Research Article

Changes in Soluble-N in Forest and Pasture Soils after Repeated Applications of Tannins and Related Phenolic Compounds

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Tannins (produced by plants) can reduce the solubility of soil-N. However, comparisons of tannins to related non-tannins on different land uses are limited. We extracted soluble-N from forest and pasture soils (0–5 cm) with repeated applications of water (Control) or solutions containing procyanidin from sorghum, catechin, tannic acid, β -1,2,3,4,6-penta-*O*-galloyl-D-glucose (PGG), gallic acid, or methyl gallate (10 mg g⁻¹ soil). After eight treatments, samples were rinsed with cool water (23°C) and incubated in hot water (16 hrs, 80°C). After each step, the quantity of soluble-N and extraction efficiency compared to the Control was determined. Tannins produced the greatest reductions of soluble-N with stronger effects on pasture soil. Little soluble-N was extracted with cool water but hot water released large amounts in patterns influenced by the previous treatments. The results of this study indicate hydrolyzable tannins like PGG reduce the solubility of labile soil-N more than condensed tannins like sorghum procyanidin (SOR) and suggest tannin effects will vary with land management. Because they rapidly reduce solubility of soil-N and can also affect soil microorganisms, tannins may have a role in managing nitrogen availability and retention in agricultural soils.

1. Introduction

Tannins are reactive secondary metabolites produced by plants that affect important biological, chemical, and physical processes in soil and couple primary productivity to biogeochemical cycles [1–4]. Tannin effects on decomposition and nitrogen availability in soil have been a subject of research for more than fifty years [5, 6]. However, development of strategies for use of tannins as soil management tools has lagged, in part because few studies have specifically related them to improving plant productivity or soil fertility. Early tannin research was conducted on temperate agricultural soils [7–9], while recent work has concentrated more on their role in forest ecosystems [10–12] and tropical soils [13–15]. These studies, however, have tended to emphasize the impacts of tannins on microbially mediated processes rather than on the more immediate abiotic interactions between tannins and soil and have made little attempt to frame their findings into the context of landscape effects.

Tannins are believed to affect the nitrogen cycle through several direct and indirect mechanisms that reduce rates of net mineralization or nitrification. Some tannins are directly toxic to plants or microorganisms [16, 17] but their effects vary with particular tannin chemistry or among taxonomic groups [18]. Some tannins or related phenolic compounds are used by soil microorganisms as substrates increasing microbial demand for nitrogen and immobilization in microbial biomass [2, 12, 19]. Tannins can also reduce rates of mineralization or decomposition by affecting the activity of enzymes [20, 21] or by forming complexes with other proteins or organic nitrogen compounds via reversible non-covalent processes such as hydrogen bonding and hydrophobic interactions (cf. [2, 22, 23]). The availability of the nitrogen sequestered in tannin-protein complexes varies among species of plants, taxa of microorganisms, or even among strains of mycorrhizae [11, 24–28]. Tannins and related phenolics may also affect soil-N through interactions with inorganic soil fractions [3, 23, 29]. For example, tannin-related

phenolic compounds can interact with nitrite, produced during nitrification, to form more recalcitrant organic forms in a process termed nitrosation [30–32].

Our earlier studies revealed some tannins were rapidly sorbed by soil and reduced the solubility of labile soil-N [33, 34]. Significant amounts of retained tannin-C remained in soil even after repeated rinses with hot water [33]. A single application of a gallotannin (β -1,2,3,4,6-penta-*O*-galloyl-D-glucose) produced a persistent reduction in the solubility of organic-N not observed with gallic acid, its simple monomeric constituent, suggesting the rapid formation of stable complexes with soil [33]. Tannic acid also influenced the recovery and composition of Bradford-reactive soil protein, associated with glomalin, produced by arbuscular mycorrhizae [35]. These observations suggested plant tannins are capable of affecting critical soil ecosystem processes such as formation of soil organic matter and rates of nutrient cycles and thus may have a role in managing nitrogen availability and retention in soil.

This report is a portion of a two-part study designed to expand the body of basic information about the effects of tannins and related non-tannin phenolics on soil organic matter and nutrient cycling. In part 1, we reported patterns of sorption of phenolic-C and showed Appalachian forest and pasture soils had a high affinity and a fixed capacity for tannins while related phenolic compounds were retained less [34]. This work summarizes the effects of repeated applications of chemically well-defined hydrolyzable and condensed tannins (polymers) and related non-tannin phenolic substances (monomers) on the solubility of soil-N. We compared surface soil from pasture to soil from the surrounding woodlands to gain insight into the magnitude of change associated with conversion from woodlands to silvopasture and assessed their potential significance on a landscape basis. Our ultimate goal is to gather and develop information needed to devise new management strategies that use the phenolic compounds added by plant residues, leachates, livestock manure or, from intentional amendments, to achieve desired agronomic or environmental goals.

2. Materials and Methods

2.1. Sample Collection and Preparation. Surface soil (0–5 cm) was collected from four farm units in Southern West Virginia, each with areas in forest (mixed deciduous or pine) and pasture use as described in greater detail by Halvorson and Gonzalez [36] and Halvorson et al. [34]. Each sample consisted of a composite of 10 soil cores (6.35 cm in diameter) collected along transects. In the lab, composite samples were sieved (2 mm), dried at 55°C, and stored until further analysis.

2.2. Soil Properties. Bulk density (BD) was determined gravimetrically from intact soil cores [36]. Soil chemical properties were determined for each composite sample (Table 1). Soil pH and electrical conductance (EC) were measured by electrode (1 : 1 soil : water). Total inorganic-N (TIN) was estimated as the sum of water extractable inorganic-N plus KCl extractable-N remaining in soil after the first extraction

reported in previous work [33]. Total soil-C content was determined by dry combustion [37] with a FlashEA 1112 NC Analyzer (CE Elantech, Lakewood, NJ). The texture of the soil samples was determined by hydrometer (Midwest labs, Omaha, NE, <http://www.midwestlabs.com/>). Cation exchange capacity (CEC) was measured at the inherent soil pH by exchange with cobalt hexamine trichloride [38–40].

2.3. Effects of Test Compounds on Extraction of Soil-N. As part of a larger study [34], soluble-N extracted from soil was determined by difference after each of eight repeated applications of aqueous treatment solutions followed by a sequential extraction with cool (23°C) and hot (80°C) water [41, 42].

2.3.1. Test Compounds. Soil samples were treated with deionized water (Control) or 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 [43]. Our representative condensed tannin was a polymeric flavonoid-based procyanidin isolated from sorghum grain [*Sorghum bicolor* (L.) Moench] (SOR) [44]. We also evaluated tannic acid (TA), a common 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 flavonoid catechin (CAT), the phenolic acid gallic acid (GA), and its ester, methyl gallate, (MG) (Figure 1, Table 2).

2.3.2. Procedure. Samples of soil (2.5 g) were weighed into Oak Ridge centrifuge tubes and treated with 25 mL of deionized water (Control) or with 25 mL of 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. Supernatants were analyzed for soluble-N with a Shimadzu TOC-VCPN analyzer equipped with a TNM-1 module (Shimadzu Scientific Instruments, Columbia, MD). The treatment application step was repeated seven more times by adding an additional 25 mL of Control or treatment solution to the soil pellet, shaking, and extracting as above. After the final treatment, all samples were extracted with 25 mL of cool (23°C) water and assayed again. Finally, more water (25 mL) was added to soil samples, which were then vortexed, incubated overnight in a hot water bath (16 hrs, 80°C), and assayed for soluble-N as before.

Data were corrected to account for any nitrogen added from the treatments, or carryover from the previous treatment step. Treatment effects were determined for absolute values (mg kg⁻¹ soil) but the amount of net soluble-N extracted by treatment solutions was also expressed relative to the Control and used to determine if treatments decreased (net Treatment < net Control) or increased (net Treatment > net Control) extraction of soluble-N

TABLE 1: Selected soil properties adapted from Halvorson et al. [34]. Mean (SEM), *n* = 4.

Land use	BD [†]	pH(1:1)	EC (μS cm soil ⁻¹)	TIN [‡] (mg N kg soil ⁻¹)	Total C (mg C g soil ⁻¹)	Total N (mg N g soil ⁻¹)	CEC (cmolc kg soil ⁻¹)	Sand %	Silt %	Clay %
Forest	0.95 (0.03)	4.47 (0.26)	103 (13)	17.3 (3.4)	56.0 (3.5)	4.0 (0.2)	9.8 (1.9)	73.0 (3.1)	20.5 (2.2)	6.5 (1.0)
Pasture	1.14 (0.09)	5.27 (0.08)	151 (14)	26.5 (4.4)	42.9 (2.4)	4.5 (0.4)	10.1 (1.0)	69.0 (6.0)	25.0 (5.3)	6.0 (0.8)

[†] Bulk density (BD) for the study was determined gravimetrically.

[‡] Total inorganic-N (TIN) was estimated as the sum of water extractable inorganic-N plus KCl extractable-N remaining in soil [33].

FIGURE 1: Chemical structures for (a) (+)-catechin (CAT), (b) gallic acid (GA), (c) methyl gallate (MG), (d) sorghum procyanidin (SOR), (e) β -1,2,3,4,6-penta-*O*-galloyl-D-glucose (PGG), and (f) tannic acid (TA). The structure shown for tannic acid is a representative molecule for tannic acid, an imprecisely defined mixture of galloyl esters [44]. Figure redrawn from [34].

TABLE 2: Details about treatment compounds [34].

Treatment	Class	Source	Compound characteristics					Solution characteristics [†]			
			MW [†]	C [‡] (%)	N [‡] (%)	C (g mol ⁻¹)	K _{ow} [§]	Soluble-C (mg kg ⁻¹ soil)	Soluble-N (mg kg ⁻¹ soil)	Phenolics [#] (mmol GA equiv kg ⁻¹ soil)	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	5141	0.1	37.2	4.4
Gallic acid, certified (GA)	Phenolic organic acid	Fisher Scientific, Pittsburgh, PA	170	47.7	0.106	85	0.3	4739	0	52.6	3.3
Tannic acid, certified (TA)	Mixture of gallotannins	Fisher Scientific, Pittsburgh, PA	902	49.4	0.142	474	ND	5042	2.5	30.7	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	5037	0.7	26.1	5.1
(+)-Catechin hydrate, >98% (CAT)	Flavonoid	Sigma, St Louis, MO	290	61.6	0	180	2.4	6067	1.0	32.1	5.6
[(4 β - > 8)-epicatechin ₁₅ -(4 β - > 8)-catechin] (Sorghum procyanidin) (SOR)	Polymeric flavonoid	[<i>Sorghum bicolor</i> (L.) Moench]	4624	48.6	0.094	2880	0.002	5189	8.2	13.7	6.0

[†] Number average molecular weight for TA estimated by RP-HPLC by Hagerman (Personal Communication) and used to calculate weighed average gC mol⁻¹.

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

[§] Octanol-water partition coefficients (K_{ow}) 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 soil application⁻¹.

[#] Determined by the Modified Prussian Blue assay.

from soil. We determined percentage change in soluble-N attributable to treatments, $\Delta\text{Sol-N}$, as

$$\Delta\text{Sol-N} = 100 * \frac{(\text{Sol-N}_{\text{trt}} - \text{Sol-N}_{\text{control}})}{\text{Sol-N}_{\text{control}}}, \quad (1)$$

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

2.4. Statistical Analysis. Significant effects of test compounds and land use were identified by analysis of variance (ANOVA) with SAS 9.1 and PROC MIXED using a model that accounted for both fixed (land use, treatment) and random (sample location) effects [47, 48]. We used the KR (Kenward-Roger) option to calculate degrees of freedom and selected covariance structures to minimize Akaike's Information Criterion. Assumptions of data normality were evaluated and appropriate data transformations identified with SAS/ASSIST. We assumed a value of 5% as the minimum criterion for significance. Significant main effects were separated by pairwise comparisons among means, adjusted by the Tukey-Kramer method. The SLICE option in PROC MIXED was used to test significant Treatment $\times$ Use interaction. Significant deviation of $\Delta\text{Sol-N}$ from zero, indicative of a meaningful change in soluble-N 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 air-dry soil basis.

3. Results

3.1. General Patterns. Multiple applications of phenolic solutions produced overall extraction pattern for soluble-N shown in Figure 2. The first of the eight cycles had the greatest effect, extracting 50–60% of the cumulative total amount, while each of the seven subsequent applications removed incrementally less N. The rinse with cool water after the treatment cycles extracted little additional soluble-N but the final incubation in hot water released a relatively large pulse of soluble-N from all treatments and the control. The distinct patterns of $\Delta\text{Sol-N}$, established with the first treatment application, generally varied little with subsequent phenolic treatments or cool water rinse but showed some differences between forest and pasture soils (Figures 3(a) and 3(b)).

3.2. Treatment with Phenolic Compounds. The amount of soluble-N extracted by the first treatment cycle varied with simple main effects of Treatment and Use. Soluble-N was comparable for most treatments with amounts from samples treated with MG slightly greater than the Control (Table 3). In contrast, both TA and PGG reduced amounts of soluble-N. Pasture soil produced more soluble-N than forest soil. Extraction efficiency of treatments compared to the water Control, $\Delta\text{Sol-N}$, varied as a Treatment $\times$ Use interaction. Treatment with MG significantly increased the amount of soluble-N from forest soil by about 19% compared to 9% for pasture soil (Figure 4). Treatment with CAT did not affect

FIGURE 2: Soluble-N extracted from 0–5 cm soil with eight treatment cycles of water (Control) or treatment solutions (10 mg g^{-1} soil) followed by extractions with cool (CW) and hot (HW) water. Values are the mean of 4 farms $\times$ 2 uses (forest and pasture), $n = 8$. Treatment abbreviations are defined in Figure 1.

soluble-N from pasture or forest soil. Gallic acid produced no effect on forest soil, but reduced soluble-N from pasture soil by 19%. Soluble-N was reduced from both soil types by SOR, TA, and PGG, but decreases were stronger in pasture soil. Greatest reductions resulted from the PGG treatment, which lowered soluble-N from forest and pasture by 24 and 34%, respectively.

Patterns established with the first treatment cycle persisted throughout the seven subsequent applications of phenolics and thus cumulative extractions, after all eight treatment cycles, varied with main effects of Treatment and Use (Table 3). While MG slightly increased soluble-N, amounts were significantly reduced by GA, TA, and PGG treatments. Pasture soil yielded more soluble-N than forest soil. Cumulative treatment $\Delta\text{Sol-N}$ varied as a Treatment $\times$ Use interaction. Eight treatment cycles with MG extracted 13% more soluble-N from forest soil than water alone but had little effect on pasture soil. By contrast, treatment with CAT reduced soluble-N by about 9% for both soils. Soluble-N was also reduced by the other phenolic compounds but treatment effects were discernibly stronger for pasture soil. Greatest reductions, with PGG, resulted in 28 and 40% less soluble-N, than the water control, from forest and pasture, respectively.

3.3. Cool and Hot Water Extractions. Only small amounts, $5.1 \pm 0.2 \text{ mg kg}^{-1}$, of soluble-N, were extracted by cool water, after the final treatment cycle, limiting the interpretive value

TABLE 3: Soluble-N (mg kg^{-1} soil) extracted with treatment solutions[†].

Trt	Single treatment		Cumulative after treatment 8		Cool water		Hot water		Final total	
	Forest	Pasture	Avg. ($n = 8$)	Forest	Pasture	Avg. ($n = 8$)	Forest	Pasture	Forest	Pasture
H ₂ O	70 (1)	94 (10)	82 (7)AB	147 (4)	186 (19)	166 (11)AB	4.9 (0.3)	5.8 (0.8)	107 (4)	191 (27)
MG	83 (3)	102 (10)	93 (6)A	166 (6)	193 (19)	179 (11)A	5.5 (0.5)	5.7 (0.5)	98 (5)	170 (26)
CAT	70 (1)	91 (8)	81 (6)AB	137 (3)	165 (14)	151 (9)BC	4.2 (0.4)	4.8 (1.0)	91 (3)	166 (24)
SOR	65 (1)	82 (8)	74 (5)BC	136 (3)	154 (13)	145 (7)BC	6.2 (0.3)	5.4 (0.5)	95 (94)	129 (8)
GA	69 (1)	75 (7)	72 (3)BC	131 (3)	131 (12)	131 (6)CD	5.5 (0.4)	5.0 (0.4)	107 (3)	148 (19)
TA	61 (1)	70 (5)	66 (3)C	110 (2)	116 (9)	113 (5)DE	5.0 (0.7)	4.5 (0.7)	86 (3)	106 (9)
PGG	53 (2)	61 (6)	57 (3)D	105 (3)	110 (9)	108 (4)E	4.2 (0.2)	4.0 (0.5)	78 (4)	99 (8)
Avg. ($n = 28$)	67 (2)Y	82 (4)X		133 (4)Y	151 (8)X		5.1 (0.2)	5.0 (0.2)	95 (2)Y	144 (9)X
									233 (6)Y	300 (16)X

[†] 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 are arithmetic average (standard error) ($n = 4$). Main effects of land use (column averages, $n = 28$) or treatments (row averages, $n = 8$) are denoted by capital letters (Tukey's HSD, $P < 0.05$).

FIGURE 3: Percent change in soluble-N extractions attributable to treatments, $\Delta\text{Sol-N}$ for (a) forest and (b) pasture soils calculated with (1) ($n = 4$). Treatment abbreviations are defined in Figure 1.

of intertreatment $\Delta\text{Sol-N}$ comparisons (Table 3). Variation among treatment effects was less than 2 mg kg^{-1} soil but samples treated with PGG yielded less cool water soluble-N than others.

In contrast, hot water extracted large amounts of soluble-N that varied with simple main effects of Treatment and Use (Table 3). The greatest amount of soluble-N, extracted from the Control treatment, was comparable to the amount extracted by the preceding eight treatment cycles (Table 3). Significantly less soluble-N was extracted with hot water from TA- and PGG-treated samples than other treatments. Pasture produced about 50% more soluble-N than forest soil.

Hot water $\Delta\text{Sol-N}$ varied as an interaction between Treatment and Use (Figure 5). Gallic acid had no effect on hot water extractions from forest soil, compared to the Control, but reduced soluble-N from pasture samples by 22%. Previous treatments with MG and CAT reduced soluble-N similarly from forest and pasture soils, by 10 and 14%, respectively. Reductions in soluble-N, observed for samples treated with SOR, TA, or PGG, were greater in pasture than forest soil. Hot water soluble-N was reduced from samples previously treated with PGG, by 27% for forest soil and by 47% for pasture.

3.4. Cumulative Extraction of Soluble-N. Final cumulative soluble-N extracted by eight treatment cycles and subsequent cool and hot rinses differed by Treatment and Use (Table 3). Treatment effects segregated into two groups with less total soluble-N extracted from soils treated with TA or PGG.

Pasture soil yielded an average of 300 mg kg^{-1} soluble-N or about 29% more than forest soil.

Final cumulative $\Delta\text{Sol-N}$ varied as an interaction between Treatment and Use (Figure 6). In both forest and pasture soils, $\Delta\text{Sol-N}$ was highest from samples treated with MG and lowest from samples treated with TA or PGG. The repeated treatments with MG did not significantly influence cumulative extraction of soluble-N from forest or pasture soil. Forest soil was also unaffected by GA but soluble-N was reduced from pasture soil by 25% (nearly 100 mg kg^{-1}). Treatments with CAT reduced soluble-N similarly from both land uses by an average of 11% (about 37 mg kg^{-1}). Tannins produced the greatest reductions of soluble-N with significantly stronger effects on pasture soil. The condensed tannin, SOR, reduced cumulative extractions of soluble-N from forest and pasture soils by 8 and 23%, respectively, (21 and 93 mg kg^{-1}). Tannic acid, reduced soluble-N from forest soil by 22% (58 mg kg^{-1}) and by 40% (156 mg kg^{-1}) from pasture soil. The hydrolyzable tannin, PGG, produced greatest reductions, 27 and 43% from forest and pasture soils, respectively, (71 and 176 mg kg^{-1}).

4. Discussion

When expressed on a landscape basis, the size of the pool of soil N affected by tannins and other phenolic compounds appears significant. Water alone (Control) extracted the equivalent of 33 and 54 kg of soluble-N ha^{-1} from 0–5 cm of forest and pasture soils with the first treatment cycle

FIGURE 4: Treatment $\times$ Use interactions for $\Delta\text{Sol-N}$ after the first application of treatment solutions (10 mg g^{-1} soil). Within each land use, letters denote differences among treatments. Differences between uses are denoted by numbers. Filled symbols denote significant deviations from the control. Treatment abbreviations are defined in Figure 1.

(Tables 1 and 3). Compared against these baseline values, the first treatment with MG increased losses of soluble-N from forest and pasture soils by $5\text{--}6 \text{ kg N ha}^{-1}$ while PGG conserved 8 and 19 kg N ha^{-1} in forest and pasture samples, respectively. These reductions of soluble-N were strongly correlated with the amount of phenolic treatment-C sorbed by the soil [34] (Figure 7).

Incremental extractions of nitrogen after eight successive treatment applications were small suggesting a majority of the most labile pool of soil-N had been removed (Figure 2). The difference between Control extractions, equivalent to 70 and 106 kg ha^{-1} from forest and pasture soils, and treatment extractions infer the magnitude of the soil-N pool most responsive to the phenolic inputs. These suggest the eight treatments with MG mobilized an additional 19 and 7 mg N kg^{-1} forest or pasture soil, compared to water, or 9 and 4 kg N ha^{-1} . Conversely, PGG reduced the solubility of labile soil-N by 41 and 76 mg kg^{-1} in forest and pasture soils, respectively, equivalent to retention of 21 and 43 kg N ha^{-1} .

Negligible extractions of soluble-N with cool water, exhibiting only small differences among treatments, were unexpected (Table 3). We had, instead, anticipated relatively large releases of soluble-N from samples that had previously retained N once the treatments were omitted.

In contrast to cool water, hot water released large amounts of soluble-N, accounting for between 40 and 50% of the cumulative extraction, suggesting it originated from a

FIGURE 5: Treatment $\times$ Use interactions for $\Delta\text{Sol-N}$ calculated for hot water extractions (not cumulative). Within each land use, letters denote differences among treatments. Differences between uses are denoted by numbers. Filled symbols denote significant deviations from the control. Treatment abbreviations are defined in Figure 1.

FIGURE 6: Treatment $\times$ Use interactions for $\Delta\text{Sol-N}$ calculated for cumulative extractions. Within each land use, letters denote differences among treatments. Differences between uses are denoted by numbers. Filled symbols denote significant deviations from the control. Treatment abbreviations are defined in Figure 1.

FIGURE 7: Relationship between $\Delta\text{Sol-N}$ and sorbed treatment-C after the first application of treatments. Data from forest and pasture soils are denoted by filled and open symbols, respectively. Values for treatment-C are from [34]. Treatment abbreviations are defined in Figure 1.

different pool of soil organic matter. The hot water-soluble pool from forest soil, 51 kg N ha^{-1} , was less than half that from pasture soil, 110 kg N ha^{-1} . Hot water-extractable soil-N is thought to be primarily composed of unspecified forms of organic-N, particularly amino-N and amides, 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 [41, 42, 49]. In a previous experiment, Halvorson et al. [33] found organic-N comprised more than 80% of soluble-N extracted by hot water after a single treatment with GA, TA or PGG.

The patterns of treatment effects observed for hot water soluble-N, along with those observed for the preceding extractions, suggest phenolic compounds, like MG, may simply affect the efficacy of the extraction process while others including the tannins and CAT somehow increase the ability for organic-N to resist hydrolysis or physically restrain it in the soil matrix. While eight treatments with MG increased soluble-N in supernatants by an average 7 kg N ha^{-1} (Table 3), hot water soluble-N from samples treated with MG was reduced by 8 kg N ha^{-1} (Figure 5). Thus, final cumulative extractions from MG-treated forest and pasture soils, though distinct from each other (129 compared to 211 kg N ha^{-1}), were not appreciably different from cumulative extractions with water (Figure 6). In contrast, the PGG treatment reduced soluble-N in treatment supernatants, the cool water rinse, and the final hot water incubation with reductions of hot water soluble-N equivalent

to 14 and 53 kg N ha^{-1} from forest and pasture (Figures 4 and 5, Table 3). All together, PGG reduced soluble-N from the forest soil by 71 mg kg^{-1} soil or 35 kg ha^{-1} and from pasture soil by 170 mg kg^{-1} soil or 97 kg ha^{-1} .

Reductions in N availability by condensed tannins are attributed to formation of protein-tannin complexes with soil protein that are more recalcitrant than those formed with hydrolyzable tannins in part because they are less available to microorganisms as substrate [10, 50–52]. However, the strong reductions in solubility of labile soil-N, observed for TA and PGG, suggest hydrolyzable tannins may play a more prominent role in abiotic immobilization of organic soil-N than previously thought. A study by Hagerman et al. [45] concluded PGG, a nonpolar tannin (Table 2), precipitated with bovine serum albumin (BSA) by forming a hydrophobic coat around the protein while the more polar, SOR, formed hydrogen-bond cross-links between BSA molecules.

In addition to precipitation, polymerization resulting from condensation reactions between phenolic and amino-containing compounds in solution can occur. Oxidation of phenolics, forming semiquinones and quinones, can be carried out biotically by the polyphenol oxidase enzymes and/or abiotically by redox reactions with manganese and iron oxides [53, 54]. When PGG is oxidized, it forms covalently linked complexes with protein [55].

Additions of some tannins/phenolics to the soil resulted in the dissolution of Mn oxides, evinced by increased Mn content in the supernatant [56]. Mobilization of other soil metals such as Fe, Al and Si by tannins has also been observed [29, 57]. During the dissolution of Mn oxides, the Mn may be reduced from insoluble Mn(III and IV) to soluble Mn(II), which is available for plants. Phenolic compounds could be oxidized, forming quinones and semiquinones, the latter a highly reactive radical that readily self-polymerizes or copolymerizes with other compounds [58]. Tannic acid, gallic acid, and other polyhydroxy phenols with OH–OH in the orthopositions are known to be highly effective in the dissolution of Mn in soils with high Mn oxides content [59] or in synthetic Mn oxide [60]. Thus, the lower soluble-N extracted with PGG, GA, or TA solutions compared to other compounds might be due in part to redox reactions with soil Mn together with oxidation of these organic compounds into quinones or semiquinones and formation of “humic-like” polymers with amino-containing compounds that were retained in the soil matrix.

The significant interactions between treatment and land use, observed for $\Delta\text{Sol-N}$, indicated the effects of phenolic plant inputs were of greater consequence in land managed as pasture than the surrounding woodlands (Figures 4–6). Variations in soil microbial community composition, related to land use, have been suggested to explain variations in the mineralization of tannin-protein complexes [13]; however this study and our related work [33] also suggest land use can affect the initial reactions between tannins and soil.

The effects of tannins on soil organic matter and nutrient cycling have important implications for livestock production in mixed systems such as silvopastures that include a mixture of forages together with browse and overstory tree species [61–64]. Appalachian silvopasture soil typically differs from

the surrounding unmanaged woodland because it is limed to increase soil pH, receives additional N-inputs from fertilizers and manure, and can develop greater bulk density due to compaction by livestock (Table 1). In addition, transitioning from either forest or pasture to a silvopastoral mixed stand, containing both forages and overstory, may affect soil nitrogen pools by redistributing the patterns of nutrients in soils and biomass. Decreases in soil C and N have been associated with afforestation, especially in the case of pines [65]. Two years after conversion from a mixed hardwood woodland, Staley et al. [66] reported losses of organic-C and -N from West Virginia silvopasture soil of 17 and 9%, respectively, which they attributed to litter decomposition.

Along with their effects on soil organic matter and the availability of nitrogen, tannins and related phenolic compounds can interact with important metals in soil, such as Ca, Mn, Al, and Fe, probably through chelation and oxidation/reduction reactions [67, 68]. Interactions between metals and phenolic compounds may inhibit or promote plant growth in forest soil. 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 Al on roots [69] and in soil [10, 26]. In addition tannins affect nutrient value of forages (e.g., [70, 71]) and animal health [72, 73]. Thus, tannins can link plant productivity, ruminant physiology, pathogen survival, and environmental quality in agroecosystems.

The results of this study indicate hydrolyzable tannins like PGG can quickly reduce the solubility of labile soil-N more than condensed tannins like SOR and suggest tannin effects will vary with land management. 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 [33]. Further work is required to determine the persistence of tannin effects as they are degraded by soil microorganisms or other soil biota [74], physically lost by leaching, or chemically oxidized after interacting with soil metals [67, 68].

Tannins and other phenolic compounds affect a number of important biological, chemical, and physical processes in plants animals and soil. Studies such as this improve our understanding of the effects of natural phenolic inputs on soil organic matter and nutrient cycling and will ultimately lead to new management strategies. Future research on tannins and other plant polyphenolics in soil ecosystems should be focused towards understanding their effects on plant productivity and soil function. Functional definitions linking specific tannin chemistry to soil processes are required that can also serve as a rationale for comparing tannins. Research remains hampered by a lack of standardized methods that simplify the extraction, identification, and quantification of tannins from plants and soil and that can be adapted to field measurements. Experimental field work will remain difficult until suitable model tannins can be identified that are available in reasonable quantities and expense.

Because they are chemically and biologically active, tannins appear to have the potential to be used to improve nitrogen retention by soils but additional work is needed to

determine how long their effects can last and whether the retained N is readily available to crop or forage plants. The effects of tannins on nutrient cycling will likely be influenced by specific tannin chemistry [13, 75, 76], vary with tannin concentrations, and the quantity and quality of soil N [77].

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