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Carbon and nitrogen dynamics in a forest soil amended with purified tannins from different plant species

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Abstract

Tannins are purported to be an important factor controlling nitrogen cycling in forest ecosystems, and the ability of tannins to bind proteins in protein–tannin complexes is thought to be the primary mechanism responsible for these effects. In this study, we examined the influence of well-characterized tannins purified from five different plant species on C and N dynamics of a forest soil A horizon. Tannic acid, a commonly used and commercially available hydrolyzable tannin (HT), and cellulose were also included for comparison. With the exception of tannins from huckleberry (*Vaccinium ovatum*), the amendments increased respiration 1.4–4.0 fold, indicating that they were acting as a microbial C source. Tannic acid was significantly more labile than the five purified tannins examined in this study. All treatments decreased net N mineralization substantially, through greater N immobilization and decreased mineralization. The six tannins inhibited gross ammonification rates significantly more than cellulose. This suggests that added tannins had effects in addition to serving as an alternative C source. Tannins purified from Bishop pine (*Pinus muricata*) were the only tannins that significantly inhibited potential gross nitrification rates, however, rates were low even in the control soil making it difficult to detect any inhibition. Differences in tannin structure such as condensed versus HTs and the hydroxylation pattern of the condensed tannin B-ring likely explain differences observed among the tannin treatments. Contrary to other studies, we did not find that condensed tannins were more labile and less inhibitory than HTs, nor that shorter chained tannins were more labile than longer chained tannins. In addition to supporting the hypothesis that reduced N availability in the presence of tannins is caused by complexation reactions, our data suggests tannins act as a labile C source leading to increased N immobilization.

Keywords: Tannin; Nitrogen cycling; Mineralization; ^{15}N isotope dilution; ^{13}C NMR

1. Introduction

Tannins are purported to influence ecosystem processes such as organic matter degradation, nitrogen cycling, soil formation and successional dynamics (Kuiters, 1990; Howard and Howard, 1993; Schimel et al., 1998; Preston, 1999; Kraus, 2003b). In woody species, foliar tannin concentrations commonly range from 15 to 25% dry weight (Mansfield et al., 1999; Preston, 1999; Yu and Dahlgren, 2000; Booker and Maier, 2001; Osier and Lindroth, 2001; Ossipova et al., 2001). Through both litter deposition and foliar leaching (Schofield et al., 1998; Hernes et al., 2001), soils in tannin-rich plant communities receive appreciable tannin inputs. By definition tannins have the ability to precipitate proteins, which suggests that tannins influence biogeochemical processes by interacting with organic N compounds in soil organic matter.

Tannins produced by plants are divided into two major classes called condensed and hydrolyzable tannins (HT) (Fig. 1). Condensed tannins (CT), also referred to as proanthocyanidins, are composed of flavan-3-ols joined with C–C bonds. The monomers of CT are distinguished by the number of OH groups on the B-ring. Procyanidins (PC) have a di-hydroxy B ring while prodelphinidins (PD) have a tri-hydroxy B-ring. HT are further grouped into gallotannins and ellagitannins that are composed of gallic acid or hexahydroxydiphenic acid esters, respectively, united by ester linkages to a central sugar moiety. Even within these groups differences between individual tannins arise from

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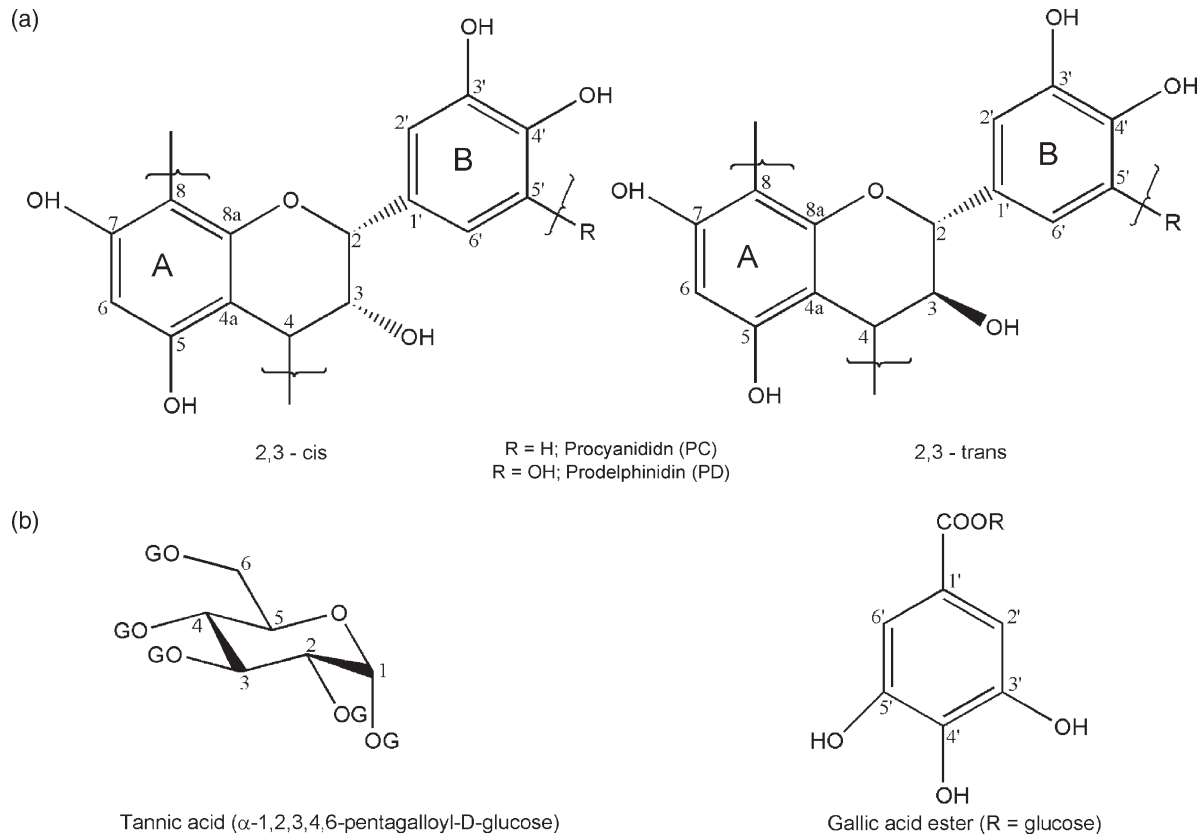

Fig. 1. Structures of (a) condensed and (b) hydrolyzable tannins.

variations in chain length, position of intermonomer linkages (e.g. C4–C6 or C4–C8 linkages in CT), stereochemistry, branching extent and substitution pattern. Gymnosperms and monocots produce only CT, while dicots can produce CT and/or HT (Bate-Smith, 1977; Haslam, 1988). Because tannins encompass a diverse group of compounds with distinct chemical structures it is likely that the different structural features confer disparate reactivities in soil (Hagerman et al., 1998). Previous work has shown that structural characteristics of tannins (CT vs. HT, PC vs. PD, *cis* vs. *trans*) are related to their tannin protein precipitation capacity and chemical reactivity (Kumar and Horigome, 1986; Porter and Woodruffe, 1984; Hagerman et al., 1998; Kraus et al., 2003a).

Nitrogen availability often limits net primary production in temperate forest ecosystems (Nadelhoffer et al., 1985; Vitousek and Howarth, 1991), therefore it is important to understand tannin influences on N cycling. While changes in nutrient dynamics are often measured in response to added tannins, the mechanism by which this occurs is not clear (Fierer et al., 2001). Tannins may decrease mineral N pools: (1) by sequestering proteins in protein–tannin complexes that are resistant to mineralization; (2) by complexing or deactivating microbial enzymes; (3) by inhibiting microbial activity through direct toxicity; and/or (4) by acting as a C source which can increase microbial N immobilization and decrease N mineralization from N-containing soil organic matter.

A number of studies have shown that litter materials high in tannins and/or polyphenols are associated with slower decomposition and N mineralization rates (Palm and Sanchez, 1991; Oglesby and Fownes, 1992; Constantinides and Fownes, 1994; Handayanto et al., 1997; Kalburtji et al., 1999; Driebe and Whitham, 2000). Other studies have attempted to clarify the specific role of tannins on soil processes by working with purified tannins (Basaraba and Starkey, 1966; Lewis and Starkey, 1968; Benoit et al., 1968; Benoit and Starkey, 1968a,b; McCarty and Bremner, 1986; Schimel et al., 1996,1998; Bradley et al., 2000; Fierer et al., 2001). Early work showed that leaf proteins combined with polyphenols are much more resistant to microbial decomposition than unaltered proteins (Handley, 1954, 1961; Davies et al., 1964; Benoit et al., 1968; Lewis and Starkey, 1968; Howard and Howard, 1993). More recently, Bradley et al. (2000) found that adding tannins purified from *Kalmia* (*Kalmia angustifolia* L.) and balsam fir (*Abies balsamea* (L.) foliage to black spruce (*Picea mariana* (Mill.) B.S.P.) humus reduced mineral N availability. Their results indicated that tannins decreased N mineralization by binding to and sequestering organic N sources rather than by suppressing microbes or by stimulating immobilization. A number of studies demonstrated tannin effects on nitrification, while

others found no effects (Baldwin et al., 1983; Olson and Reiners, 1983; Fierer et al., 2001). Schimel et al. (1996, 1998) found that balsam poplar (*Populus balsamifera*) tannins inhibited microbial activity, N-fixation, and respiration in Alaska taiga and thereby reduced mineral N pools. Fierer et al. (2001) reported that the higher molecular weight fractions bound N-containing substrates and reduced mineral N pools while lower molecular weight tannins (tetramers and smaller) appeared to act as substrates or toxins.

Considering that tannins are complex polyphenols that are chemically heterogeneous, it is not surprising that reports on the effects of tannins on C and N cycling differ. Thus, there is a need to account for the structural characteristics of tannins when considering their effects on soil processes. In most studies on tannins the structural characteristics of the tannins were not reported, nor were differences between tannins with different structures specifically examined. The objective of this study was to examine how different purified tannins alter soil C and N mineralization and to determine if tannins with diverse structures differentially influence nutrient dynamics. To accomplish this, we purified and characterized foliar tannins with various structures from five different plant species, added them to a mineral soil (A horizon) and examined the effects on C respiration, microbial biomass and N turnover.

2. Materials and methods

2.1. Study site

Plant and soil materials were collected from the Mendocino Ecological Staircase located on the northern California coast about 200 km north of San Francisco. The Ecological Staircase consists of terraces formed from the action of wave cutting, sea level fluctuation and tectonic uplift followed by eolian deposition of sand resulting in soils ranging from 100,000 to 500,000 years old (Jenny et al., 1969; Merritts et al., 1991). Soil fertility and pH decrease on the older terraces resulting in stunted forests (<3 m tall) referred to as pygmy forests.

Plant foliage was collected from five species growing in pygmy forest ecosystems located on the three oldest terraces (Table 1). Foliage was composited from 10 to 15 individuals of each species. Previous work at this site found that plants growing on these highly acidified (pH < 4) and low fertility soils (Spodosols and Ultisols) contain high concentrations of CT and total phenolics (Northup et al., 1995a).

Mineral soil (A horizon, 0–10 cm) was collected from eight different locations on the youngest and most fertile terrace (Inceptisols) under a stand of tall Bishop pine (*Pinus muricata* D. Dons). Samples were composited, sieved through a 2 mm screen, homogenized and stored at 2 °C. The soil pH (H_2O) was 5.0, and C and N contents were 28.7 and 1.77 g kg^{-1}, respectively. We chose soil from the youngest terrace because it does not receive litter with high tannin concentrations compared to older soils and because it has higher rates of N turnover (Yu et al., 2003).

Table 1
Species names and foliar phenol and condensed tannin concentrations for second year foliage

Common name	Latin name	Extractable phenols (mg g^{-1})[a]	Extractable condensed tannins (mg g^{-1})[a]	Residual condensed tannins (mg g^{-1})[a]
Bishop pine	*Pinus muricata*	144	198	15
Huckleberry	*Vaccinium ovatum*	237	174	9
Manzanita	*Arctostaphylos nummularia*	268	71	7
Rhododendron	*Rhododendron macrophyllum*	221	79	7
Salal	*Gaultheria shallon*	225	283	27

[a] Extractable (50:50 acetone:water) total phenols determined using the Folin Ciocalteu assay (Scalbert et al., 1989), extractable and residual CT determined using the acid butanol assay (Porter et al., 1986). Tannins purified from the species of interest were used as standards.

2.2. Tannin purification and characterization

Tannins were purified following the methods described by Schimel et al. (1996). Briefly, the plant material was freeze dried and ground in a Wiley mill (0.5 mm). The material was washed several times with methylene chloride and then extracted with acetone–water (80:20) followed by 100% methanol. After rotoevaporation to remove solvents, the acetone- and methanol-extractable fractions were combined and washed with hexane. The extracted material was loaded on a Sephadex LH-20 column, washed with methanol–water (50:50) to remove low molecular weight phenolics and flavonoids, and then washed with acetone–water to remove the bonded tannin fraction from the column. The eluted acetone–water solution was rotary evaporated and freeze dried.

Total C and N concentrations of the purified tannins were determined by dry combustion (Carlo Erba analyzer). Structural characteristics of the purified tannins were determined by ^{13}C NMR spectroscopy as described by Kraus et al. (2003a). A minor modification was made to increase the precision of integral areas within the C2–C3 region. Rather than measuring the integral heights, areas were measured by photocopying this region of the spectrum, then cutting according to the integral regions, and weighing the pieces.

2.3. Tannin addition rate study

Tannins purified from huckleberry and manzanita foliage were added directly to soil at rates of 0, 2, 5, 10, 20 and 40 mg g^{-1} soil. Tannins purified from huckleberry foliage were 100% CT while tannins from manzanita foliage

Table 2
Characterization of the purified tannins

Common name	% C	% CT [a]	% PC[b]	% CIS[c]	Average chain length[d]	P:T ratio (μg μg^{-1})[e]
Bishop pine	56.9	100	22	76	9.1	0.70
Huckleberry	57.7	100	97	86	3.7	0.69
Manzanita	59.2	41	32	ND	ND	0.51
Rhododendron	50.6	68	88	77	4.3	0.59
Salal	49.9	100	37	79	4.6	0.58
Tannic acid	43.5	0	n.a.[f]	n.a.	n.a.	1.52

[a] % CT, percent condensed tannin versus hydrolyzable tannin.
[b] % PC, percent procyanidin versus prodelphinidin CT monomer units.
[c] % CIS, percent *cis* versus *trans* stereochemistry at CT C2-C3, ND, no determination due to interference of hydrolyzable tannins.
[d] Average chain length (number of monomeric units) for condensed tannins.
[e] Protein precipitation capacity, P:T, mass of bovine serum albumin precipitated per mass tannin. Previously determined for these species, see Kraus et al. (2003).
[f] n.a., not applicable, tannic acid does not contain condensed tannins.

consisted of 41% CT and 59% HT (Table 2). A concentration of each tannin type comprised one treatment. Treatments were replicated three times. Soil moisture content was adjusted to 55% of soil water holding capacity to provide favorable conditions for microbial activity. Bulk soil samples were pre-incubated at 20 °C for 1 week to allow microbial respiration to stabilize. Following this pre-incubation, aliquots of soil (5.0 g dry weight equivalent) were weighed into individual test tubes, mixed thoroughly with the appropriate tannin treatments and placed in sealed 460 ml Mason jars fitted with septa. Each jar contained 2 ml of water to maintain soil moisture content. Samples were incubated for 54 days at 20 °C. Carbon dioxide evolution was measured every 3–7 days during the course of the incubation by sampling the headspace (1 ml) and analyzing the CO_2 concentration using an infrared gas analyzer. Jars were opened and vented occasionally to keep CO_2 concentrations below 1%.

At time zero and after 54 days, soils were extracted with 30 ml of 2.0 M KCl and gravity filtered through pre-washed Whatman #42 filter paper. Extracts were analyzed for mineral N (NH_4^+ and NO_3^-) by conductimetric analysis (Carlson, 1978, 1986). Net N mineralization (change in concentration of NH_4^+ and NO_3^-) was calculated by subtracting the mineral N concentration determined at time zero from the mineral N concentration of the soils incubated for 54 days.

2.4. Tannin source study

The effect of tannin source on C and N dynamics was evaluated using eight different treatments: control (no addition), five purified tannins (Bishop pine, huckleberry, manzanita, rhododendron, salal), tannic acid and cellulose Tannic acid (Fisher, Lot #922178), is a commercially available HT. Cellulose (J.T. Baker, Inc, Lot #C33706) was included to represent the effects of an added carbon source. A treatment level of 10 mg tannin g^{-1} soil was chosen for this experiment. This concentration was shown to reduce N mineralization in our preliminary rate study, and was within the range of tannin addition (10–45 mg tannin g^{-1} soil) utilized in similar studies (Lewis and Starkey, 1968; Schimel et al., 1996; Bradley et al., 2000; Fierer et al., 2001). While senescent foliage typically contains 100–250 mg tannin g^{-1}, this amount of tannin is appreciably diluted upon mixing with mineral soil. Therefore, a 10 mg tannin g^{-1} soil addition is an ecologically realistic amount. Cellulose was added at a C-equivalent weight (10.9 mg cellulose g^{-1} soil) assuming a tannin C content of 50% and a cellulose C content of 44.4%.

Treatments were applied and soils were incubated for 32 days as described above for the tannin addition rate experiment, with the exception that 6.5 g of soil dry weight equivalent was used and that each treatment was replicated five times. A total of 240 test tubes were divided into six sets (eight amendments × five replications × six sets = 240) for determination of: (1) CO_2 evolution, net N mineralization, non-fumigated total organic C (TOC) and non-fumigated dissolved organic N (DON); (2) chloroform fumigated TOC and DON; (3) time zero ^{15}N for gross ammonification; (4) 24 h ^{15}N for gross ammonification; (5) time zero ^{15}N for potential gross nitrification; and (6) 48 h ^{15}N for potential gross nitrification. Following the incubation period, samples were analyzed as described below to assess N turnover rates and microbial biomass. In addition, time zero mineral N concentrations were measured on 10 unamended soil samples.

Mineral N concentrations (NH_4^+ and NO_3^-) were determined at the beginning and end of the incubation by extraction with 0.5 M K_2SO_4 (30 ml per test tube) as described above. Gross ammonium and nitrate production and consumption rates were determined using ^{15}N pool dilution techniques (Hart et al., 1994). For gross ammonification, ^{15}N labeled NH_4SO_4 (0.3 ml, 9.82 Atom% ^{15}N) was added to samples at a rate of 10 μg N g^{-1} dry soil. For potential gross nitrification, in addition to adding ^{15}N labeled KNO_3 (0.3 ml, 9.65 Atom% ^{15}N) at a rate of 10 μg N g^{-1} dry soil, unlabeled NH_4SO_4 (20 μg N g^{-1} soil) was added to ensure the presence of an NH_4^+ pool to undergo nitrification.

Control soils received 0.3 ml of distilled–deionized water to maintain the same water content as the ^{15}N-spiked soils. Isotope additions were made to paired soil samples; one soil was extracted within 1 min with 30 ml 0.5 M K_2SO_4 and the other was extracted after an incubation of 24 h for $^{15}NH_4^+$–N measurements and 48 h for $^{15}NO_3^-$–N measurements. Although a 48 h incubation time may allow some re-mineralization of added $^{15}NO_3^-$, in preliminary trials we found that we could not detect a change in the NO_3^- pool during shorter time periods. Mineral N in the extracts was measured conductimetrically as described above. Isotopic N levels were determined by concentrating 20 ml samples using the acid trap/diffusion method described by Stark and Hart (1996). Because NH_4^+ concentrations in the tannic acid ^{15}N-spiked samples incubated for 24 h were low (5–8 μg N in 20 ml), unlabeled NH_4^+ (50 μg N) was added to these vials to ensure sufficient N concentrations for reliable isotope analysis. Samples were analyzed for ^{15}N on an isotope-ratio mass spectrometer (Europa Scientific, Crew, UK). Gross NH_4^+ and NO_3^- production and consumption rates were calculated using equations given by Davidson et al. (1991). The addition of NH_4^+ and NO_3^- in the pool dilution assay may cause an overestimation of gross consumption but are useful to compare across treatments (Hart et al., 1995).

Microbial biomass C and N were determined using the chloroform fumigation extraction technique with a 48 h fumigation time (Horwath and Paul, 1994). Soils extracts (30 ml 0.5 M K_2SO_4) were analyzed for TOC using a UV-persulfate carbon analyzer (Techmar-Dohrman, Phoenix 8000). Total dissolved N was determined by persulfate digestion followed by determination of NO_3^-–N (Yu et al., 1994). Microbial biomass C and N were calculated by subtracting total C and N of non-fumigated from those of fumigated samples. We did not correct for extraction efficiency but instead report biomass C and N as the 'flush' of C and N released from fumigated versus non-fumigated samples (Fierer et al., 2001).

2.5. Statistical analyses

The effect of tannin concentration and tannin source on soil C and N variables were analyzed by one-way ANOVA. Significant differences between treatments were evaluated using Tukey's test of least significant differences ($P < 0.05$). All analyses were performed using Systat version 7.0 (SPSS Inc., 1997).

3. Results

3.1. Tannin characterization

Carbon concentrations for the purified tannins ranged between 500 and 550 mg g^{-1}. Nitrogen concentrations in the purified materials were below the detection limit for the Carlo Erba analyzer (<0.3 mg g^{-1}). The structural characteristics of the purified tannins determined by ^{13}C NMR are given in Table 2. These results are similar to those found for tannins previously purified from these species (Kraus et al., 2003a). It is important to note that this method provides average structural characteristics, and that the purified samples contain a mixture of tannins with different chain lengths, hydroxylation patterns and stereochemistry. Purified tannins from Bishop pine, huckleberry, and salal contained only CT, each with varying amounts of PC versus PD and *cis* versus *trans* units. Average CT chain length varied between 3.7 for huckleberry and 9.1 for Bishop pine. Rhododendron and manzanita contained both CT and HT structures. Due to interference by HT peaks, CT chain lengths and % *cis* stereochemistry were not determined for manzanita tannin. In qualitative terms, the % *cis* stereochemistry in the manzanita tannin appeared to be high, similar to the value of 84% reported for a previous preparation (Kraus et al., 2003a). Chain length and stereochemistry were calculated for rhododendron, however, the values may be slightly affected by HT interference. We estimated this effect by assuming the same peak shapes as for huckleberry tannin with similar characteristics, and assigning the rest to HT. This gave somewhat lower values for % CIS and chain length (71 and 3.5%, respectively), than reported in Table 2.

3.2. Tannin addition rate study

At tannin addition rates of 5–40 mg tannin g^{-1} soil, respiration increased significantly in samples amended with tannins from manzanita foliage (Fig. 2a). In contrast, huckleberry tannins did not significantly increase C mineralization relative to the control (rate = 0), even at addition rates as high as 40 mg g^{-1} soil.

Relative to the control, net N mineralization over the 54 day incubation period decreased significantly for all treatments (Fig. 2b). Net N mineralization was negative for soils amended with manzanita tannins and there were no differences between rate treatments. Nitrogen mineralization also decreased significantly in the presence of huckleberry tannins, and changes were similar for addition rates between 5 and 40 mg tannin g^{-1} soil.

3.3. Influence of tannins on C mineralization

In the tannin source study, C respiration rates decreased considerably during the first 2 weeks (Fig. 3). Tannic acid additions resulted in the greatest increase in C mineralization rates relative to the control, followed by manzanita, salal, Bishop pine and cellulose treatments. The rhododendron treatment had significantly greater rates of respiration than the control during the first 10 days, but thereafter respiration rates were similar to the control. As was observed in the rate study (data not shown), huckleberry tannins increased C mineralization slightly during the first few days of the incubation but subsequently the C respiration rate was not significantly different from the control.

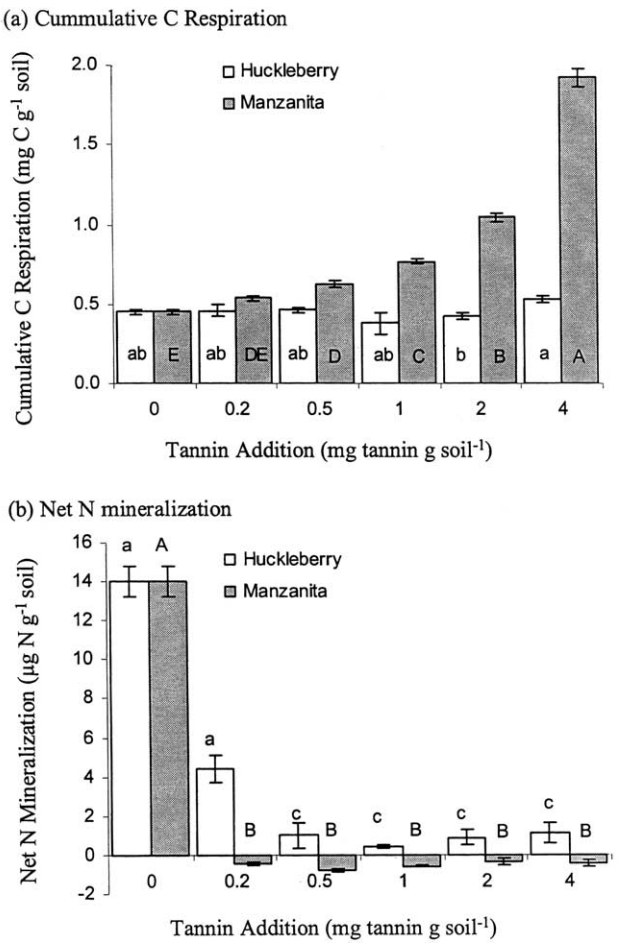

Fig. 2. Cumulative CO_2–C production (a) and net N mineralization (b) during the 54 d incubation experiment for huckleberry and manzanita tannins applied at different rates. Treatments associated with the same lower or upper case letters are not significantly different ($P > 0.05$). Vertical bars indicate ± 1 SE, $n = 3$.

Cumulative CO_2–C mineralized during the 32 day incubation experiment reflects the same trends as the 54 day incubation rate data (Fig. 4). All of the additions except huckleberry tannins significantly increased total C released. The additions of manzanita, salal and Bishop pine tannins almost doubled the amount of C released while tannic acid resulted in a 4-fold increase. Cellulose and rhododendron treatments produced a 1.8-fold and 1.4-fold increase in C mineralization, respectively.

Assuming that the total increase in respired C relative to the untreated control resulted from mineralization of the added amendment, 16.5% of the added C from tannic acid was metabolized to CO_2 during the 32 day incubation experiment. Using the same assumptions, less than 5% of the added C was metabolized in the Bishop pine, manzanita, rhododendron, salal and cellulose treatments. Except for the small amount of C that was mineralized during the first few days, it appears that huckleberry tannins were not used as a C source.

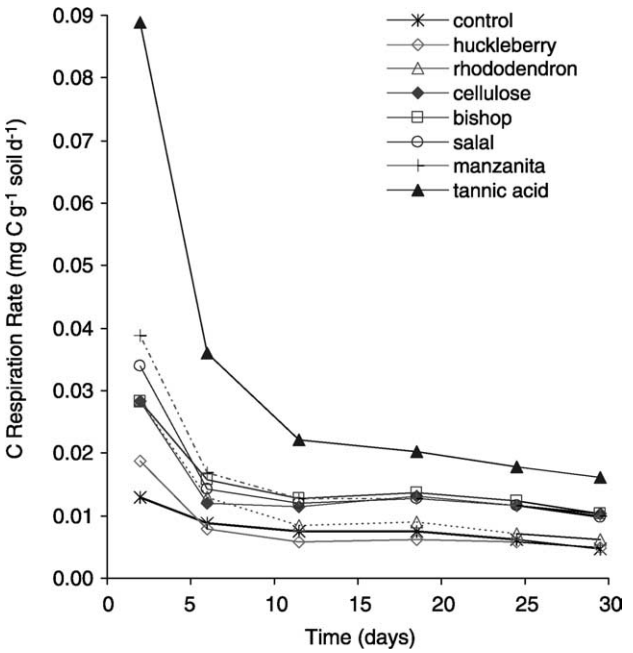

Fig. 3. Respiration rates during the 1 month incubation experiment for treatments with 10 mg tannins g^{-1} soil additions. The standard error associated with each data point is less than 0.001, $n = 5$.

3.4. Influence of tannins on N mineralization

Mineral N concentrations at the start of the experiment were 1.04 µg NH_4^+–N g^{-1} soil and 5.47 µg NO_3^-–N g^{-1} soil. Over the 32 day incubation experiment, mineral N concentrations only increased in the control soil (Fig. 5). Additions of the five purified tannins and cellulose were associated with a 70–80% decrease in NH_4^+ concentration compared to the time zero NH_4^+ concentration, while

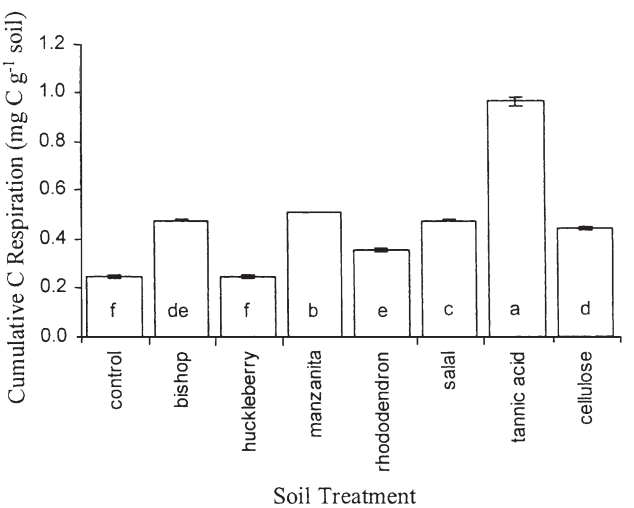

Fig. 4. Cumulative CO_2–C production during the 1 month incubation experiment for treatments with 10 mg tannin g^{-1} soil additions. Treatments associated with the same lower or upper case letters are not significantly different ($P > 0.05$). Vertical bars indicate ± 1 SE, $n = 5$.

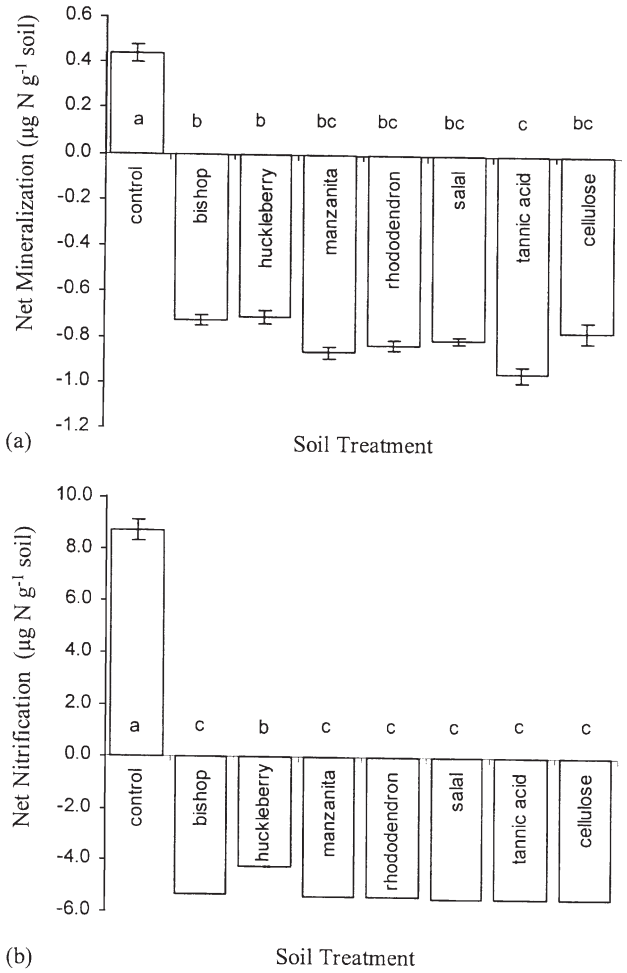

Fig. 5. Net N mineralized during the 1 month incubation experiment for treatments with 10 mg tannin g^{-1} soil additions: (a) NH_4^+–N and (b) NO_3^-–N. Treatments associated with the same lower or upper case letters are not significantly different ($P > 0.05$). Vertical bars indicate ±1 SE, $n = 5$.

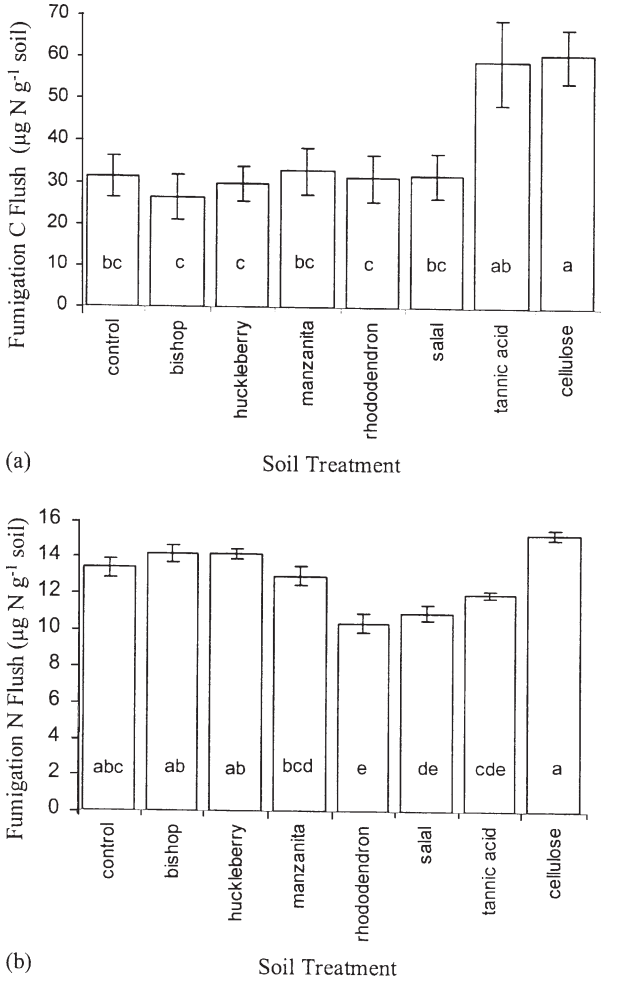

Fig. 6. Microbial biomass measured 1 month after the 10 mg tannin g^{-1} soil treatment additions: (a) microbial biomass C presented as the TOC chloroform fumigation flush, (b) microbial biomass N presented as the DON chloroform fumigation flush. Treatments associated with the same lower or upper case letters are not significantly different ($P > 0.05$). Vertical bars indicate ±1 SE, $n = 5$.

the tannic acid treatment appeared to immobilize over 90% of the time zero NH_4^+. Except for the huckleberry treatment that had NO_3^- concentrations of 1.67 μg N g^{-1} soil, virtually all of the NO_3^- was lost by day 32 in the amended soils. Because the soil was well aerated, we assumed that denitrification was minimal and therefore losses of NH_4^+ or NO_3^- are attributed to microbial uptake or conversion of NH_4^+ to NO_3^-.

3.5. Influence of tannins on microbial biomass C and N

No significant change in microbial biomass C was measured relative to the control in the five purified tannin treatments (Fig. 6a). In contrast, both tannic acid and cellulose treatments resulted in significant microbial biomass increases as reflected in a doubling of the TOC flush. Differences in microbial biomass N were not as great as differences in microbial biomass C and showed different trends than microbial biomass C (Fig. 6b). Relative to the control, rhododendron and salal treatments had significantly lower microbial biomass N.

3.6. Influence of tannins on gross NH_4^+ production/consumption

Gross ammonification rates were significantly lower in all of the amendmended soils (Fig. 7). Relative to the control, the gross NH_4^+ production rate was decreased 32% by cellulose, 50% by manzanita tannins, and about 70% by the other amendments. The gross NH_4^+ consumption rate was increased significantly by all of the amendments (Fig. 7). Tannic acid and cellulose increased the gross NH_4^+ consumption rate 15–20 fold while manzanita tannins increased consumption rate 12.5-fold. In the tannic acid treatment, greater than 90% (8.3 μg N g^{-1} soil) of the NH_4^+ added was consumed during the 24 h incubation period. Amendment with Bishop pine, rhododendron and salal tannins showed similar increases in NH_4^+ consumption

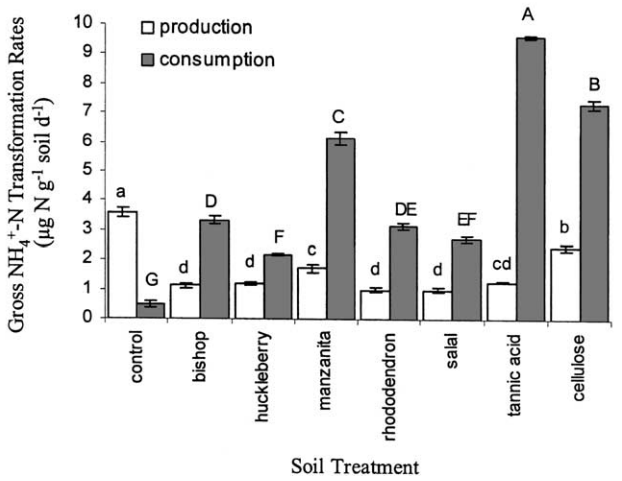

Fig. 7. Gross NH_4^+ production and consumption rates measured 1 month after the 10 mg tannin g^{-1} soil treatment additions using isotope pool dilution techniques. Treatments associated with the same lower or upper case letters are not significantly different ($P > 0.05$). Vertical bars indicate ± 1 SE, $n = 5$.

(5.6–6-fold), and huckleberry tannins increased consumption the least (4.4-fold).

3.7. Influence of tannins on NO_3^- production/consumption

All soil samples maintained a significant pool of NH_4^+ (>7.5 μg N g^{-1} soil) throughout the 48 h incubation, thus NH_4^+ was available for nitrification. Nevertheless, potential gross nitrification rates were low compared to gross ammonification rates even in the control soil (Fig. 8; 0.13 μg NO_3^-–N g^{-1} soil day^{-1}). These rates are lower than those measured in the field ($\sim$0.5 μg NO_3^-–N g^{-1} soil day^{-1}) at the same study site (Yu et al., 2003). Only addition of Bishop pine tannins, which resulted in no measurable gross NO_3^- production, was significantly different from the control. Consumption of NO_3^- was much lower than consumption of NH_4^+–N (0.13 vs. 3.59 μg N g^{-1} soil day^{-1} in the control soil). Addition of the five purified tannins had no significant effect on NO_3^- consumption, while tannic acid and cellulose additions increased gross NO_3^- consumption rate by about 3.5-fold relative to the control.

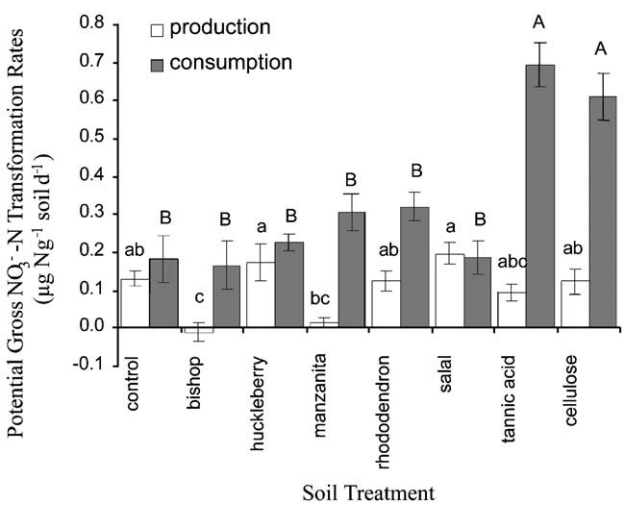

Fig. 8. Potential gross NO_3^- production and consumption rates measured 1 month after the 10 mg tannin g^{-1} soil treatment additions using isotope pool dilution techniques. Treatments associated with the same lower or upper case letters are not significantly different ($P > 0.05$). Vertical bars indicate ± 1 SE, $n = 5$.

4. Discussion

4.1. Tannin addition rate study

Results from our rate experiment indicate that tannin additions as low as 2 mg g^{-1} soil (0.2%) may significantly reduce net N mineralization. Amendment with huckleberry tannins did not significantly increase C respiration, even at rates as high as 40 mg g^{-1} soil, indicating that tannins derived from huckleberry foliage were not used as a microbial C source. In contrast, tannins purified from manzanita foliage at rates of 5 mg g^{-1} soil or greater significantly increased C respiration. No tannins reduced soil C respiration, indicating that these tannins were not inhibitory or toxic to the microbial community.

Previous studies that examined tannin effects on soil nutrient dynamics used different tannin sources, different tannin concentrations and different soils (e.g. organic vs. mineral soil). For example, Lewis and Starkey (1968) added six different tannins (3 HT, 3 CT) to A horizon forest soil at a rate of 10 mg g^{-1} soil; Schimel et al. (1996) applied balsam poplar CT at rates of 20 and 50 mg g^{-1} soil to taiga O horizon soil; Bradley et al. (2000) added 30 mg g^{-1} soil *kalmia* and balsam fir CT over an 8 week period to black spruce humus; and Fierer et al. (2001) added balsam poplar CT fractions at a rate of 45 mg g^{-1} soil to taiga Oa and Oe horizon material. Due to differences in experimental design, comparison of results among studies is difficult. Nevertheless, as was found here, all of these studies reported significant reductions in N mineralization while the effects on C mineralization were more variable.

4.2. Influence of tannins on C mineralization

Tannins, like lignin and other polyphenols, are generally assumed to be resistant to decomposition, however, our results show some tannins are readily degraded. Some microbes are known to use tannins as a sole C source (Lewis and Starkey, 1968, 1969; Scalbert, 1991; Makkar et al., 1994; Bending and Read, 1996, 1997; Gamble et al., 1996; Field and Lettinga, 1992; Bhat, 1998). In this study, except for the huckleberry tannins, added tannins increased soil respiration relative to the control soil which suggests that these tannins were acting as a microbial C source. In fact, amendment with Bishop pine and salal tannins increased C mineralization as much as cellulose, an easily metabolized

C-source, while tannic acid and manzanita tannins were significantly more degradable than cellulose. Other studies also found a wide variability in tannin decomposability. Lewis and Starkey (1968) examined the decomposition of six tannins over 60 days and found that four of them underwent appreciable decomposition (>27%) while two showed little CO_2 release. In contrast, Schimel et al. (1996) found that balsam poplar tannins decreased soil respiration 40% over a 30 day incubation period. These inconsistent results indicate that different types of tannins are processed differently by soil microbes.

Some reports indicate that lower molecular weight tannins are more easily biodegraded than higher molecular weight tannins (Lewis and Starkey, 1968; Field and Lettinga, 1992; Fierer et al., 2001). Fierer et al. (2001) separated purified tannins from balsam poplar into different size fractions and found that the smaller tannins (<3 monomers) generally increased C mineralization, especially during the first 10 days after addition. In contrast, the higher molecular weight fractions showed no evidence of being metabolized and even inhibited microbial activity in some soils. In our study, only a small percentage (<17%) of the added C was released as CO_2 during the 32 day incubation, and most of this C release occurred during the first week of incubation. Based on ^{13}C NMR characterization of the purified tannins, average CT chain length was about five monomers (Table 2). Thus, the initial flush of C may reflect microbial degradation of the more labile lower molecular weight tannins found in the tannin mixtures. The decrease in respiration over time may also result from the added tannins being less available for microbial degradation as they became sorbed to soil particles or reacted with organic matter. However, because there was also a decrease in degradation of added cellulose, it seems more likely that microbial activity was limited by N availability or other factors. Large increases in gross NH_4^+ consumption rates (Fig. 7) in the presence of all added amendments support the idea that the microbial community was N limited.

Among the purified tannins included in this study average CT chain length ranged between 3.7 and 9.1. Assuming that increased C respiration indicates microbial degradation of the added amendments, in contrast to the findings of Fierer et al. (2001) we did not find that purified tannins containing shorter chained CT were more labile than those containing longer chained tannins. Samples amended with huckleberry tannins that had the lowest average chain length (3.7) showed no increase in C respiration while amendment with Bishop pine tannins with the longest average chain length (9.1) increased C respiration almost 2-fold in the 1 month incubation experiment.

HTs are often reported to be more susceptible to microbial attack than CT (Handley, 1954; Lewis and Starkey, 1968; Field and Lettinga, 1992; Bhat et al., 1998). We found that tannic acid, which is a pure HT, had greater lability than the other amendments. Cumulative C respired from soils receiving tannic acid was 4-fold greater than from control soils, and at least 2-fold greater than that associated with additions of the five other tannins and cellulose (Fig. 4). However, addition of manzanita tannins that contain about 59% HT had only a small increase in C mineralization compared to the other tannin treatments. Samples with rhododendron tannin additions that contain about 32% HT had significantly lower C mineralization than samples treated with Bishop pine and salal tannins that contain only CT. Therefore, our data do not support the generalization that all HT are more easily degraded than CT. Rather, it appears that commercially available tannic acid specifically acts as a readily available microbial C source.

Stereochemistry is another structural characteristic that may affect tannin reactions. Within the group of CT, monomer units can be made up of PC or PD units with two or three hydroxyl groups on the B-ring, respectively. There is evidence that PD monomers are more labile and more susceptible to chemical transformation by abiotic processes than PC monomers (Hernes et al., 2001). Our study included three pure CT with mixtures of PD and PC. Huckleberry tannins that had the highest proportion of PC units (97%) had no affect on C mineralization. Bishop pine (22% PC) and salal (37% PC) tannin treatments had similar C mineralization rates. The purified rhododendron tannins contained 68% CT with 77% PC units, and soils amended with these tannins had significantly lower C mineralization than the Bishop pine and salal tannin treatments. Although these comparisons are limited, our data support the suggestion that PC units are less labile than PD units.

4.3. Influence of tannins on N mineralization

All of the additions decreased net N mineralization relative to the control (Fig. 5). Based on ^{15}N pool dilution data, the decrease in NH_4^+ resulted both from a decrease in production and an increase in consumption (Fig. 7). Results from the NO_3^- pool dilution data are less clear; only Bishop pine and manzanita treatments had a marked reduction in NO_3^- production, and only tannic acid and cellulose treatments significantly increased consumption rates relative to the control.

An increase in N consumption (i.e. immobilization) is expected with the addition of a C source that stimulates microbial activity. This was evident in the cellulose treatment where addition of an available C source increased respiration and microbial biomass C about 2-fold, and resulted in significantly greater consumption of mineral N (Figs. 7 and 8). Tannic acid, which appears to be more labile than cellulose as reflected in the higher rates of C mineralization and similar increase in microbial biomass C, also exhibited a large increase in gross mineral N consumption (Figs. 5, 7 and 8). Although addition of huckleberry tannins did not increase C respiration nor microbial biomass relative to the control, net N mineralization was greatly reduced due to both an increase in NH_4^+

consumption and a decrease in NH_4^+ production. However, treatments that received huckleberry tannins had significantly lower net nitrification and gross NH_4^+ consumption than the other tannin treatments, suggesting that increased lability of the tannin is associated with greater effects on N availability.

The addition of a labile C source can reduce gross N mineralization due to a reduction in microbial decomposition of other N-containing soil organic matter (Schimel et al., 1992). Lower NH_4^+ production rates were seen in all of the amended soils suggesting such an inhibition mechanism (Fig. 7). However, that cellulose was less effective at lowering gross NH_4^+ production than were tannins, suggests that the functional link between tannins and N cycling is different than for cellulose. There were no decreases in microbial respiration or in microbial biomass C with the tannin amendments; therefore, it seems unlikely that the tannins had a direct toxic effect on the microbial community. Similarly, because binding of microbial exoenzymes would lead to a decrease in microbial respiration and biomass, it appears that microbial enzyme activity remained unaffected. Thus, while some of the decrease in the available N pool is likely due to the addition of a C source which can increase microbial immobilization and decrease N mineralization from N-containing soil organic matter, it appears that the tannins are also inhibiting production of NH_4^+ by sequestering organic N sources. It has been suggested that immobilization of N into complex organic structures could occur through reaction of amino groups with quinones derived from the tannin B-ring, with greater reactivity expected for PD tannins and basic amino acids (Hernes et al., 2001). These results are consistent with the conclusions of Bradley et al. (2000) and Fierer et al. (2001) whose data also indicated that tannins inhibit N mineralization by binding extracellular substrates.

4.4. Influence of tannins on nitrification

Inhibition of nitrification could be an important factor which helps conserve ecosystem N by maintaining mineral N as NH_4^+ which is less prone to leaching than NO_3^- and which is not lost by denitrification. Although we saw no significant effect of most added tannins on potential gross nitrification rates (Fig. 8), the rate of potential nitrification in the soil was low to begin with ($0.13\ \mu g\ NO_3^- – N\ g^{-1}$ soil day^{-1}). Because of the low rate, it was difficult to detect reductions in nitrification due to treatment effects. Testing these purified tannins in a soil that has higher ambient nitrification rates would help elucidate whether these particular tannins inhibit nitrification.

While some studies have reported that tannins inhibit nitrification (Rice and Pancholy, 1973; Lodhi and Killingbeck, 1980; Baldwin et al., 1983; Olson and Reiners, 1983), others found no effects (McCarty and Bremner, 1986; Clein and Schimel, 1995; Schimel et al., 1996; Fierer et al., 2001). Rice and Pancholy (1973) found that tannins (CT, CT + HT mixtures and tannic acid) at concentrations as low as 2 ppm inhibited *Nitrosonomas* and *Nitrobacter*. Lodhi and Killingbeck (1980) reported a large (93%) inhibition of *Nitrosonomas* by plant and soil extracts containing CT. Baldwin et al. (1983) partitioned CT from a forest floor using aqueous methanol extraction and measured a reduction in nitrate production when this material was added to soil. However, in these cases the reduction in nitrification may be due to decreased NH_4^+ availability rather than to inhibition of nitrification itself (Basaraba, 1964; McCarty and Bremner, 1986).

In contrast, McCarty and Bremner (1986) found that adding five different tannins to mineral soil at concentrations up to $0.25\ mg\ g^{-1}$ along with $(NH_4)_2SO_4$ had no effect on nitrification. These tannin additions are much lower than the rates used in this study ($2–40\ mg\ tannin\ g^{-1}$ soil). More recent studies which added tannins at rates of $20–50\ mg\ g^{-1}$ mineral soil or humus materials also found no evidence that tannins specifically inhibit nitrifier activity (Schimel et al., 1996; Fierer et al., 2001). Although at this time it appears that tannins do not specifically inhibit nitrification, studies using an array of tannins and soils are needed to confirm this notion.

4.5. Influence of tannins on C and N dynamics

Significant differences among the six tannins examined in this study were apparent. While tannic acid additions increased C mineralization by more than twice that of manzanita, salal, Bishop pine and rhododendron tannins, the addition of huckleberry tannins showed no increase in C mineralization. These results contrast with those of Schimel et al. (1996) that found additions of balsam poplar tannins inhibited soil respiration rates by 40%. The specific structures of the tannins included in the purified tannin mixtures should account for these differences. While Fierer et al. (2001) found that shorter chained balsam poplar CT were more labile and less inhibitory than the longer chained CT, we found the opposite trend in lability. From our data it appears that other characteristics such as CT versus HT and PC versus PD content of the tannins also influence tannin reactivity. Although tannic acid, the only pure HT included in this study, did show greater lability than the other tannins, manzanita and rhododendron tannins that contain 59% and 32% HT, respectively, were not significantly more labile than the pure CT. These three tannins that contain varying amounts of HT all had similar inhibitory effects on net and gross mineralization rates. Thus, our data do not support the generalization that HT are more labile and less inhibitory than CT. As discussed above, there is some indication that CT containing PD units are more labile than those containing PC units.

Overall, it appears that tannic acid is significantly more labile than the five purified tannins examined in this study. Soils amended with tannic acid had much greater C mineralization rates, and tannic acid was the only tannin

that resulted in significantly greater microbial biomass C and gross NO_3^- consumption relative to the control. In a previous study we also demonstrated that tannic acid had a much higher (2–5-fold) protein precipitation capacity than nine other purified tannins (Kraus et al., 2003a). Because tannic acid is commercially available it is often used to study the effects of tannins on ecosystem processes. However, this tannin may not be representative of tannins commonly found in coniferous forests and we believe that its inclusion as a representative HT has lead to some biases regarding the reactivity of HT versus CT.

5. Conclusions and implications

Plants greatly influence soil nutrient dynamics by the chemical quality of the litter they produce (Melillo et al., 1982, 1989; Aber et al., 1990), and tannins have specifically been implicated as an important factor regulating nutrient cycling in forest ecosystems (Kraus et al., 2003b). In this study, the ability of tannins to significantly decrease N mineralization was notable at addition rates as low as 2 mg g^{-1} soil (0.2%). Added at a rate of 10 mg g^{-1} soil, all of the amendments decreased net N mineralization. Because the six tannins were significantly more inhibitory of gross ammonification rates than cellulose, we believe that a factor other than the addition of a labile C source was responsible for the inhibition. Notably, addition of huckleberry tannins did not increase C mineralization yet still decreased gross NH_4^+ production. Thus, our investigation supports the hypothesis that tannins reduce N availability by sequestering organic N sources (Schimel et al., 1996; Bradley et al., 2000; Fierer et al., 2001), in addition to increasing N immobilization when serving as a C source.

While decreased rates of nutrient cycling are usually associated with decreased site quality, slower rates of nutrient release can help improve synchrony between mineralization and uptake and thereby reduce ecosystem nutrient losses (Handley, 1961; Zucker, 1983; Horner et al., 1987, 1988; Kuiters, 1990; Northup et al., 1995b, 1999; Berendse, 1998; Driebe and Whitham, 2000). In infertile ecosystems that have fewer nutrients to lose, reduced mineral release rates may provide an important nutrient conservation mechanism. Furthermore, the production of elevated levels of foliar polyphenols and tannins in some plant species appears to give them a competitive advantage by altering nutrient cycling processes to the point where forest dynamics are altered (Kraus et al., 2003b and references therein).

While litter quality influences soil processes, soil conditions in turn can affect litter chemistry. A number of studies have found that plant tannin concentrations are greater in plants growing in low fertility soils (Gebauer et al., 1998; Penuelas and Estiarte, 1998; Booker and Maier, 2001; Kraus, 2003c). Moreover, besides evidence that soil conditions influence plant tannin concentrations, plants may produce different types of tannins under different environmental conditions (Howard and Howard, 1993; Gallet and Lebreton, 1995). Determining how edaphic and environmental conditions interact with the production of specific tannin types and quantities would help elucidate how structure relates to the role of tannins in ecosystem function. Soil conditions (e.g. nutrient concentrations, pH, mineralogy, and microbial community) are also likely to influence how tannins are processed in soil. Fierer et al. (2001) demonstrated that soils respond differently to tannin additions. These authors surmised that differences in soil microbial communities, perhaps shaped by prior exposure to different litter qualities, may account for the different ways in which tannins alter C and N mineralization among soil types.

The results from this study and others (Hernes et al., 2001; Fierer et al., 2001; Kraus et al., 2003a) demonstrate that tannin structure is an important factor influencing C and N dynamics and emphasizes a need to improve our understanding of the interactions among tannin structure, tannin quantity and soil processes. Because tannin structure strongly affects how these compounds interact in soil, generalization about tannin influences on ecosystem processes should consider different types of tannins obtained from natural systems.

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