



# Influence of balsam poplar tannin fractions on carbon and nitrogen dynamics in Alaskan taiga floodplain soils

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Accepted 8 May 2001

## Abstract

The feedbacks between plant and soil processes play an important role in driving forest succession. One poorly understood feedback mechanism is the interaction between plant secondary chemicals and soil microbes. In the Alaskan taiga, changes in nutrient cycling caused by balsam poplar (*Populus balsamifera*) secondary chemicals may affect the transition from alder (*Alnus tenuifolia*) to balsam poplar on river floodplains. We examined the effects of four poplar condensed tannin fractions on N cycling in alder and poplar soils. Tannins were added to forest floor samples from both poplar and alder sites. Samples were incubated for 1 month in the laboratory with soil respiration rates measured over the course of the incubation. At the end of the incubation we measured both net and gross nitrogen mineralization and nitrification, microbial biomass C and N, and the activity of various exoenzymes. In all soils, tannin additions reduced N availability, however, the mechanisms differed depending on the molecular weight of the tannin and the native soil microbial community. Low molecular weight tannin fractions served as a labile C source in poplar Oi, poplar Oe, and alder Oe horizons but were toxic to microbes in alder Oi. High molecular weight tannin fractions appeared to act primarily by binding extracellular substrates and thus limiting C and N mineralization, with the strongest effects observed in the alder soils. © 2001 Elsevier Science Ltd. All rights reserved.

**Keywords:** Succession; Nitrogen cycling; Mineralization; Tannin; Secondary chemicals; Balsam poplar; Taiga

## 1. Introduction

Disturbance and succession dominate the landscape structure of the Alaskan boreal forest (Viereck et al., 1986). The major disturbance regimes in interior Alaska include fire, leading to secondary succession, and river migration, leading to primary succession (Zasada, 1986). During primary succession, river bars are colonized by willow (*Salix*) and *Equisetum* species, which are replaced by thinleaf alder (*Alnus tenuifolia*) within roughly 10 years. After approximately 50 years, balsam poplar (*Populus balsamifera*) reaches canopy dominance with white spruce (*Picea glauca*) dominating on floodplain sites over 150 years old (Viereck et al., 1993).

The transition from alder to poplar is of specific interest because it is associated with major changes in soil carbon and nitrogen dynamics. Alder fixes large amounts of N relative to plant demand, so soil in alder dominated sites has abundant nitrogen with high rates of N turnover and high nitrification rates (Schimel et al., 1998). As poplar comes to

dominance in alder sites, the soil microbial community shifts from being C limited to N limited, reducing rates of mineralization and nitrification with  $\text{NH}_4^+$  becoming the dominant form of inorganic nitrogen (Schimel et al., 1998).

The changing availability of  $\text{NH}_4^+$  provides the most important constraint on N cycling during the alder-poplar transition by affecting plant uptake, microbial immobilization, and nitrification (Clein and Schimel, 1995). A key mechanism controlling this change in soil  $\text{NH}_4^+$  availability seems to be production of secondary carbon compounds by poplar (Schimel et al., 1996, 1998). In Alaska, balsam poplar tissues may contain high concentrations of condensed tannins (76 mg g<sup>-1</sup> dry wt.; Schimel et al., 1996) and other simple phenolics (80–120 mg g<sup>-1</sup> dry wt; Cates, unpublished data). While some poplar compounds (including simple phenolics) act as microbial substrates (Clein and Schimel, 1995), enhancing microbial growth and immobilization, poplar tannins act as inhibitors of microbial activity, inhibiting N-fixation, respiration, and N mineralization (Schimel et al., 1996). The mechanism(s) behind this inhibition of soil microbial activity by poplar tannins is not presently clear. However, other studies have reported that tannins are able to inhibit microbial activity through direct toxic effects (Scalbert, 1991; Field and Lettinga,

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1992; Schulz et al., 1992), by complexing extracellular enzymes (Benoit and Starkey, 1968; Scalbert, 1991), and by complexing protein substrates (Swain, 1979; Bradley et al., 2000).

The effects of condensed tannins on microbial activity in soil are probably more complex than shown by initial experiments performed in Alaska (Clein and Schimel, 1995; Schimel et al., 1996). Tannins are not a uniform entity. While condensed tannins are all made from a limited group of polyhydroxyflavan monomers that have similar chemical properties, the degree and nature of polymerization varies (Waterman and Mole, 1994). The monomers can bind in different ways to produce a variety of linear and branched structures. Thus, the size and molecular weight of condensed tannins can vary dramatically (Waterman and Mole, 1994). Depending on the degree of polymerization, tannins may be processed by different groups of microorganisms (Lewis and Starkey, 1968; 1969; Gamble et al., 1996; Bhat et al., 1998). Also, soil microbial communities may adapt to specific tannin chemistries during forest succession (Cowley and Whittingham, 1961). Thus, it is not surprising that studies of tannin effects on soil microbial processes have produced somewhat variable conclusions. By not considering the size of tannins and their effects on different microbial communities, our full understanding and ability to generalize tannin effects across ecosystems have been limited.

We initially had two alternative hypotheses for the behavior of different tannin fractions. As small molecules can be taken up by microbes, while large molecules generally cannot, one possibility was that the light fractions would behave qualitatively differently than the heavy fractions. Thus, the light fractions might act within microbial cell as either substrates (as suggested by Sugai and Schimel, 1993), or toxins (Bhat et al., 1998). Heavy fractions, on the other hand, would act primarily by binding extracellular proteins (Kumar and Horigome, 1986). Alternatively, since all tannins have affinity for proteins, there might be more consistent effects acting through extracellular protein binding. In this case we would predict similar effects for all tannins, though the effects would be stronger with the heavy tannins, which bind more strongly to proteins (Kumar and Horigome, 1986).

In this paper we further develop the earlier work done by Schimel et al. (1996, 1998) to address two main questions: (1) do different fractions of balsam poplar tannins have similar effects on soil processes and organisms?, and (2) are microbial communities in poplar soil more tolerant to poplar tannins than microbial communities in alder soils? To answer these questions, we fractionated bulk poplar tannin extract into four molecular weight classes and added each fraction to the Oi and Oe horizons of alder and poplar soil. We then evaluated the effects of different tannin fractions on carbon and nitrogen metabolism in alder and poplar soils and tried to evaluate the mechanisms causing the effects.

## 2. Materials and methods

### 2.1. Study sites

Forest floor samples were collected from the Bonanza Creek Long Term Ecological Research (LTER) area, approximately 30 km SW of Fairbanks, Alaska (64° 45'N, 148° 18'W). The balsam poplar (*P. balsamifera*) samples were collected from three sites on the Tanana River floodplain with a dominant overstory vegetation of balsam poplar and a few scattered alder and white spruce (*P. glauca* (Moench) Voss). The thin-leaf alder (*A. tenuifolia*) samples were collected from three sites on the Tanana River floodplain at which alder is the dominant vegetation, though there are a few young poplars. Information on the vegetation, soils, and climates at both of these sites has been documented by Van Cleve et al. (1993a). Two forest floor horizons were collected in each community. The Oi horizons were composed primarily of the partially decomposed litter from the previous fall, and the underlying Oe horizons were composed of 2–3 year old litter (Wagener and Schimel, 1998). Samples from the same horizon and vegetation type were composited to provide a single sample. Selected soil characteristics are described in Table 1. All samples were collected in July of 1997 and stored at –20° C for 5 months prior to the start of the experiment. While freezing may have caused some changes in the microbial community, these soils regularly freeze, and freezing was necessary to prevent extensive decomposition and loss of labile C.

### 2.2. Tannin extraction and fractionation

The tannin fractions were prepared as described in Schimel et al. (1996) with the following modifications. Freeze dried balsam poplar leaves (1 kg) were finely ground in a Wiley Mill and extracted three times with 500 ml hexane and the hexane discarded. The remaining plant material was extracted with 500 ml acetone/water (70/30) three times. The acetone/water extracts were combined, and concentrated by roto-evaporation. This concentrated extract was extracted with 100% ethyl acetate three times in a separatory funnel. The ethyl acetate fractions were combined and roto-evaporated to dryness. This material was Fraction 1. The remaining acetone/water fraction was concentrated and

Table 1  
Selected properties of the soil samples used in the experiment. TAE = tannic acid equivalents, BQE = bisulfited quebracho equivalents. Means with 1 SE in parentheses ( $n = 3$ )

	C:N ratio	Total phenolics (mg TAE g <sup>-1</sup> dry soil)	Condensed tannins (mg BQE g <sup>-1</sup> dry soil)
Poplar Oi	24.7 (3.5)	72.7 (8.2)	106.4 (12.3)
Poplar Oe	24.15 (4.3)	3.2 (0.4)	not detectable
Alder Oi	14.7 (2.9)	54.3 (6.7)	0.9 (0.1)
Alder Oe	17.6 (3.8)	5.8 (0.3)	not detectable

Table 2

Tannin fractions from balsam poplar, composition of each fraction, and estimates of percent of total tannin weight, and molecular weight

Tannin fraction	Chain length units	Estimated % of total tannin weight	Estimated molecular weight
1	monomer, dimer, trimer	15	200–700
2	trimer, tetramer	19	700–1000
3	tetramer, pentamer, hexamer, heptamer	41	1000–2000
4	heptamer and above	25	> 2000

loaded onto a LH 20 Sephadex Chromatography Column. The column was eluted with MeOH/water (50/50) followed by acetone/water (70/30). The acetone/water fraction was concentrated and loaded onto a clean LH 20 Sephadex column for further fractionation. This fraction was eluted three times with 100% ethanol. The ethanol extracts were collected and concentrated using roto-evaporation; this was Fraction 2. The extract on the LH 20 column was then eluted with MeOH (100%) three times, the extracts were combined and then concentrated; this was Fraction 3. The extract in the LH 20 column was then eluted with acetone/H<sub>2</sub>O (70/30) three times, and the eluates concentrated; this was Fraction 4.

All four fractions were analyzed using thin layer chromatography to determine their composition. TLC separates oligomers of tannins by molecular weight. Since oligomers come in discrete molecular weights, this produces a series of spots on the TLC plate, each corresponding to a tannin of a particular degree of polymerization. Degree of polymerization is determined by the distance the material travels relative to the solvent front ( $R_f$  value). The solvent system for TLC analyses was toluene/acetone/formic acid (3/6/1). Detection of components in each fraction was by UV light.

Extractions of 1 kg freeze dried leaves yielded 55 g condensed tannin. General characteristics of the four fractions (Table 2) were noted during extraction, fractionation, TLC analysis, and also from the literature. Overlaps between each of the fractions occurred, but the overlapping component, based on TLC analysis, for each fraction was an insignificant amount. The overlapping component was just visible on the TLC plates in each case. Fractions were sorbed onto silica gel and dried by rotary evaporation for handling and application to soils (Schimel et al., 1996).

### 2.3. Incubation

The four soils were thawed and adjusted to 50% of water saturation in order to maximize microbial activity. The alder and poplar Oi litters were macerated with a knife so each piece of litter was close to 1 cm<sup>2</sup>. Samples (approx. 4.5 g dry weight equivalent) of each litter type were weighed into 21 individual 230 ml glass canning jars for a 10 day equilibration period at 20°C. After 10 days, six replicate samples of each soil type (a total of 24 jars) were destructively harvested and analyzed as described below to obtain initial time point data. Five treatments were applied to the remaining samples with three replicates per treatment/soil type

combination (a total of 60 jars). The five treatments consisted of the four tannin fractions and a control (silica gel that had been treated with the same solvent systems used to extract the tannins). The tannins were thoroughly mixed into each sample with a ratio of 45 mg tannin g<sup>-1</sup> soil. The jars were sealed and incubated for 1 month at 20°C. After this incubation, samples were analyzed using the full suite of assays described below to evaluate rates of C and N cycling.

### 2.4. Chemical and microbiological analyses

The C/N ratios of the soil samples were determined by combustion in a Fisons NA1500 C/N analyzer. Total phenolic content of the samples was determined using the Folin-Ciocalteu method as described in Waterman and Mole (1994). Condensed tannin content was measured using the proanthocyanidin method of Porter et al. (1986). Absorbance values at 550 nm were converted to bisulfited quebracho equivalents (mg BQE g<sup>-1</sup> soil) using the calibration curve constructed by Martin and Martin (1982).

During the course of the incubation, CO<sub>2</sub> concentrations in each jar were measured periodically by sampling the headspace with a glass syringe and analyzing CO<sub>2</sub> on a gas chromatograph (Shimadzu model 14 with a 2 m Porapak Q column running at 45°C with a thermal conductivity detector). The jars were vented after each sampling to keep headspace CO<sub>2</sub> concentrations from exceeding 1–2%. Total C respired was calculated by integrating the amount of CO<sub>2</sub> respired over the course of the incubation.

At the beginning and end of the incubation, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations were determined by extraction with 0.5 M K<sub>2</sub>SO<sub>4</sub> and subsequent analysis of the extract on a Lachat autoanalyzer. Ammonium was analyzed using the diffusion method (Lachat method #31-107-06-5-A, Milwaukee, WI) and nitrate was analyzed using Griess-Ilovsay reaction after Cd reduction (Lachat method #12-107-04-1-B, Milwaukee, WI). Net mineralization was calculated as the change in total inorganic N during the course of the incubation, while net nitrification was the change in NO<sub>3</sub><sup>-</sup> pools.

The <sup>15</sup>N pool dilution technique was used to calculate gross NH<sub>4</sub><sup>+</sup> production and consumption rates (Hart et al., 1994). <sup>15</sup>N labeled NH<sub>4</sub><sup>+</sup> was added to paired soil samples (10 µg N g<sup>-1</sup> dry soil to all soil types except for alder Oe which received 100 µg N g<sup>-1</sup> dry soil), with one sample extracted immediately with 0.5 M K<sub>2</sub>SO<sub>4</sub> and the other

sample extracted after a 24 h incubation. Ammonium in the extracts was measured as described above. The extracts were diffused for  $^{15}\text{NH}_4^+$  (Brooks et al., 1989) and analyzed at the University of Illinois (Nuclide 3-60-RMS mass spectrometer; Mulvaney et al., 1990). Gross  $\text{NH}_4^+$  production and consumption rates were calculated using the equations from Kirkham and Bartholomew (1954).

Microbial biomass C and N were measured using the chloroform fumigation-extraction technique with a 24 h fumigation time (Vance et al., 1987). The extracts were analyzed for total C and total N using a persulfate digestion technique (Doyle et al., submitted). Microbial biomass carbon and nitrogen content were calculated by subtracting the total C and N in unfumigated samples from that in fumigated samples. Biomass C and N values were *not* corrected for extraction efficiency and thus represent a 'flush' of C and N instead of the total microbial biomass.

Nitrification potentials, an assay for nitrifier biomass, were determined using the short-term chlorate slurry inhibition assay (Belser and Mays, 1980).  $\text{NO}_2^-$  concentrations in the slurry were measured every 2 h during a 6 h incubation.

Extracellular enzyme assays were conducted by preparing a sample homogenate with 0.5 g litter (dry wt. equivalent) and 50 ml of 50 mM Na-acetate solution, pH 5.0. The mixture was thoroughly blended with a tissue homogenizer and the assays were conducted immediately. Endocellulase activity was determined by viscometric analysis of carboxymethylcellulose breakdown with four readings per 3 h incubation (Almin et al., 1967).  $\beta$ -glucosidase activity was assayed using the *p*-nitrophenyl method as described

by Eivazi and Tabatabai (1988). Phenol oxidase and peroxidase activity were assayed as by Sinsabaugh et al. (1992).

## 2.5. Statistical analyses

Treatment effects were analyzed by three way ANOVA using soil type, horizon, and tannin treatment as the sets of variables. General ANOVAs were performed using Systat 7.0 for Windows (1997). ANOVAs with Fisher's LSD test were used to examine the effects of the individual tannin fractions on microbial activity within a litter type. Since we used a single bulk soil sample rather than field replicates, the soil type variable is strictly limited to the soil sample used, rather than to that soil type broadly in the field. However, we believe that the patterns and dynamics observed here are representative of those found in natural forest stands.

## 3. Results

### 3.1. $\text{CO}_2$ flux

While respiration rates differed dramatically among soils from the different plant communities, the patterns within each soil horizon were somewhat similar. The control Oi soils had respiration rates that declined more rapidly over the course of the incubation than the control Oe soils. (Fig. 1). In the poplar Oi soil, respiration in the control treatment increased over the first 10–14 days and then declined at the end of the experiment, while in the alder Oi, respiration rates were initially high and relatively stable

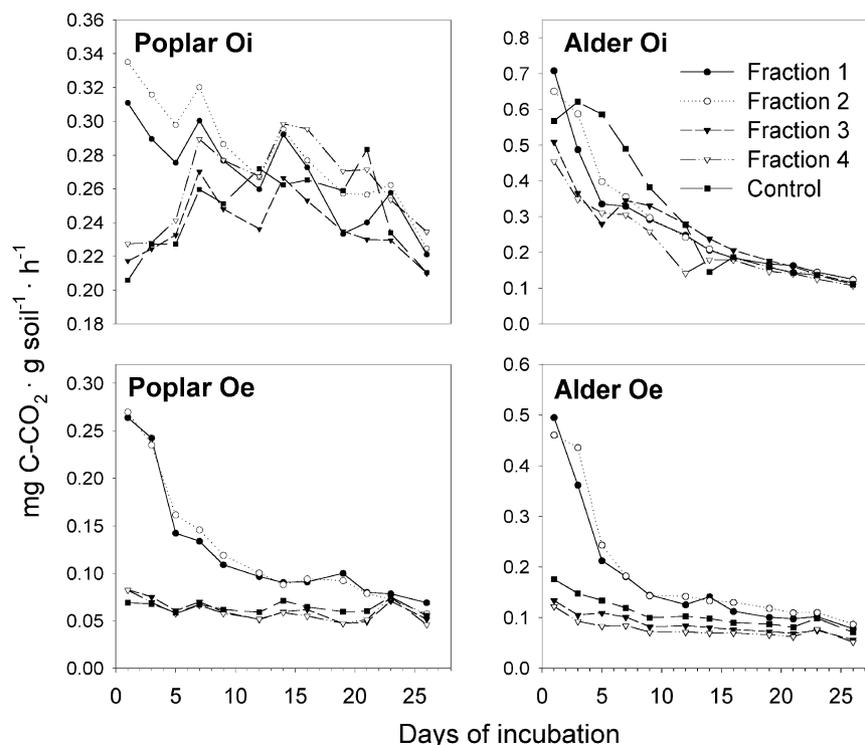


Fig. 1. Respiration rates during the 1 month incubation.  $n = 3$ .

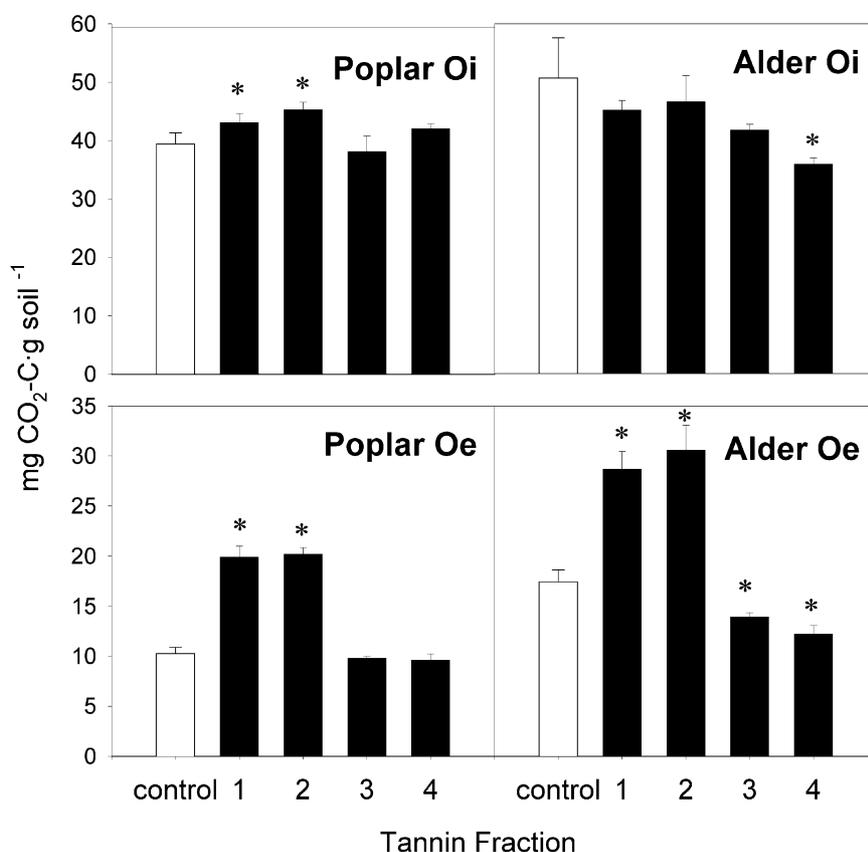


Fig. 2. Cumulative CO<sub>2</sub> production over the first 17 days of the incubation. \*Significantly different from the control ( $P < 0.10$ ). Vertical bars =  $\pm 1$  SE.  $n = 3$ .

for the first week, and then declined exponentially. Respiration rates in the poplar Oe control were low but stable over the experiment, while in the alder Oe, respiration rates started somewhat higher than the poplar Oe but declined over the course of the experiment. In all four soil types, the effect of the tannins on CO<sub>2</sub> flux was most apparent during the first 2 to 3 weeks of the 4 week incubation period (Fig. 1). Tannin fractions 1 and 2 significantly stimulated respiration in all the soils except alder Oi, in which they somewhat inhibited respiration. Fractions 3 and 4 had only slight effects on respiration in the poplar soils, but somewhat inhibited respiration in the alder soils.

Cumulative CO<sub>2</sub> production was analyzed over the first 17 days of the incubation for statistical analyses (Fig. 2). In poplar Oi soil there was a strong tannin effect on respiration ( $P < 0.001$ ) with the light fractions causing an increase in CO<sub>2</sub> flux relative to the heavy fractions and the control ( $P < 0.05$  for both). Respiration in poplar Oe soil also showed a significant tannin effect ( $P < 0.001$ ) with fractions 1 and 2 causing a very large increase in respiration ( $P < 0.001$  for both). Respiration with fractions three and four was essentially indistinguishable from the control. The alder Oi soil showed a slight tannin effect ( $P = 0.073$ ) with fraction 4 depressing respiration relative to the control ( $P = 0.047$ ). The tannin fractions strongly affected respiration in alder Oe soil ( $P < 0.001$ ) with fractions 1 and 2 inducing higher respiration rates than the other treatments

( $P < 0.001$  in all cases). Fractions 3 and 4 had respiration rates significantly lower than the control ( $P = 0.04$  and  $0.01$ , respectively).

We calculated the total increase in respiration due to adding fractions 1 or 2 to these soils, by integrating over the entire incubation period. These values were 4.75, 9.82, and 12.26 mg C-CO<sub>2</sub> g<sup>-1</sup> soil, for the poplar Oi, poplar Oe, and alder Oe. In the alder Oi, tannins reduced respiration so this calculation could not be done. We added 45 mg tannin g<sup>-1</sup> soil, and condensed tannins average approximately 62% C (Waterman and Mole, 1994). If we assume that the stimulation of respiration is all from tannin C, that all the added tannin material was metabolized, and that the unrespired tannin went into microbial biomass, this would generate C-use efficiencies for the light fractions of 83, 65, and 56% for poplar Oi, poplar Oe, and alder Oe soils. Given other measures of C-use efficiency for phenolic materials (e.g. 59–21%, Sugai and Schimel, 1993), these numbers seem implausibly high, suggesting that a significant portion of the tannins added to the soils were not fully metabolized. Either not all of the tannin on the silica gel was available to microbes or the added tannins were adsorbed by soil organic matter.

### 3.2. Net N mineralization/immobilization

In all soils, the control soils showed net mineralization, with alder soils mineralizing at least an order of magnitude more N

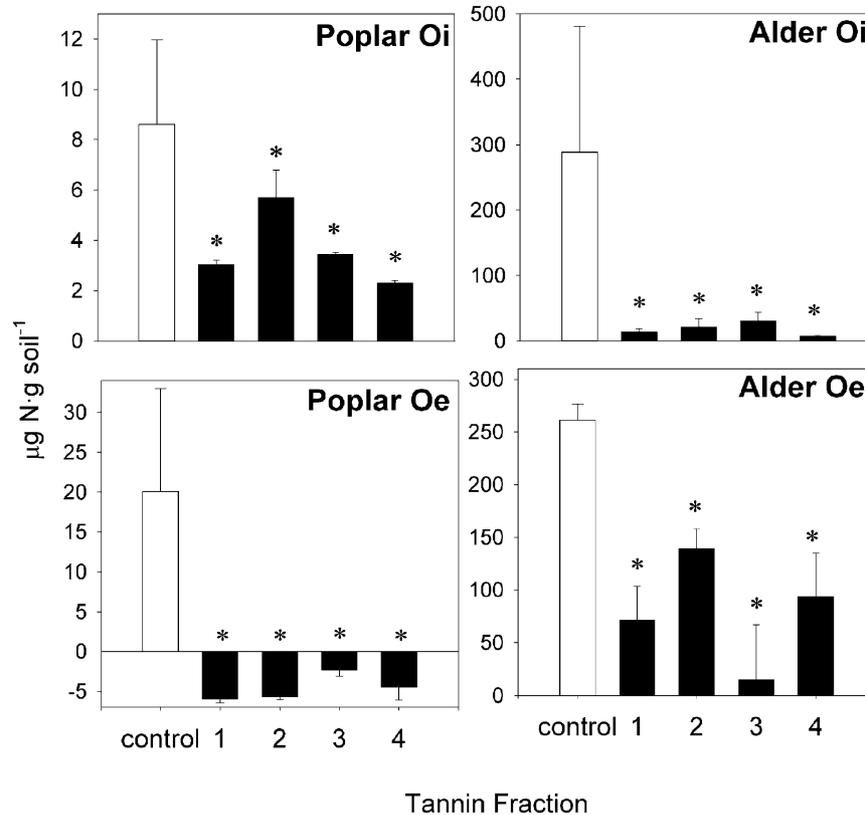


Fig. 3. Net N mineralized ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) during the 1 month incubation. \*Significantly different from the control ( $P < 0.10$ ). Vertical bars =  $\pm 1$  SE.  $n = 3$ .

than the poplar soils (Fig. 3). For example, alder Oi control soil mineralized close to  $300 \mu\text{g N g}^{-1}$  soil over the experiment, while poplar Oi soil mineralized less than  $10 \mu\text{g N g}^{-1}$  soil. All tannin fractions significantly reduced mineralization ( $P = 0.081$  across all soil types), in the case of poplar Oe actually shifting to net immobilization. There were no obvious differences between tannin fractions in their effects on net mineralization. However, the magnitude of the tannin effects on N mineralization varied by soil type. Net mineralization in the alder Oe and poplar Oi soils was reduced an average of 69 and 57%, respectively, while in the alder Oi and poplar Oe soils net mineralization was reduced by an average 94% and greater than 100%, respectively.

Only alder Oe soil had any measurable  $\text{NO}_3^-$ ,  $\text{NO}_3^-$  production, or nitrification potential (data not shown). In this soil, all the mineralized N was converted to  $\text{NO}_3^-$ , and there was net  $\text{NH}_4^+$  consumption over the assay. There was no evidence for a tannin effect on net nitrification. While net mineralization was lower in all the tannin treatments than in the control, there does not seem to have been a specific inhibition of nitrification by tannins; there was no accumulation of  $\text{NH}_4^+$  and nitrification potentials were unaffected by tannin additions ( $P = 0.794$ ).

### 3.3. Gross mineralization/immobilization

Gross  $\text{NH}_4^+$  production and consumption rates were measured at the beginning and end of the experiment. In

almost all cases, measured  $\text{NH}_4^+$  consumption rates were similar to, but slightly higher than  $\text{NH}_4^+$  production, despite the fact that in all soils, with the exception of alder Oe,  $\text{NH}_4^+$  accumulated over the experiment. In both Oi control soils, gross turnover rates increased over the experiment (Fig. 4), while in both Oe soils, turnover rates declined over the experiment. In both cases, the shifts were greater in the alder soils than in the poplar. At the end of the incubation, turnover rates were greatest in the alder Oi (ca.  $50 \mu\text{g N g soil}^{-1} \text{d}^{-1}$ ), somewhat lower in the alder Oe ( $22\text{--}38 \mu\text{g N g soil}^{-1} \text{d}^{-1}$ ), and lower yet in the poplar soils, ( $15\text{--}18 \mu\text{g N g soil}^{-1} \text{d}^{-1}$ ). Tannin effects on  $\text{NH}_4^+$  production and consumption were variable across soil types and tannin fractions. In the poplar Oi, fractions 1 and 2 reduced  $\text{NH}_4^+$  turnover significantly ( $P < 0.03$  for both production and consumption), while fractions 3 and 4 did not significantly alter turnover rates. In the poplar Oe, gross  $\text{NH}_4^+$  production and consumption were significantly lowered with fraction 2 ( $P = 0.03$  and  $0.02$ , respectively). In the alder Oi, all tannin fractions significantly reduced gross  $\text{NH}_4^+$  production rates ( $P < 0.03$  in all cases), but only fractions 2 and 4 significantly reduced consumption rates ( $P = 0.06$  in both cases). In the alder Oe, fractions 3 and 4 reduced  $\text{NH}_4^+$  turnover rates, ( $P < 0.07$ ).

### 3.4. Microbial biomass flush C ( $C_{mic}$ ) and N ( $N_{mic}$ )

In both Oi control soils,  $C_{mic}$  declined in the control treatment during the incubation, though the decline was not

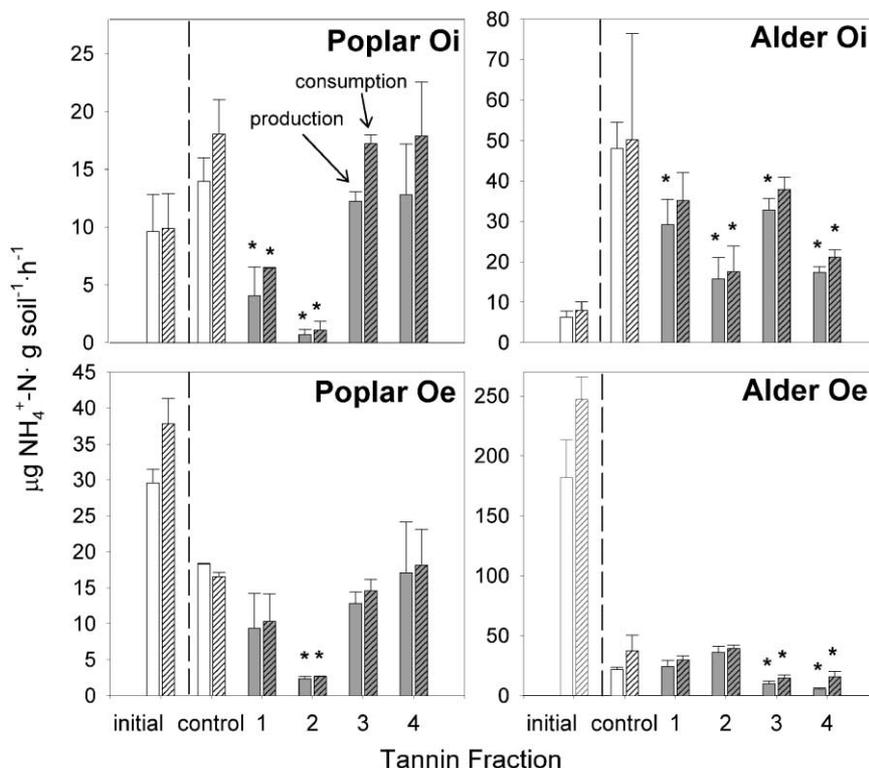


Fig. 4. Gross  $\text{N-NH}_4^+$  production and consumption rates as measured by isotope pool dilution before and after 1 month of incubation at  $20^\circ\text{C}$ . \*Significantly different from the control ( $P < 0.10$ ). Vertical bars =  $\pm 1$  SE.  $n = 3$

significant in the alder Oi (Fig. 5). In both Oe soils, however,  $C_{\text{mic}}$  increased significantly. Across all soil types there were significant effects of the tannin fractions ( $P = 0.017$ ) on  $C_{\text{mic}}$ . In poplar Oi,  $C_{\text{mic}}$  was significantly higher in fractions 1, 2, and 4 than in the control. In poplar Oe,  $C_{\text{mic}}$  was reduced by all fractions ( $P = 0.03$ ). In alder Oi,  $C_{\text{mic}}$  was greatly reduced by all four tannin fractions ( $P < 0.001$  in all cases). In alder Oe, fractions 1 and 2 enhanced  $C_{\text{mic}}$  ( $P < 0.05$ ), while fractions 3 and 4 decreased  $C_{\text{mic}}$  ( $P < 0.12$ ).

Treatment effects on  $N_{\text{mic}}$  showed some similarities and some differences to the treatment effects on  $C_{\text{mic}}$  (Fig. 6). In the poplar Oi,  $N_{\text{mic}}$  was not significantly affected by tannin additions. In the poplar Oe, however,  $N_{\text{mic}}$  was significantly reduced relative to the control ( $P = 0.02$ ). In both alder Oi and Oe, all the tannin fractions reduced  $N_{\text{mic}}$  relative to the control (Fig. 6;  $P < 0.03$  in all cases). In the alder Oi, tannins caused a decrease in biomass even from the initial value, while in the alder Oe, tannins merely limited the increase in biomass N.

The initial C/N ratio of the microbial biomass flush was greater than 25 in both Oi soils (Fig. 7), while it was less than eight in both of the Oe soils. In all control soils except the poplar Oe, the ratio declined over the experiment dropping below 15 in the Oi soils, and to only three in the alder Oe. In the poplar Oe, the biomass flush C/N ratio increased to approximately 14, a ratio similar to that of the Oi soils. Tannin treatments generally increased the

C/N ratio of microbial biomass relative to the control ( $P = 0.10$ ), especially the lighter tannin fractions (Fig. 7). Over the course of the incubation, tannin additions to alder Oi soil resulted in less of a decrease in C/N ratio compared to the control ( $P < 0.03$  in all cases). The tannin effect on C/N biomass change in alder Oe was also significant ( $P = 0.001$ ) with tannin fractions 1–3 resulting in a higher C/N ratio of the microbial population ( $P < 0.04$  in all cases).

### 3.5. Enzyme activities

The extracellular enzymes assayed,  $\beta$ -glucosidase, endocellulase, phenol oxidase, and peroxidase, had different rates of activity depending on soil type (Table 3). In three-way ANOVAs, activities were always significantly higher in the Oi horizon ( $P = 0.099$  for peroxidase, but  $P < 0.001$  for the other three enzymes). Both enzymes involved in carbohydrate breakdown were significantly higher in the alder soils ( $P < 0.01$  and  $P = 0.08$  for endocellulase and  $\beta$ -glucosidase, respectively). Alternatively, the ligninolytic enzymes showed no significant differences between plant communities ( $P > 0.15$  for phenol oxidase and peroxidase).

Tannin additions generally did not affect enzyme rates (Table 3). There were a few significant differences but there was little consistent pattern. The only overall significance in the three-way ANOVA was that the tannin fraction

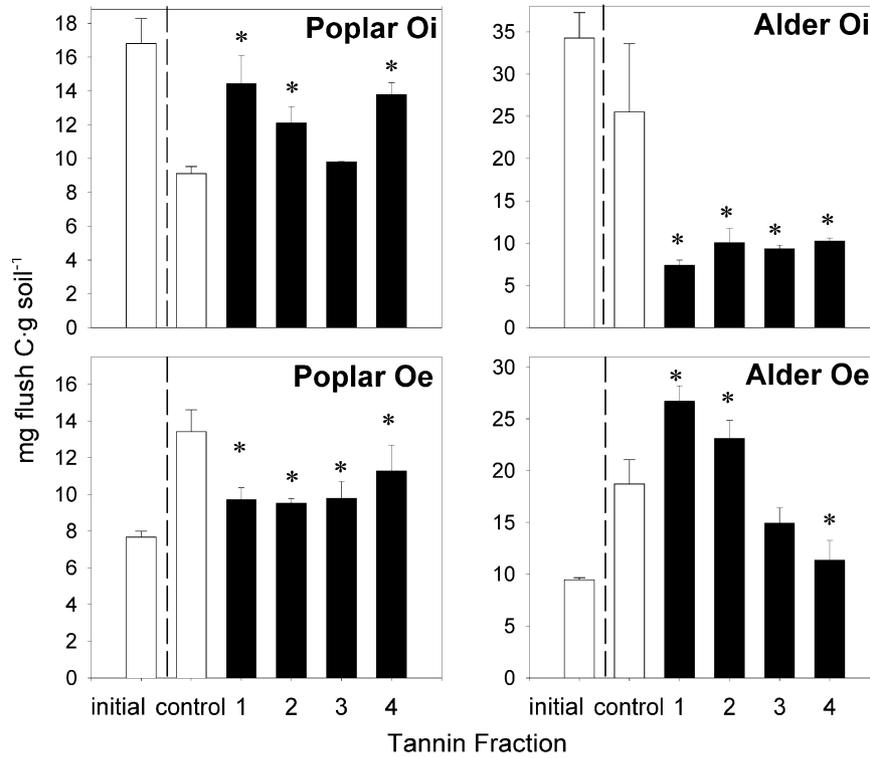


Fig. 5. Biomass C (chloroform fumigation flush) before and after the 1 month incubation. \*Significantly different from the control ( $P < 0.10$ ). Vertical bars =  $\pm 1$  SE.  $n = 3$ .

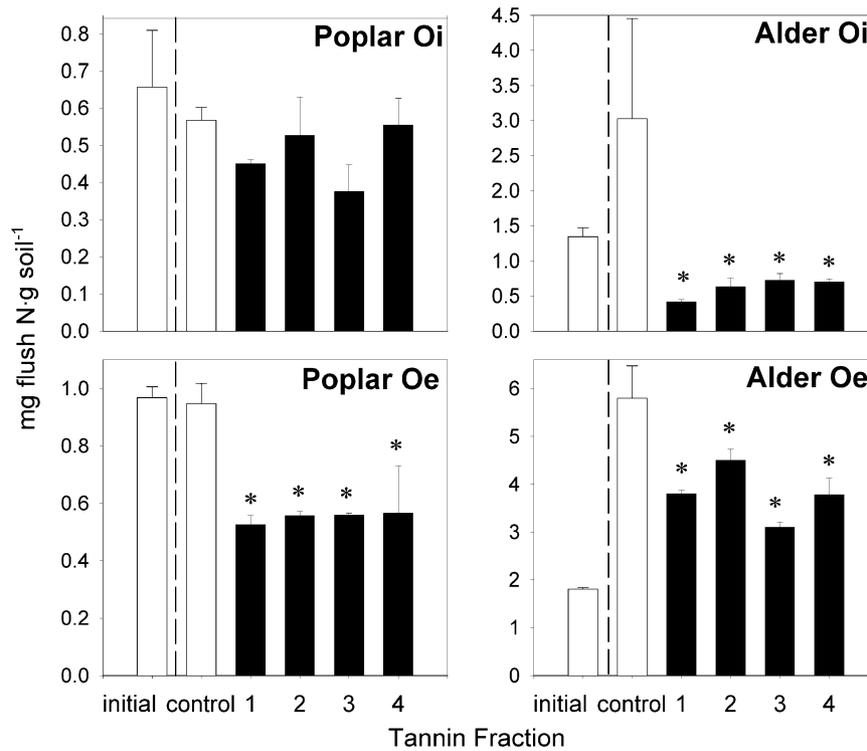


Fig. 6. Biomass N (chloroform fumigation flush) before and after the 1 month incubation. \*Significantly different from the control ( $P < 0.10$ ). Vertical bars =  $\pm 1$  SE.  $n = 3$ .

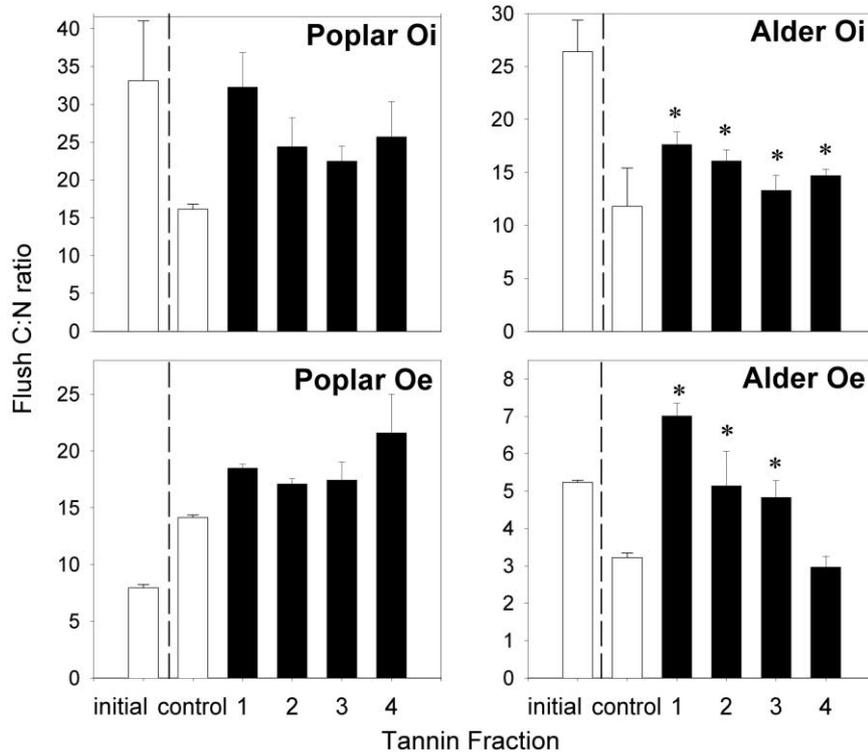


Fig. 7. C:N ratio of biomass (chloroform fumigation flush) before and after 1 month long incubation. \*Significantly different from the control ( $P < 0.10$ ). Vertical bars =  $\pm 1$  SE.  $n = 3$ .

1 treatment had higher phenol oxidase activity than the other treatments ( $P = 0.012$ ), but that effect was only individually significant in the alder Oi. In the poplar Oe the fraction 4 treatment had a lower phenol oxidase activity than the control. In the alder Oe, both fractions 1 and 2 elevated  $\beta$ -glucosidase relative to the control. No other effects were significant.

#### 4. Discussion

##### 4.1. Methodological considerations

Before discussing our specific data, we believe that it is necessary to discuss the  $^{15}\text{NH}_4^+$  pool dilution method of measuring gross rates of  $\text{NH}_4^+$  production (mineralization)

Table 3  
Mean extracellular enzyme activity (units  $\text{g}^{-1}$  soil  $\text{h}^{-1}$ ). SE in parentheses. \*Value significantly different from control ( $P < 0.10$ )

Soil type	Tannin fraction	Endocellulase	Phenol Oxidase	Peroxidase	$\beta$ -Glucosidase
Poplar Oi	control	4501 (393)	9.29 (1.23)	1.40 (0.99)	2136 (255)
	1	3597 (311)	7.36 (1.23)	0.87 (0.40)	2135 (99)
	2	4893 (750)	3.98 (0.44)	1.93 (0.32)	2820 (589)
	3	3781 (372)	6.34 (0.43)	0.81 (0.57)	1932 (300)
	4	4376 (257)	5.80 (1.19)	0.74 (0.52)	2904 (256)
Alder Oi	control	24489 (14573)	4.02 (0.92)	0.85 (0.05)	2323 (356)
	1	20400 (7431)	9.23* (1.53)	2.09 (0.51)	2858 (74)
	2	8622 (1780)	3.36 (0.69)	1.09 (0.31)	2011 (55)
	3	35809 (13105)	3.68 (0.56)	2.48 (0.44)	2679 (14)
	4	13843 (3500)	4.01 (0.68)	2.82 (0.77)	1884 (266)
Poplar Oe	control	195 (85)	0.49 (0.11)	1.70 (0.55)	936 (130)
	1	386 (47)	0.84 (0.16)	0.13 (0.01)	793 (166)
	2	1064 (656)	0.60 (0.29)	0.90 (0.30)	731 (86)
	3	182 (54)	0.48 (0.14)	1.64 (1.09)	585 (21)
	4	82 (24)	0.07* (0.03)	1.03 (0.17)	701 (236)
Alder Oe	control	643 (526)	1.00 (0.13)	1.80 (0.33)	1014 (6)
	1	403 (97)	0.90 (0.02)	0.72 (0.23)	1318* (227)
	2	836 (194)	1.10 (0.05)	0.08 (0.03)	1591* (99)
	3	187 (18)	0.92 (0.12)	0.54 (0.20)	946 (105)
	4	100 (12)	0.74 (0.12)	1.27 (0.27)	1033 (107)

and consumption because the interpretation of these data affects the conclusions from this study. There has been some controversy regarding what exactly drives measured gross mineralization, and what is actually measured. Pool dilution may not measure the overall breakdown of soil organic matter and growth of microbial biomass but rather the microbial cycling and recycling of small pools of highly labile, N-rich compounds. Thus, pool dilution may be more of a measure of microbial cellular, rather than soil organic matter, processes. There are a number of lines of evidence that support this hypothesis. First, production and consumption rates are almost always very closely linked, even in soils that are either mineralizing or immobilizing rapidly (e.g. Schimel et al., 1992; Norton and Firestone, 1996, this study). Second, the rates of gross N turnover have generally been found to be very high relative to rates of C metabolism, high enough that some have argued that the rates of C and N turnover cannot be reconciled. For example, Hart and Stark (J. Stark, personal communication) used gross mineralization and respiration data to estimate that the C/N ratio of microbial substrates were on the order of 4–10. Such values are plausible if they represent the cycling of a small highly labile organic matter pool, but harder to accept if they were to represent the breakdown of bulk soil organic matter. In our control samples, we measured CO<sub>2</sub> production/gross mineralization rates of 18.5, 3.7 in poplar Oi and Oe and 5.8, and 5.0 in alder Oi and Oe soils. With the exception of the poplar Oi, these values also indicate a relatively low C/N ratio substrate driving gross N mineralization. Finally, Clein and Schimel (1995) found that gross turnover was maximized when microbes had an optimal balance of C and N, which we believe is also easier to explain if pool dilution measurements are indicative of cellular rather than soil organic matter processes.

#### 4.2. Tannin effects

While tannin effects varied greatly among soils, the four different fractions could usually be clustered into two size groups: the light fractions (fractions 1 and 2; molecular weights <1000) and the heavy fractions (fractions 3 and 4; molecular weights >1000). In most soils the light fractions appeared to be metabolized as substrates for microbes, while the heavier fractions showed no evidence of being metabolized and commonly inhibited microbial activity. However, these patterns varied across soils with the greatest differences appearing with the light fractions.

In the poplar Oi soil, respiration rates and microbial biomass were significantly increased relative to the control (Figs. 2 and 5), while net mineralization was reduced (Fig. 3). These data indicate that the tannins were used as substrates. These compounds decreased gross N turnover, but this is not necessarily indicative of inhibiting microbial activity. Clein and Schimel (1995) proposed that in a N-limited soil, adding N would increase microbial N turnover. The converse would be that adding C, and exacerbating N

limitation, would reduce N turnover. Since these soils showed net mineralization, one could argue that they were not N limited. However, the mineralization rates were by far the lowest of any of the soils tested, and the ratio of C mineralized to N mineralized was the highest. The ratio of total CO<sub>2</sub> produced to net N mineralization was 4600, while the ratio of CO<sub>2</sub> produced to gross N mineralization was 18.5. These high ratios are strongly suggestive of microbial N limitation (Clein and Schimel, 1995). Thus, we feel that the reduction in gross turnover is consistent with the light fractions acting as microbial substrates in the poplar Oi.

In the poplar Oi, the heavy fractions had limited effects on respiration rates, biomass C, biomass N, and gross N turnover. The only process that was significantly affected by the heavy fractions was net N mineralization, which was reduced. These results suggest that the likely mechanism of action was through binding N-rich substrates outside the cell. Heavier tannin fractions are known to be better at complexing proteinaceous substrates (Kumar and Horigome, 1986), which would reduce net mineralization. If one accepts our hypothesis that pool dilution measurements are indicative of cellular N turnover, then it is possible that binding some soil substrates would not necessarily substantially reduce gross N turnover. If only proteinaceous substrates were complexed then this also might not substantially affect overall soil C dynamics during the assay.

The effects of tannin additions on the poplar Oe soil were similar to those in the poplar Oi. Respiration was significantly enhanced by the light fractions (Figs. 1 and 2), the soil shifted from net mineralization to net immobilization (Fig. 3), and gross NH<sub>4</sub><sup>+</sup> turnover rates were reduced (Fig. 4). These results strongly suggest that the light fractions were substrates. Surprisingly though, the increases in biomass C and N that occurred in the control treatment were blocked by the light fractions. The reason for this is unclear, though it might involve mixed effects. Perhaps the tannins were used as substrates, but also bound extracellular N-rich compounds. The heavy fractions did not alter respiration significantly, but limited the biomass increase over the experiment and induced net N immobilization. These data are consistent with heavy tannins binding N-containing substrates. As with the poplar Oi soil, the reduction in net N mineralization did not correspond with a concomitant reduction in gross N turnover.

Alder soils responded differently to the tannin fractions than did the poplar soils, and the two alder soil horizons responded distinctly from one another. Most dramatically, in the alder Oi, all the tannin fractions appeared to be toxic or inhibitory. The four tannin fractions reduced respiration, caused a significant decline in microbial biomass C and N, almost eliminated net mineralization, and showed some evidence of reducing gross NH<sub>4</sub><sup>+</sup> turnover rates. However, it is not clear if the mechanisms behind the inhibition of microbial activity by the tannin fractions were the same. It is possible that the light fractions acted as cellular toxins, with the heavy fractions acting by complexing substrates in the soil.

In the alder Oe soil, the light fractions appeared to act as microbial substrates, as they did in the poplar soils. They enhanced respiration and microbial biomass C, while lowering net N mineralization. The heavy fractions again, were inhibitory, reducing respiration rates, microbial biomass C and N, gross turnover, and net N mineralization. There were no specific effects on nitrification; essentially all the N mineralized was converted to  $\text{NO}_3^-$ . These results are consistent with the conclusions of Schimel et al. (1996); Clein and Schimel (1995) who found no evidence that tannins caused a specific inhibition of nitrifier activity.

The evidence of these experiments and others (Gamble et al., 1996) strongly suggests that low molecular weight tannin fractions can be taken up and act within microbial cells. This allows them to be used as C substrates by microbes that are adapted to them (apparently all soils but the alder Oi), and also to act as toxins by complexing cellular proteins (Field and Lettinga, 1992), as appeared to be the case in the alder Oi. The heavy fractions, on the other hand, are unlikely to have been taken up by microbes (Field and Lettinga, 1992). The effects were likely to be due to interactions with extracellular molecules, either as substrates or exoenzymes. As we showed no significant inhibition of exoenzymes responsible for cellulose and lignin degradation, we conclude that any effects of the heavy tannin fractions resulted primarily from binding substrates. These tannin fractions consistently reduced net mineralization and tended to reduce microbial biomass N (with the sole exception of fraction 4 in the poplar Oi). The greatest reductions were in the mineral N-rich alder soils and weakest in the mineral N-poor poplar Oi soil. The heavy tannin effects on C dynamics were weaker, with limited effects on respiration and biomass C. Taken together, these results suggest that heavy fractions bound N-containing substrates and reduced N availability to microbial cells. These results are very similar to those of Bradley et al. (2000) who concluded that the main effect of *Kalmia angustifolia* tannins was to sequester N substrates. While the gross turnover data may seem inconsistent with this result, we believe that it is possible, if not likely, that pool dilution measures turnover through the microbial cells, rather than breakdown of organic matter, as mentioned above. If this is the case, then tannin binding of N-containing substrates should have limited effects on N turnover kinetics.

If tannins were binding nitrogenous substrates, one might predict that they would also bind exoenzymes, as has been shown by other research (Benoit and Starkey, 1968; Field and Lettinga, 1992). There are a number of possible reasons why we did not observe significant effects of tannins on individual extracellular enzyme activities. First, in 26 of 32 assay combinations (Table 3), the enzyme activities in tannin fractions 3 and 4 were slightly lower than the control, though the differences were not individually significant. Thus, there may have been a trend of limited inhibition by the heavy tannin fractions, while the light fractions showed no evidence of a pattern (half the rates were lower than the

control, half higher). Given the large variability in the enzyme data, only large effects would have been observable on each enzyme rate. Tannins must reach some threshold concentration to fully precipitate enzymes, and until that level is reached, there is very limited inhibition (Juntheikki and Julkunen-Titto, 2000). We may not have reached that level, resulting in very limited or no inhibition. Finally, some enzymes (including  $\beta$ -glucosidase) retain activity even when they are precipitated (Scalbert, 1991; Juntheikki and Julkunen-Titto, 2000), and thus very little inhibition would occur even with the enzymes fully complexed by tannins. Thus, we believe that the exoenzyme data are still consistent with the hypothesis that the heavy tannin fractions bind to substrates.

The varying effects of tannins in the different soils raises the question of why the soils responded differently to the different tannin fractions. We hypothesize that this results in part, from differing histories of exposure to tannins and the existence of microbial communities that are adapted to their chemical environment. In the field, poplar soils are naturally exposed to high concentrations of tannins and phenolic compounds (Table 1; Schimel et al., 1996), and so may be adapted to this input. While the alder Oi soil has a relatively high total phenolic content (Table 1), the exposure to tannins, particularly poplar derived tannins, would be minimal, possibly resulting in a community that was not adapted to dealing with poplar tannins, and so resulting in strong inhibitory effects by all the poplar tannin fractions. Why, then, would the alder Oe community be tolerant of the light poplar tannin fractions and capable of using them as substrates? We hypothesize that the alder Oe community is exposed to phenolics being leached out of the Oi, and released from lignin breakdown (alder has a higher lignin content than poplar; Van Cleve et al., 1993b). This might select for alder Oe microbes that would be capable of processing low molecular weight poplar tannins.

#### 4.3. Ecological implications

Earlier work on the role of poplar secondary chemicals suggested that chemical interactions affecting soil nutrient cycling are complex and may affect the dynamics of the transition from alder to poplar dominance in the Alaskan taiga (Schimel et al., 1998). Poplar is known to release a variety of condensed tannins, simple phenolics, and other labile substrates that may inhibit the breakdown of soil N compounds and concomitantly stimulate microbial growth and N uptake (Schimel et al., 1996; Clein and Schimel, 1995). This paper has taken the next step by analyzing the mechanisms by which balsam poplar tannins affect N cycling in soil. The effects were mixed. We found that small tannins (tetramers and smaller) have strong direct biological effects, acting as substrates (in most of the soils) or as toxins (the alder Oi). The larger fractions, on the other hand, appeared to have primarily biochemical effects, complexing substrates in the soil. All the fractions affected N availability, but since N availability is already low

in poplar soils, the effects were weaker in this soil than in the alder soils. As the heavier fractions account for 66% of the total balsam poplar mix, the substrate binding effects are likely to be more important than the substrate/toxin effects of the lighter fractions. There are several explanations that could support this hypothesis. First, the results of Schimel et al. (1996), who worked with bulk poplar tannins, are more similar to the results we saw for the heavy fractions. Second, the heavy fractions make substrates unavailable and are thus likely to have longer-lasting effects than the light fractions. While the light fractions may act as substrates once the community adapts, phenolics have relatively low substrate use efficiencies (Sugai and Schimel, 1993) so the amount of biomass produced and the amount of N immobilized should be relatively low from this modest C input. Additionally, microbial biomass can be turned over rapidly, limiting the time scale of effects. Thus the light fractions may be a part of the labile C inputs that Clein and Schimel (1995) identified as important in controlling microbial processes in the alder to poplar transition, but we think that they are unlikely to constitute the bulk of that material.

Our work focussed on inorganic N dynamics, yet there is growing evidence that, in boreal systems, organic N may be important in overall N cycling and plant nutrition (Näsholm et al., 1998). However, we believe that the patterns of tannins reducing N availability that we observed would hold true for available organic N as well. First, by complexing proteins, tannins would simply reduce the supply of amino acids for plant uptake. Second, by acting as substrates, the lighter tannins would still act as a sink for microbially available N, which would include amino acids.

#### 4.4. Conclusions

Condensed tannins, as a broad class of chemicals, can have varied effects on soil processes. As has been shown in other studies (Kumar and Horigome, 1986; Bradley et al., 2000) tannins with differing degrees of polymerization have different effects on microbial processes. Thus we predict that in cases where tannin-rich species invade communities (either through normal successional processes or as the introduction of exotics), if the dominant tannins are highly polymerized then the effects should be predictable: a reduction of net mineralization through substrate binding, with otherwise limited effects on overall C cycling or microbial communities directly. If however, invading species produce smaller chain tannins, the effects will be less predictable and will depend on the prior exposure of the soil community to related molecules. If the microbial communities have had limited exposure to tannins, they may be more likely to prove toxic, causing shifts in microbial community structure as well as changes in C and N cycling.

#### Acknowledgements

We thank Allen Doyle for invaluable assistance in meth-

ods development and sample processing. This work was supported by a grant from the US National Science Foundation's Ecology Program and the National Science Foundation Long Term Ecological Research Program.

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