

NOTE / NOTE

Interactive effects of condensed tannin and cellulose additions on soil respiration

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Abstract: Plant polyphenolics are receiving increased attention for their influences on belowground processes. Tannins are of particular interest because of their predominance in natural systems, their wide variation in both quality and quantity, and their protein-binding abilities. Current theory holds that simple phenolics increase microbial activity by acting as carbon substrates, while larger tannins decrease microbial activity by binding with organic nitrogen such as proteins. Here, we present results from a simple microcosm experiment that demonstrates that the influence of condensed tannins on soil respiration depends on the availability of additional carbon substrates. We purified tannins from trembling aspen (*Populus tremuloides* Michx.) and crossed three levels of tannin additions with three levels of cellulose additions in laboratory microcosms. Soil respiration was measured over 36 days. In the absence of cellulose, high amounts of condensed tannins increased cumulative soil respiration. In the presence of abundant cellulose, condensed tannins decreased cumulative soil respiration. The positive and negative effects of purified tannins on soil respiration are time dependent, such that initial respiration is likely tannin induced, while later respiration is cellulose induced and tannin limited.

Résumé : Les polyphénols végétaux suscitent un intérêt grandissant à cause de leur influence sur les processus du sol. Les tannins sont particulièrement intéressants à cause de leur prédominance dans les systèmes naturels, de leur grande variabilité tant en qualité qu'en quantité et à cause de leur capacité à se lier aux protéines. La théorie courante veut que les composés phénoliques simples augmentent l'activité microbienne en agissant comme source de carbone tandis que les tannins plus volumineux diminuent l'activité microbienne en se liant à l'azote organique comme les protéines. Nous présentons ici les résultats d'une simple expérience en microécosystèmes qui démontrent que l'influence des tannins condensés sur la respiration du sol dépend de la disponibilité de sources additionnelles de carbone. Nous avons purifié des tannins de peuplier faux-tremble (*Populus tremuloides* Michx.) et combiné trois niveaux d'apport de tannins et trois niveaux d'apport de cellulose dans des microécosystèmes en laboratoire. La respiration du sol a été mesurée pendant 36 jours. En l'absence de cellulose, de fortes quantités de tannins condensés ont augmenté la respiration cumulative du sol. En présence de beaucoup de cellulose, les tannins condensés ont diminué la respiration cumulative du sol. Les effets positifs et négatifs des tannins purifiés sur la respiration du sol sont fonction du temps de telle sorte que la respiration initiale est probablement induite par les tannins tandis que, par la suite, la respiration est induite par la cellulose et limitée par les tannins.

[Traduit par la Rédaction]

Introduction

Although polyphenolics are best known for their herbivore and pathogen defense roles in living leaves, they also have several important impacts on terrestrial nutrient cycling (Hättenschwiler and Vitousek 2000). Tannins are a subset of polyphenolics that have received special attention in the soil nutrient cycling literature due to their ability to influence a host of belowground processes, including litter decomposi-

tion, nutrient cycling, microbial activity, humic acid formation, and pedogenesis (Cadisch and Giller 1997; Kraus et al. 2003). Tannins are defined, in part, by their ability to bind to proteins (Bate-Smith 1975) and consequently have large influences on belowground nitrogen cycling by forming recalcitrant complexes with organic nitrogen (Neirop et al. 2006). Likewise, they can also complex with carbohydrates and cellulose to form recalcitrant organic fractions (Horner et al. 1988).

Trembling aspen (*Populus tremuloides* Michx.) is a widespread, genetically diverse canopy species that is a dominant component of many North American forests. Previous work in our laboratory has shown that the condensed tannin content of senesced leaves can vary from about ~3% to 13% dry mass (Lindroth et al. 2002; Madritch et al. 2006). Genetically mediated variation in condensed tannin production in *Populus* species can be a strong predictor of belowground functioning (Schweitzer et al. 2004) and different aspen genotypes can vary widely in decomposition rates (Madritch

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et al. 2006). Natural aspen forest soils receive a range of condensed tannin inputs that is determined, in part, by the overlying aspen genotype. Although aspen forests vary in the quantity of condensed tannins, we have little understanding of how varying amounts of polyphenolics interact with other carbon substrates to influence belowground processes.

Simple phenolics are credited with increasing respiration by acting as carbon substrates, while tannins are typically attributed with retarding decomposition and microbial activity through either protein binding or outright toxicity (Horner et al. 1988; Schimel et al. 1996, 1998; Harborne 1997; Bradley et al. 2000; Fierer et al. 2001). However, several recent studies suggest a positive effect of tannins, or fractions thereof, on soil respiration when compared with controls (Kraus et al. 2004; Kanerva et al. 2006; Orwin et al. 2006). For instance, tannins purified from different species can have a range of positive influences on soil respiration, ranging from no detectable effect to doubling the amount of total carbon respired (Kraus et al. 2004). Likewise, Orwin et al. (2006) showed that adding commercially available tannin to soil microcosms induced as much respiration as did cellulose additions. To date, experimental studies have investigated microbial activity in response to varying levels of either cellulose or tannin additions (Schimel et al. 1996; Kraus et al. 2004) or to a single level of carbon substrate addition consisting of various combinations (Orwin et al. 2006). Despite these very useful studies, simple factorial experiments varying the quantity of both tannin and cellulose additions are lacking. Here, we present the results of a microcosm experiment that mimicked the natural range of tannin and cellulose inputs from *P. tremuloides* to forest soils. Our results demonstrate the interactive effects of varying amounts of condensed tannin and cellulose inputs on soil respiration.

Methods

We purified condensed tannins from trembling aspen using methods derived from Hagerman and Butler (1980, 1994). *Populus tremuloides* contains primarily condensed, and no detectable amount of hydrolysable, tannins (Bryant et al. 1987; Lindroth et al. 1993). In June 2005, aspen leaves were collected from the Pine Island State Wildlife Area near Portage, Wisconsin, USA, and then freeze-dried and finely ground in a Wiley mill. Ground leaf material was washed three times with ethyl ether before extracting three times with 70:30 acetone–water. The acetone–water extract was then rotary evaporated to remove all acetone and filtered to remove particulates. The aqueous extract was then loaded onto LH-20 Sephadex that had been equilibrated in 50% ethanol. Low molecular mass phenolics and nontannin fractions were eluted from the Sephadex by exhaustively washing with ethanol until two consecutive ethanol rinses tested negative for phenolics using a modified Prussian Blue test (Graham 1992). Tannins were then removed from the Sephadex using 70:30 acetone–water washes until a Prussian Blue test gave negative results. The acetone–water extract containing purified tannins was then rotary evaporated, frozen, and freeze-dried to yield dry condensed tannins. Sephadex LH-20 binds strongly to phenolic groups, and this method yields purified complex tannins (Hagerman and

Butler 1980, 1994) with low amounts of impurities that are most likely simple phenolics. To confirm our purification methods, tannins were analyzed with high-performance thin-layer chromatography based on thin-layer chromatography methods used by Hagerman (2002) and Fierer et al. (2001). Tannic acid was used to provide an estimate of molecular mass, and we considered compounds with a molecular mass less than that of tannic acid to be nontannin, low molecular mass fractions. Based on this method, our purified samples contained greater than 95% condensed tannins.

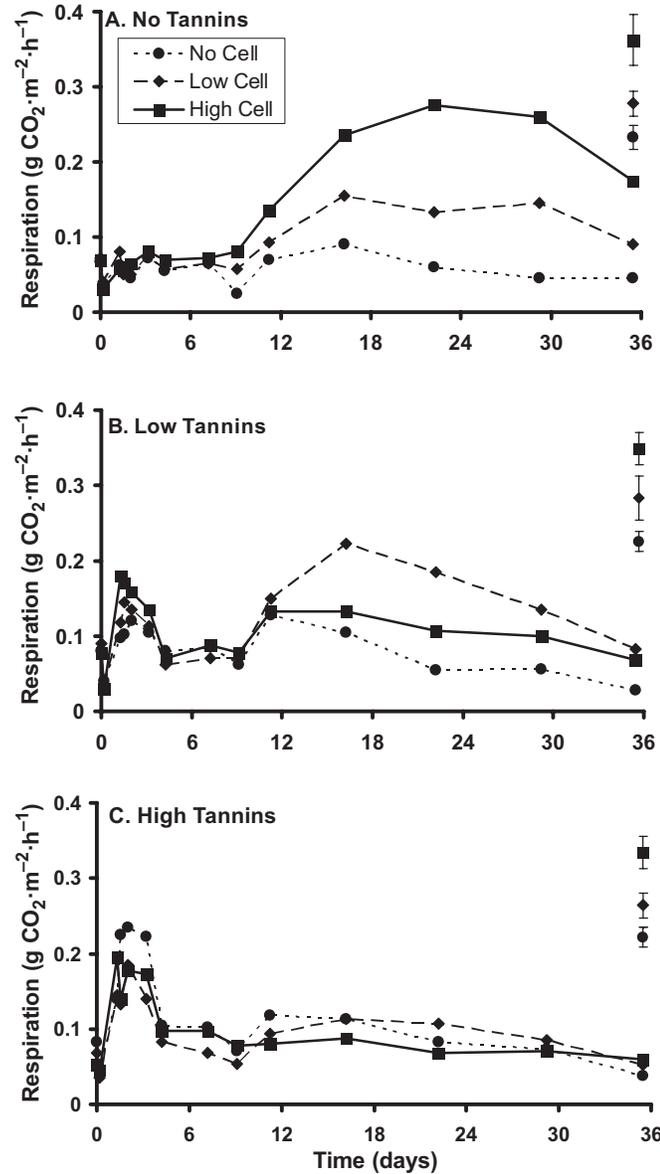
Each soil microcosm consisted of a 5 cm × 18 cm cylinder of clear acrylic tubing with a fiberglass mesh bottom leading into a funnel. Cylinders were capped with a rubber seal. All microcosms received 100 g of air-dried silt loam field soil collected from an undisturbed aspen forest in southern Wisconsin. Soil was sieved and well mixed before being placed into microcosms and allowed to equilibrate for 1 week at 22 °C and 80%–90% relative humidity in a Percival incubator. These conditions were kept constant throughout the experiment. Tannin treatments consisted of 0, 0.1, and 0.2 g additions per microcosm and were crossed with 0, 0.5, and 1.0 g cellulose additions (3 tannin treatments × 3 cellulose treatments × 4 replicates = 36 microcosms). Tannins were dissolved in 3 mL of double-distilled water before addition and all 0 g tannin treatments also received 3 mL of double-distilled water. Cellulose treatments consisted of chopped (2 mm × 2 mm) cellulose filter paper (Fisherbrand quantitative grade). We used filter paper instead of powdered cellulose because it more closely resembles leaf litter. These amounts of tannin and cellulose additions were added at the beginning of the experiment (single additions) and were chosen based on the range of naturally occurring inputs per soil area. The 0.1 and 0.2 g tannin treatments correspond roughly to 7% and 14% tannin content in senesced leaf litter, respectively. Cellulose additions were intended to supply a realistic range of complex carbon substrates. These calculations were based on a ~1.4 g-microcosm⁻¹ upper limit for litterfall values derived from natural aspen specific leaf area (Lindroth and Hwang 1996), aspen leaf area index (Burrows et al. 2002), and aspen litterfall values (Raich and Nadelhoffer 1989; Steele et al. 1997; Davidson et al. 2002). We measured respiration at 0, 4, 31, 37, 49, 77, 102, 173, 219, 270, 390, 533, 700, and 851 h over the course of 36 days using a PP Systems soil respiration meter with a modified soil chamber.

Data were log transformed to meet the assumptions of normality before statistical analysis. Respiration data were analyzed in two complementary fashions. First, respiration data were analyzed via a repeated-measures analysis of variance (ANOVA). Then, cumulative soil respiration data were calculated and analyzed with an ANOVA. All statistical analyses were completed with SAS JMP (v. 4.0.4). Data presented in the figures are untransformed.

Results

Tannin additions induced a quick pulse of respiration that corresponded to the amount of tannins added (Fig. 1). However, the tannin-induced respiration ended within 2 weeks, after which tannins had a negative influence on soil respira-

Fig. 1. Soil respiration over time. No cell = 0 g of cellulose, low cell = 0.5 g of cellulose, high cell = 1.0 g of cellulose. No tannins = 0 g of tannins, low tannins = 0.1 g of tannins, high tannins = 0.2 g of tannins. Each line represents the average of four microcosms. Points in the upper right corner of each panel represent the mean standard error for each data set over time. See Table 1 for repeated-measures ANOVA results.



tion if cellulose was present (Fig. 1). Microcosms with low levels of tannins displayed a two-humped respiration pattern, which shows the initial tannin-induced respiration period followed by the cellulose-induced respiration period (Fig. 1B). There were no independent effects of tannins or cellulose on respiration, but both tannins and cellulose (marginally) influenced respiration over time (Table 1). Tannin and cellulose additions interacted to influence respiration (Table 1) such that cellulose increased respiration in the absence of tannins but not in the presence of high amounts of tannins.

The interactive effects of tannin and cellulose additions are also evident in the cumulative respiration data (Fig. 2).

Table 1. Repeated-measures ANOVA effects of tannin and cellulose additions and their interaction on soil respiration over time.

Effects	df	F	P
Tannin	1, 32	1.79	0.190
Cellulose	1, 32	2.75	0.107
Tannin × cellulose	1, 32	4.84	0.035
Time	13, 20	3.70	<0.001
Time × tannin	13, 20	8.89	<0.001
Time × cellulose	13, 20	2.01	0.078
Time × tannin × cellulose	13, 20	1.65	0.151

Fig. 2. Cumulative respiration over 36 days. Bars represent the average cumulative respiration for four microcosms. Different letters indicate significant differences at $P < 0.05$. Error bars represent 1 SE. See Table 2 for ANOVA results.

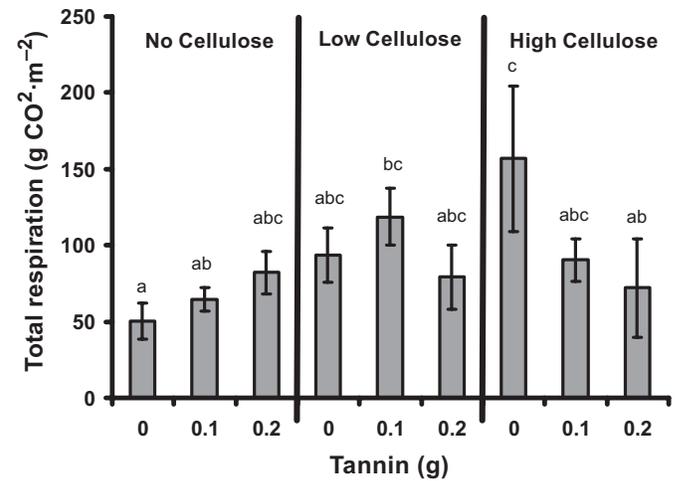


Table 2. ANOVA effects of tannin and cellulose treatments on the cumulative amount of CO₂ respired.

Effects	df	F	P
Tannin	1, 32	1.09	0.305
Cellulose	1, 32	2.90	0.098
Tannin × cellulose	1, 32	8.79	0.006

Tannins tended to increase respiration when no cellulose was present but decreased respiration when large amounts of cellulose were present (Fig. 2). This pattern is confirmed by a large tannin by cellulose interaction effect on the total amount of carbon dioxide respired (Table 2). Microcosms with low cellulose treatments displayed no clear effect of tannins on cumulative respiration (Fig. 2) because total respiration values do not account for the apparent temporal variation in tannin activity displayed in Fig. 1B.

Discussion

While previous research has shown that qualitative differences among tannins can influence microbial activity (Kraus et al. 2004), our results demonstrate that quantitative variation in tannins, purified from a single species, can increase or decrease cumulative soil respiration depending on the

presence of alternative carbon substrates. In this simple model system, condensed tannins increased soil respiration in the absence of an alternative carbon source but decreased cellulose-induced soil respiration.

The initial tannin-induced flush of carbon dioxide (Figs. 1B and 1C) could have been caused by several non-mutually exclusive mechanisms. First, respiration may have been elevated initially due to the presence of simple phenolics in our tannin additions. Although the tannins we used were purified, the condensed tannin extracts contained small amounts (<5%) of low molecular mass fractions that were probably easily decomposed. Kanerva et al. (2006) reported a similar initial pulse of respiration that was induced by low molecular mass phenolics during the first 2 weeks of incubation. Second, at least some portion of larger, more complex tannins was probably catabolized by microbes. Kraus et al. (2004) demonstrated that even complex tannins can induce short-term soil respiration. In their experiment, regardless of qualitative differences among tannins, tannin-induced respiration lasted for 12 days (Kraus et al. 2004). Both of these mechanisms probably accounted for at least some of the tannin-induced respiration that we observed within the first 2 weeks, after which only recalcitrant tannin fractions remained.

The cellulose-induced period of soil respiration occurred after the tannin-induced period because of the time required for the microbial community to colonize the cellulose substrate. Even in the absence of any tannins (Fig. 1A), microcosms with cellulose additions still took approximately 12 days to start respiring measurably. After colonization, the effect of tannins on cellulose decomposition was consistent with the commonly held perspective that tannins retard microbial activity and thus decomposition. When tannins were absent, cellulose additions increased soil respiration; however, when large amounts of tannins were present, cellulose additions had little to no influence on respiration (Figs. 1 and 2). Tannins may have been directly toxic to some microorganisms (Field and Lettinga 1992), but they also likely influenced microbial respiration by affecting soil nitrogen availability and extracellular enzyme activity. Tannins bind organic nitrogen and make it unavailable to microbes, thereby reducing the ability of microbes to produce the extracellular enzymes necessary for cellulose decomposition. In addition, tannins may have also bound, and thereby incapacitated, cellulytic enzymes once they were produced. The inhibitory mechanisms of tannins were apparent only when large amounts of cellulose (and therefore a high demand for nitrogen) were present, suggesting that at least some of the inhibitory effects of tannins on cellulose decomposition were nitrogen mediated. Clarification of the specific mechanism of how tannins mediate cellulose decomposition will contribute to understanding of how variation in aboveground chemistry contributes to belowground nutrient cycling. The relationships that we observed were apparent only because we added several different levels of both tannin and cellulose carbon substrates in an effort to simulate natural forest floor inputs.

Several recent works have highlighted the impacts that tannins and other carbon-containing compounds have on soil processes (Kraus et al. 2004; Kanerva et al. 2006; Orwin et al. 2006). These studies have shown strong interac-

tive effects of different carbon substrates on soil processes and in some cases reported positive effects of tannins on soil microbial activity. However, the interactive effect of differing amounts of tannin and cellulose additions has received little attention. The influence of tannins in natural forests is probably time dependent; tannins may increase short-term microbial activity while retarding long-term decomposition of leaf structural material. Although untested directly in this study, the mechanism through which tannins retard cellulose-induced respiration is probably via binding with organic nitrogen or by incapacitating microbially produced enzymes. Given that tannins never enter natural soils alone, the overall effect of tannins on decomposition is likely negative, despite the fact that purified tannins may cause a transient increase in soil respiration in artificial systems.

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