4. Procedure

Samples of soil (3.0 g) were weighed into Oak Ridge polypropylene centrifuge tubes, 50 mL (nominal) and treated with 30 mL of deionized water (control) or with 30 mL of aqueous test solution to yield a final amendment of 10 mg test compound g⁻¹ soil. After reciprocal shaking at 200 rpm for 1 hour 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 carbon and nitrogen (soluble-C and -N) with a Shimadzu TOC-VCPN analyzer equipped with a TNM-1 module (Shimadzu Scientific Instruments, Columbia, MD). Sample pellets were briefly stored 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 for 16 h in a water bath (80 °C), and assayed for soluble-C and -N as before. Values for hot-water soluble-C, -N, and total phenols by Prussian Blue (PB) assay (described below), were corrected to account for carryover from the previous treatment step and are expressed on an oven-dry equivalent soil basis.

Net sorption of treatment-C by soil was calculated as:

$$\text{Net Sorption} = \text{Trt-C}_{\text{added}} - (\text{sol-C}_{\text{trt}} - \text{sol-C}_{\text{control}}) \quad (1)$$

where Trt-C_{added} is the soluble-C added in treatment solutions (Table 2), and sol-C_{trt} and sol-C_{control} are soluble-C extracted from treated and control samples respectively. Sorption was analyzed as a percent of added Treatment-C values.

Net treatment effects on soluble-N were determined as the difference between treated and control values and expressed as a percent increase or decrease relative to the control:

$$\text{Net treatment effects} = 100 \times (\text{sol-N}_{\text{trt}} - \text{sol-N}_{\text{control}}) / \text{sol-N}_{\text{control}} \quad (2)$$

A similar approach was used to analyze soluble-C extracted after the hot-water incubation.

Total phenolics in supernatants after the treatment application of test compounds and after the hot-water incubation were determined with the modified Prussian Blue assay for total phenols [41,43,44] using gallic acid as the standard. This assay is a colorimetric determination of phenolics and other oxidizable compounds and does not distinguish between tannins, and other phenolic substances, or other easily oxidized compounds such as thiols.

Table 2. Information on the test compounds added to soil from the field studies of amendments to agricultural soil at the Columbia Plateau Conservation Research Center, Pendleton, OR, USA.

Compound/Treatment	Class	Source	Compound characteristics					Treatment [¶]			
			MW [†]	C [‡] (%)	N [‡] (%)	C (g mol ⁻¹)	K _{ow} [§]	Soluble-C (mg/kg soil)	Soluble-N (mg/kg soil)	Phenolics [#] μmol GA equiv/kg	Solution pH 1mg/mL)
Methyl 3,4,5-trihydroxybenzoate, 98% (Methyl Gallate) (MG)	Phenolic organic ester	Indofine Chemical Co., Hillsborough, NJ	184	51.7	0.084	96	6.3	5122	0.6	41.7	4.4
Gallic Acid, Certified (GA)	Phenolic organic acid	Fisher Scientific, Pittsburgh, PA	170	47.7	0.106	85	0.3	4654	0.6	53.7	3.3
Tannic Acid, Certified (TA)	Mixture of gallotannins	Fisher Scientific, Pittsburgh, PA	902	49.4	0.142	474	ND	4669	2.0	30.1	3.5
β-1,2,3,4,6 penta-O-galloyl-D-glucose (PGG)	Gallotannin	Purified from Tannic Acid (Fisher)	941	49.7	0.099	492	129	4919	0.6	29.4	5.1
(+)-Catechin hydrate, >98% (CAT)	Flavonoid	Sigma, St Louis, MO	290	61.6	0	180	2.4	5810	0.7	32.6	5.6
[(4β->8)-epicatechin] ₁₅ -(4β->8)-catechin (Sorghum Proanthocyanidin) (SOR)	Polymeric flavonoid	Sorghum grain [<i>Sorghum</i> <i>bicolor</i> (L.) Moench]	4624	48.6	0.094	2880	0.002	4833	6.8	13.7	6.0

[†] Average molecular weight for TA estimated by RP-HPLC by Hagerman (unpublished observation) and used to calculate weighed average g C mol⁻¹. [‡] Total C and N were determined in triplicate with a FlashEA 1112 NC Analyzer (CE Elantech, Lakewood, NJ). [§] Octanol-water partition coefficients for PGG CAT and SOR from [45] GA and MG from Lu *et al.*, [46]. Low values correspond to hydrophilic compounds while higher values are indicative of hydrophobic ones. [¶] Supplied g⁻¹ air dry soil in a single application. Solution pH is shown at solution concentration; 10 ml of solution were applied per g soil. [#] Determined by the Modified Prussian Blue assay.

2.5. Measurements of Final CEC and Total Soil -C

The soil pellet remaining in tubes after the hot-water incubation was dried (55 °C), and a portion assayed for CEC as above. In addition, treatment effects on final soil total-C and -N were determined on composited samples that were created by combining equal portions from each block within the main plots (n = 12). Total soil-C remaining in samples after the hot water extraction was compared against predicted values, calculated as:

$$\begin{aligned} \text{Predicted final soil-C} = \\ (\text{initial soil-C} + \text{C added by phenolic treatment}) - \text{total extracted soluble-C} \end{aligned} \quad (3)$$

where total extracted soluble-C is defined as the sum of soluble-C in treatment supernatant + soluble-C removed by hot water.

2.6. Statistical Analysis

Values for the two management treatments (fallow, winter wheat) were analyzed together because the focus of this study was the interaction between phenolic treatments and soil amendments. An analysis of variance (ANOVA) was performed using SAS 9.2 and PROC MIXED procedure and a model that contained both fixed (treatment and amendment) and random (sample and block) effects [47,48]. The KR (Kenward-Roger) option was used to calculate degrees of freedom. Significant main effects and interactions were noted, but because our focus was on differences among treatments, detailed discussion of interactions was limited to those instances where they moderated the main findings or were particularly noteworthy. Multiple pairwise comparisons of means were performed using the Tukey-Kramer method. A value of 5% was selected as the minimum criterion for significance unless otherwise noted. Assumptions of normality were evaluated and appropriate data transformations identified with SAS/ASSIST. Values indicated in text and graphs are the arithmetic averages $\pm$ the standard errors of the means.

3. Results and Discussion

3.1. Sorption of Treatment-C

Significant amounts of each compound sorbed to soil but demonstrated a clear treatment by amendment interaction (Table 3). Initial sorption of treatment-C was highest for samples treated with tannins in the order PGG > TA > SOR. Sorption of tannins was greatest for plots that were amended with biosolids, manure and alfalfa, materials associated with humic substances or relatively high amino acid content. Sorption was lowest for non-amended control plots or those amended with cotton fiber, compost, sucrose or wheat residue, substances with relatively low N content (Table 1). Conversely, relatively little of the non-tannin phenolic compounds (*i.e.*, MG, GA and CAT) were sorbed and there was little effect of amendment. The percent sorption of treatment-C based on equation 1 closely matched the disappearance of total PB phenolics from the treatment solutions (Figure 2a).

Hot water extracted about 30% more soluble-C from samples treated with CAT, SOR or PGG, than the control. Soluble-C was also about 10% higher from samples treated with TA and MG, but slightly reduced in GA-treated samples compared to the control (Table 4). Detection of PB phenolics in

supernatants after the hot-water incubation step suggested that some of the extracted soluble-C was from the previous treatments (Figure 2b), assuming the phenolic treatments themselves did not increase the solubility of added amendments or resident soil organic SOM. This assumption appears reasonable given that no meaningful differences among amendments were detected in 4 of the 6 phenolic treatments. Hot water extractions were only slightly affected by amendment in GA-treated samples with the largest values from plots amended with biosolids and the smallest from plots amended with *Brassica* residue. Samples treated with PGG were most strongly affected by soil amendment with greatest increases in soluble-C observed in the non-amended control plots or those amended with cotton fiber, compost, or wheat residue and lowest from plots that received biosolids or manure.

Figure 2. Mean ($n = 96$) and standard error (a) Percent reduction of Prussian blue (PB) phenolics, and percent of treatment-C sorption (from Table 3); and (b) Soluble-C and total phenolics in hot water supernatant. Total phenolics were determined by the Prussian Blue assay and are expressed in (a) as percent of the input values from Table 2, or in (b) as $\mu\text{moles gallic acid equivalents}$.

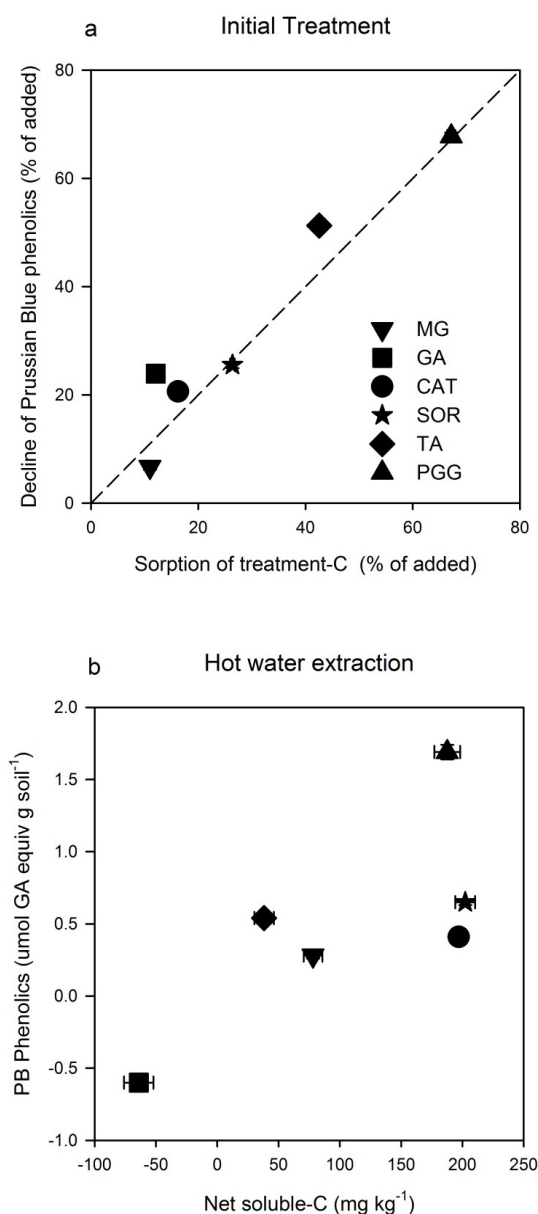


Table 3. Response of soils from the amendment field study at the Columbia Plateau Conservation Research Center, Pendleton, OR to addition of test compounds and a 1 hour shake at room temperature [†].

Amendment	Sorption of treatment-C (% of amount added) [‡]								Net Soluble-N (% difference from Control) [‡]							
	Control Soluble- C (mg/kg)	MG	GA	CAT	SOR	TA	PGG	Average (n = 48)	Control Soluble- N (mg/kg)	MG	GA	CAT	SOR	TA	PGG	Average (n = 48)
No amendment	314 (34)	10.3ab4	12.4a4	13.9a4	23.2c3	39.0c2	60.0cd1	26.5E	29 (2)	21.5a1	−13.8a3	7.8a2	1.0a2	−3.4a23	2.4a2	2.6
Cotton fiber	325 (27)	10.8ab4	11.6a4	13.9a4	22.8c3	37.9c2	59.3d1	26.1E	19 (1)	20.3ab1	−27.9a4	1.4a2	−11.8a3	−19.3a34	−10.6a3	−8.0
Compost	330 (22)	8.8a5	10.6a5	15.6a4	23.8bc3	39.8c2	64.3bcd	27.2DE	42 (4)	15.4ab1	−16.4a4	7.0a12	1.4a23	−9.6a34	−1.4a23	−0.6
Sucrose	346 (36)	10.6ab5	11.8a5	16.1a4	25.2bc3	39.3c2	61.4cd1	27.4CDE	32 (5)	17.2ab1	−20.4a4	−1.2a2	−7.9a23	−13.6a34	−6.5a23	−5.4
Wheat residue	332 (30)	10.4ab5	12.9a45	15.3a4	24.3bc3	38.9c2	62.9cd1	27.4CDE	32 (3)	18.7ab1	−18.1a4	6.4a2	−2.3a23	−10.7a34	−4.4a23	−1.7
Grass	373 (17)	10.0ab5	11.4a5	15.6a4	24.8bc3	41.8bc2	65.3bcd1	28.1CDE	27 (2)	20.4ab1	−22.0a4	4.7a2	−7.2a3	−14.8a34	−8.9a3	−4.6
Wood	425 (26)	11.4ab5	12.7a5	18.4a4	25.4bc3	39.9c2	64.5bcd1	28.7BCDE	23 (4)	19.7ab1	−25.9a4	−2.1a2	−11.9a23	−18.9a34	−9.6a23	−8.1
Brassica crop	345 (22)	10.7ab5	12.8a45	15.8a4	27.5abc3	42.8bc2	67.8bc1	29.6BCDE	64 (18)	13.7ab1	−18.4a4	3.0a12	−1.6a23	−11.8a34	−4.9a23	−3.3
Brassica residue	343 (24)	11.6ab5	12.4a45	16.3a4	26.6bc3	44.6bc2	71.9ab1	30.6BCD	55 (9)	12.6ab1	−19.0a4	4.1a12	−4.8a23	−12.5a34	−7.2a234	−4.5
Alfalfa	394 (20)	10.8ab5	12.9a5	18.7a4	29.7ab3	44.2bc2	72.5ab1	31.5ABC	119 (31)	10.0ab1	−17.7a4	3.5a12	−1.6a123	−12.6a34	−4.1a23	−3.8
Manure	428 (11)	12.6ab5	10.6a5	17.8a4	29.7ab3	49.0ab2	77.9a1	33.0AB	106 (23)	11.9ab1	−16.2a4	5.3a12	−2.8a23	−14.2a34	−6.0a234	−3.7
Biosolids	368 (15)	14.1a45	12.7a5	17.4a4	33.7a3	53.5a2	78.4a1	35.0A	136 (28)	4.5b1	−15.2a3	0.3a1	−1.8a12	−13.1a23	−6.3a123	−5.3
Average (n = 96)		11.0F	12.1E	16.2D	26.4C	42.6B	67.2A			15.5A	−19.2E	3.4B	−4.3C	−12.9D	−5.6C	

[†] Treatment solutions consisted of a water control or supplied 10 mg of methyl gallate (MG), gallic acid (GA), catechin (CAT) condensed tannin from sorghum (SOR), tannic acid (TA) or β-1,2,3,4,6-penta-O-galloyl-D-glucose (PGG) per g soil. Data for control-C and -N are arithmetic average (standard error) n = 8. Average sorption of treatment-C and net soluble-N were calculated from equations 1 and 2 respectively n = 8. [‡] Main effects of treatment (column averages, n = 96) or amendment (row averages, n = 48) are denoted by capital letters. For interactions, differences among amendments within each treatment are denoted by lowercase letters while differences among treatments within each amendment are denoted by numbers (Tukey's HSD, *P* < 0.05).

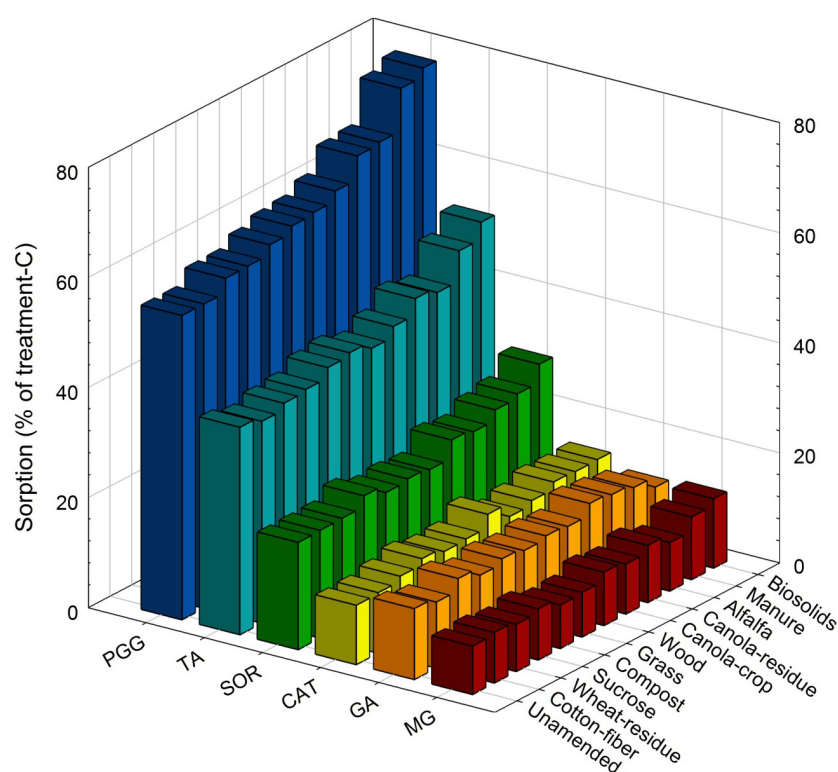
Table 4. Response of soils from Pendleton amendment field study at Columbia Plateau Conservation Research Center, Pendleton, OR to addition of treatment compounds with a 1 hour shake at room temperature followed by a hot-water treatment[†].

Amendment	Control	Treatment-C removed by hot water							Average (n = 48)	Control	Net Soluble-N							Average (n = 48)
	Soluble-C	(% change from Control) ‡								Soluble-N	(% change from Control) ‡							
	(mg/kg)	MG	GA	CAT	SOR	TA	PGG	(mg/kg)		MG	GA	CAT	SOR	TA	PGG			
No ammendment	603 (102)	16.1a3	0.0ab4	35.1a12	32.2a2	14.9a3	48.0a1	24.4	46 (9)	−12.9a2	−21.8ab23	4.2a1	−1.2ab1	−24.4a3	−21.2a23	−12.9 A		
Cotton fiber	598 (63)	16.6a23	−1.0ab4	31.6a1	28.8a12	12.7a3	38.3ab1	21.2	46 (5)	−13.4a2	−26.0abc3	1.8a1	−3.7b1	−28.2a3	−25.3a3	−15.8 ABC		
Compost	822 (123)	9.7a2	−10.2ab3	29.2a1	26.9a1	2.3a2	37.5ab1	15.9	68 (11)	−18.1a2	−32.1bc3	−2.8a1	−2.5ab1	−31.4a3	−25.6a23	−18.8 ABC		
Sucrose	751 (142)	14.0a23	−1.7ab4	29.7a1	25.8a12	11.4a3	31.4abcd1	18.4	52 (7)	−14.7a2	−24.7abc3	2.1a1	−3.9b1	−27.2a3	−26.4a3	−15.8 ABC		
Wheat residue	694 (73)	14.0a2	−6.6ab3	31.2a1	34.0a1	9.5a2	38.6ab1	20.1	53 (5)	−15.4a2	−28.7bc3	0.7a1	−0.9ab1	−28.5a3	−27.0a3	−16.7 ABC		
Grass	791 (37)	12.6a23	−9.2ab4	23.7a12	26.2a1	6.3a3	26.6abcd1	14.4	65 (3)	−16.0a2	−32.0bc3	−4.6a1	−4.8b1	−31.0a3	−29.2a3	−19.6 ABC		
Wood	890 (92)	12.8a23	−2.6ab4	26.5a1	31.1a1	4.8a34	19.4bcd12	15.3	56 (7)	−14.3a2	−25.8abc3	−0.5a1	0.7ab1	−29.9a3	−27.1a3	−16.1 ABC		
Brassica crop	707 (67)	14.1a2	−0.4ab3	31.0a1	29.7a1	12.2a2	35.6abc1	20.3	56 (5)	−14.2a2	−23.8abc3	1.5a1	−0.1ab1	−27.4a3	−27.1a3	−15.2 ABC		
Brassica residue	815 (103)	10.0a2	−13.7b3	27.4a1	26.4a1	4.0a2	29.8abcd1	14.0	69 (9)	−19.9a2	−35.7c3	−4.6a1	−9.2b1	−34.7a3	−33.0a3	−22.9 C		
Alfalfa	952 (76)	11.9a2	−7.7ab3	27.5a1	24.8a1	5.4a2	26.5abcd1	15.0	86 (9)	−15.3a2	−27.7abc3	−2.6a1	−2.9ab1	−29.5a3	−27.4a3	−17.5 ABC		
Manure	1055 (120)	9.0a23	−13.8ab4	22.5a1	20.8a12	2.0a3	12.9cd123	8.9	102 (11)	−17.5a2	−31.1bc3	−5.8a1	−5.1b1	−31.4a3	−33.5a3	−20.7 BC		
Biosolids	810 (82)	10.2a23	4.7a3	22.9a12	30.3a1	10.7a23	9.6d3	14.7	85 (8)	−15.4a3	−15.7a3	−5.8a2	9.9a1	−27.3a4	−31.0a4	−14.2 AB		
Average (n = 96)		12.6 B	−5.2 D	28.2 A	28.2 A	8.0 C	29.5 A			−15.6 B	−27.1 C	−1.4 A	−2.0 A	−29.1 D	−27.8 CD			

[†] Incubation of sample pellets with water for 16 hours at 80 °C. Data for control-C and -N are arithmetic average (standard error) n = 8. Average sorption of treatment-C and net soluble-N were calculated from equations 1 and 2 respectively n = 8. [‡] Main effects of treatment (column averages, n = 96) or amendment (row averages, n = 48) are denoted by capital letters. For interactions, differences among amendments within each treatment are denoted by lowercase letters while differences among treatments within each amendment are denoted by numbers (Tukey's HSD, $P < 0.05$).

Treatment-C, retained in the soil after the hot-water extraction, was influenced by an interaction between treatment and amendment (Figure 3). Final sorption was lowest (<15%) for non-tannin phenolics and unaffected by soil amendment. Remaining treatment-C for SOR and TA were higher, $1089 \pm 21 \text{ mg} \cdot \text{kg}^{-1}$ soil (~23%) and $1974 \pm 29 \text{ mg} \cdot \text{kg}^{-1}$ soil (~42%). Sorption of both tannins was affected by soil amendment, with more treatment-C remaining in soil amended with biosolids than in soil amended with sucrose, grass, wood, compost, wheat residue, cotton fiber or the non-amended plots. The PGG treatment was most strongly retained by soil, an average of $3158 \pm 44 \text{ mg} \cdot \text{kg}^{-1}$ soil (~63%) with retention higher in plots treated with biosolids, manure, alfalfa and canola residue compared to those treated with sucrose, wheat residue, cotton fiber, or compost.

Figure 3. Effect of treatment by soil amendment interaction on average ($n = 8$) percent of Treatment-C remaining after a hot-water (16 hrs, 80 °C) incubation.



Sorption of polyphenolic compounds may be strongly influenced by polarity of the compound and polarity of the soil [49,50]. High molecular weight polyphenols (tannins) interact with biomolecules, including proteins and other forms of organic-N [5], by both hydrogen bonding and by hydrophobic interactions. Thus the relatively high retention of PGG observed for plots amended with alfalfa, manure or biosolids might be associated with the comparatively high N content of these amendments, indicative of organic-N (Figure 3, Table 1). Hydrophobic (nonpolar) organic compounds have been reported to be preferentially sorbed by soils resulting in greater improvements to soil quality and more recalcitrant soil organic matter than hydrophilic organic compounds (e.g., [51–55]). Nonpolar PGG would also be predicted to bind most effectively to soil amended with substances like manure and biosolids because hydrophobicity is correlated with the degree of humification of the soil organic matter. Humification of organic amendments, mediated by microbial decomposition, could increase during composting or with time after application to soil [56–59]. The accumulation of comparatively

greater amounts of total soil-C in plots treated with manure or biosolids (Table 1) suggests that these amendments contained or promoted the formation of recalcitrant-C.

In contrast, less PGG would be predicted to be retained by soils amended with cotton fiber or wheat residue since they are relatively simple plant-derived polysaccharides with low-N [60,61] that have not undergone modifications from condensation or polymerization reactions in soil [53]. However, the hydrophobic PGG could still be expected to have a stronger affinity for soils amended with substances containing cellulose, organic-N, or lipids than hydrophilic SOR [62,63]. Sorption of polar SOR would be favored by hydrophilic sites in the soil [55,56,64–67]. The smaller neutral phenolic compounds such as MG and CAT may interact with soils by hydrogen bonding while the acidic GA may interact via hydrogen bonding and ionic bonds.

3.2. Effects on Soluble-N

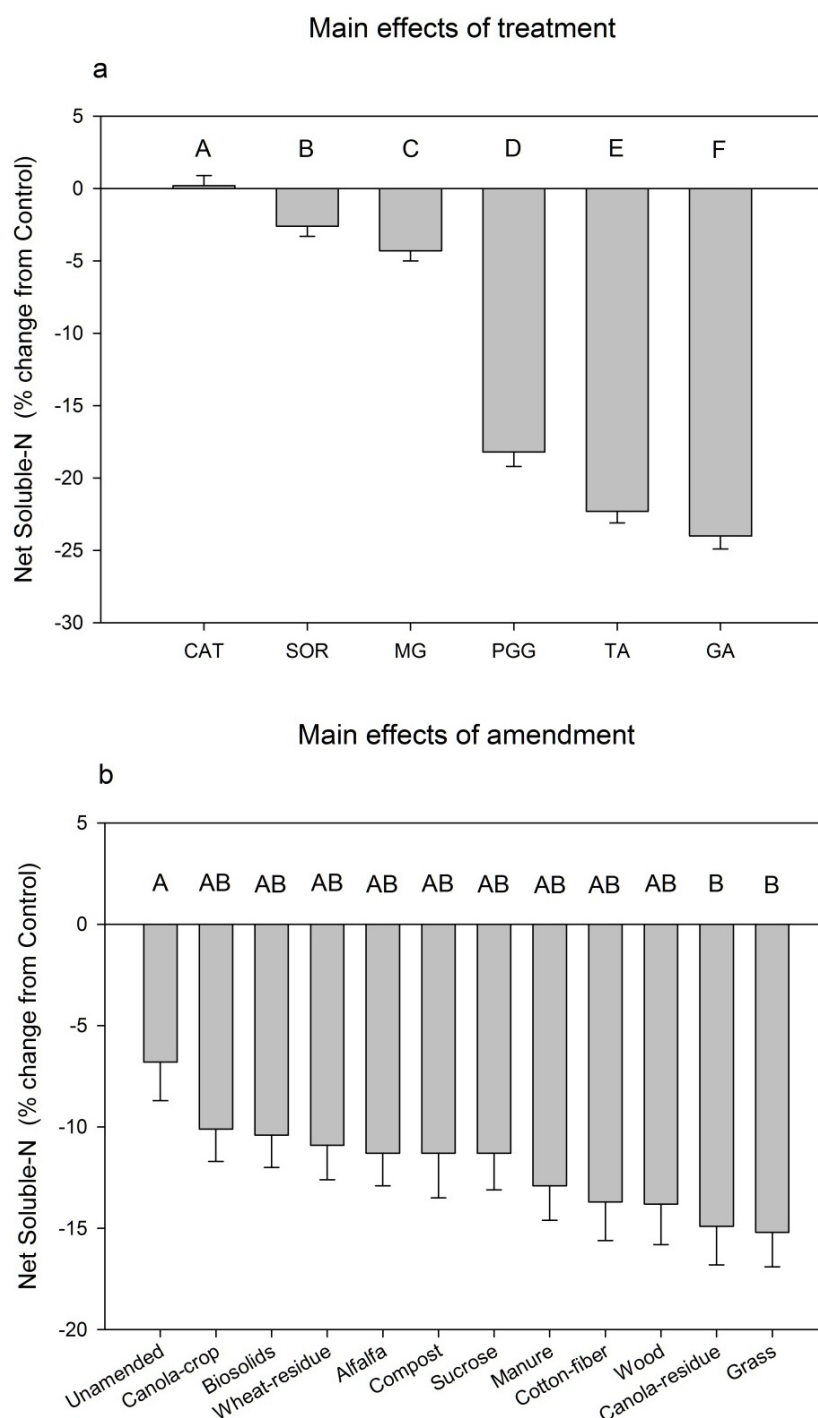
Extraction of net soluble-N from the soils was reduced by TA or GA treatment solutions but little affected by CAT, SOR, or PGG and none of these treatments were appreciably affected by soil amendment (Table 3). Conversely, MG increased extraction of soil-N, relative to water and was affected by soil amendment. Extraction of soluble-N was most increased in soil from the non-amended control plots but unaffected from plots amended with biosolids.

Soluble-N extracted by hot water was not affected by treatment with either flavonoid-derived compounds SOR or CAT (Table 4). Conversely, treatment with gallic acid-derived compounds reduced extractions, compared with control values, by about 15% for MG and by nearly 30% for GA, TA, or PGG. However, unlike the other treatments, reductions from GA-treated samples varied with soil amendment ranging from about 36% in soil amended with Brassica residue to about 16% in samples amended with biosolids.

Patterns of cumulative extraction of soluble-N were dominated by treatment effects with little influence of amendment (Figure 4). Neither the condensed tannin, SOR, nor its related flavan-3-ol monomer unit, CAT, affected extraction of soluble-N and differed from the control by less than $4 \text{ mg} \cdot \text{N} \cdot \text{kg}^{-1}$. Enhanced extractability of soluble-N, observed with the initial treatment of MG, was balanced by reductions in extractability of soluble-N following the hot-water incubation, resulting in a slight net reduction ($\sim 6 \text{ mg} \cdot \text{N} \cdot \text{kg}^{-1}$ soil). Treatment with PGG diminished cumulative soluble-N by nearly 20% ($22 \text{ mg} \cdot \text{N} \cdot \text{kg}^{-1}$) predominantly due to its strong effect on hot-water soluble-N.

Both increased extraction of soluble-N with solutions of MG and reduced extraction with TA or GA was observed in our other studies with acid soil [8,31] but the lack of response to solutions of tannins, especially PGG, was surprising. Tannins are normally thought to form complexes with proteins or organic nitrogen compounds in soil via reversible non-covalent processes such as hydrogen bonding and hydrophobic interactions, [1,5,6] but may also affect soil-N through interactions with mineral soil fractions [4,5,7]. Different responses among TA, GA and PGG suggest solubility of the soil-N was affected by solution pH or that labile pools of soil-N were dominated by forms that did not complex with tannins.

Figure 4. Average (SEM) effects of (a) phenolic treatment and (b) soil amendment on extraction of soluble-N. Differences among mean values are denoted by letters (n = 8, Tukey's HSD).



Initial increases in soluble-N by MG solutions followed by decreases in the subsequent hot water extracts suggests MG affected the efficacy of the extraction process. However, gallic acid-derived compounds appear to somehow increase the ability for organic-N to resist hydrolysis or physically restrain it in the soil matrix. Hot water-extractable soil-N is thought to be primarily composed of unspecified forms of organic N associated with soil microbial biomass and organic matter, with the remainder consisting of $\text{NH}_4\text{-N}$, generated by hydrolysis of heat-labile organic N [38,39,68].

Halvorson *et al.*, [8] previously found that treatment with PGG reduced organic-N in hot water extracts from an acid soil. The GA treatment decreased extractability of soluble-N most strongly, with significant reductions observed after both the initial treatment and the hot-water extraction, equivalent to nearly 30 mg soluble-N kg⁻¹. Cumulative effects of TA on soluble-N were intermediate to the effects of GA and PGG. Tannic acid is typically composed of a mixture of galloyl esters that could behave in part like GA or PGG.

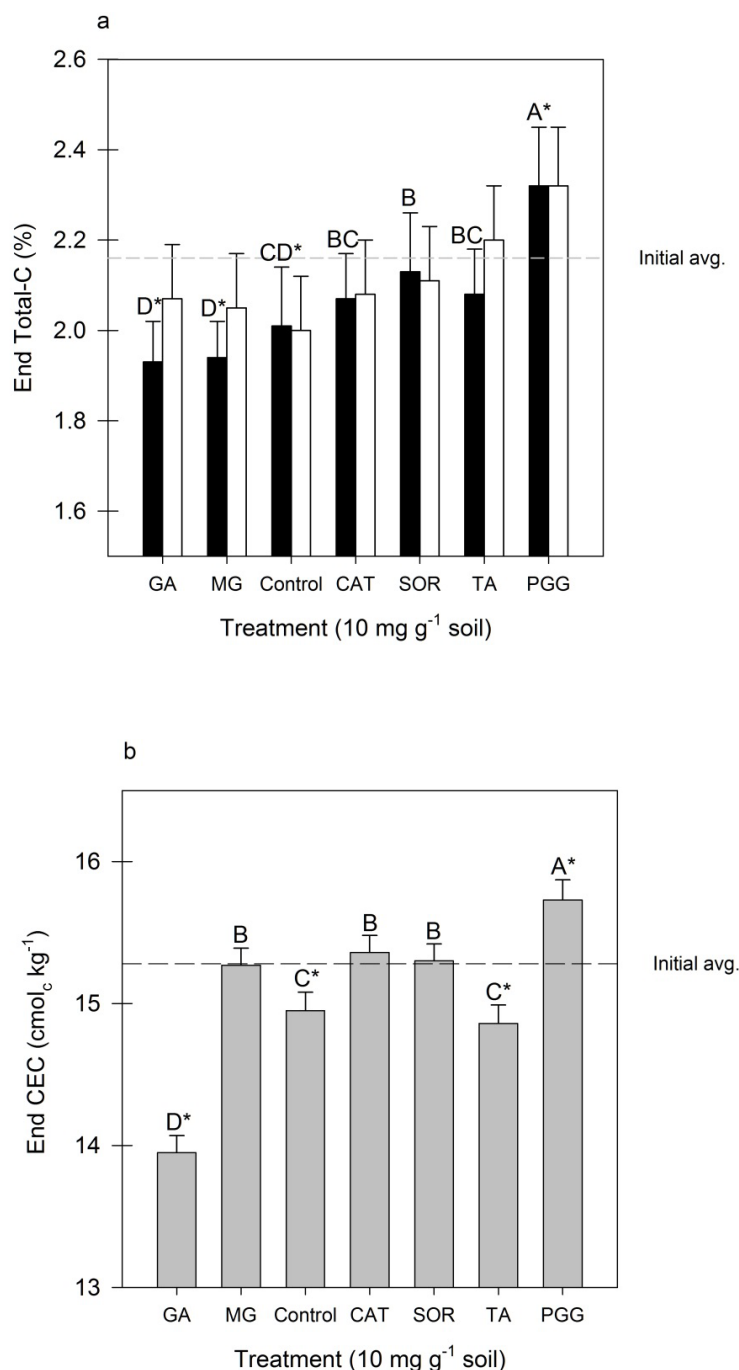
3.3. Total Soil-C and CEC

The amount of total soil-C remaining in samples at the end of the experiment varied by treatment and was higher for tannin treatments than non-tannin phenolics (Figure 5a). The samples treated with PGG gained about 8% more total C than the average initial value while those treated with MG or GA, and the control, contained about 7–9% less. Gains or losses of soil-C were assumed to be the net result of the C added by the treatments balanced against losses of soluble-C. The values for control samples and the PGG, SOR, and CAT treatments were in close agreement with values predicted with Equation (3). The average measured reduction of soil-C in control samples, 1478 ± 469 mg·kg⁻¹ soil, was comparable to cumulative extractions of water soluble-C, 1151 ± 35 mg·kg⁻¹ soil (Table 3 and Table 4). Similarly, the average measured difference between PGG-treated and control samples, 3132 ± 444 mg·kg⁻¹ soil, was close to final estimated retention of PGG-C (3158 ± 44 mg·kg⁻¹ soil) illustrated in Figure 3.

Total soil-C, measured for the MG, GA, and TA treatments, was unexpectedly less than predicted values by about 1000 mg·kg⁻¹ soil. We anticipated larger values than control for these treatments, based on positive net sorption values for all amendments (Figure 3). Less total-C than expected might be the result of physical losses during sample handling, but it is unlikely that this would vary between treatments. Losses of C might be due to evolution of CO₂ from soil following treatment with low pH solutions of MG, GA, or TA (Table 2). However the average starting soil pH was 5.88 inferring carbonates would not be present (unless occluded). Alternatively, oxidation of some phenolics could occur after interacting with soil metals particularly Mn [13,69].

Increased CEC accompanied highest predicted sorption and measured gains in total soil-C values for PGG-treated samples (Figure 5b). Reduced CEC corresponded to low predicted sorption and measured losses of soil-C in samples treated with GA but not MG. Halvorson *et al.* [9] observed a similar pattern in acid soils receiving repeated applications of the treatment compounds. A slight decrease in CEC was also observed in the control samples. Compared to these values, PGG increased soil CEC by an average of 0.8 cmolc·kg⁻¹ soil (~5.3%), while GA reduced CEC by an average of 1 cmolc·kg⁻¹ soil (~6.5%) irrespective of organic soil amendment.

Figure 5. Mean and standard error of treatment effects on: (a) Total-C in soil after the hot water incubation (black bar) compared to predicted values (open bar) calculated with equation 3; and (b) final soil cation exchange capacity. Differences among measurement means are denoted by letters (n = 8, Tukey's HSD). Significant differences from initial soil total-C are denoted by asterisk (paired t-test, $P \leq 0.05$).



4. Conclusions

The patterns of sorption observed in this study, corroborate our earlier studies by showing that tannins are more readily sorbed to soil compared to related phenolics. In addition, more tannin-C was retained by plots amended with biosolids, manure or alfalfa compared with plots amended with

sucrose, wheat residue, cotton fiber, or compost indicating that sorption can be affected by soil organic matter composition. This study also showed that reductions in the extractability of soluble-N from soil after treatment with gallic acid derived compounds, like GA, TA or PGG, were less affected by soil amendment. However, information about short-term reactions that incorporate tannin-C onto the soil matrix and immobilize soil-N must be considered together with their potential for chemical and biological degradation.

Tannin effects can be managed by adopting methods that affect the quantity or composition of phenolic plant secondary compounds in soil. Localization of the effects of tannins on soil cation exchange capacity and immobilization of soil N may be of most value in the vicinity of plant roots while greatest retention of phenolic compounds may be stratified in relation to the distribution of organic matter. Further information is needed on the stability or recalcitrance of phenolic-soil complexes or the phenolic-metal complexes that may form in soil to best manage the quantity and quality of soil organic matter and nutrient cycling in agricultural systems.

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