Biol Fertil Soils (2007) 43:303–311
DOI 10.1007/s00374-006-0106-5

ORIGINAL PAPER

# Charcoal effects on soil solution chemistry and growth of *Koeleria macrantha* in the ponderosa pine/Douglas-fir ecosystem

**Michael J. Gundale · Thomas H. DeLuca**

Received: 26 July 2005 / Revised: 10 April 2006 / Accepted: 12 April 2006 / Published online: 3 June 2006


**Abstract** We conducted laboratory and greenhouse experiments to determine whether charcoal derived from the ponderosa pine/Douglas-fir ecosystem may influence soil solution chemistry and growth of *Koeleria macrantha*, a perennial grass that thrives after fire. In our first experiment, we incubated forest soils with a factorial combination of Douglas-fir wood charcoal generated at 350°C and extracts of *Arctostaphylos uva-ursi* with and without the addition of glycine as a labile N source. These results showed that charcoal increased N mineralization and nitrification when glycine was added, but reduced N mineralization and nitrification without the addition of glycine. Charcoal significantly reduced the solution concentration of soluble phenols from litter extracts, but may have contributed bioavailable C to the soil that resulted in N immobilization in the no-glycine trial. In our second experiment, we grew *K. macrantha* in soil amended with charcoal made at 350°C from ponderosa pine and Douglas-fir bark. Growth of *K. macrantha* was significantly diminished by both of these charcoal types relative to the control. In our third experiment, we grew *K. macrantha* in soil amended with six concentrations (0, 0.5, 1, 2, 5, and 10%) of charcoal collected from a wildfire. The data showed increasing growth of *K. macrantha* with charcoal addition, suggesting some fundamental differences between laboratory-generated charcoal and wildfire-produced charcoal. Furthermore, they suggest a need for a better understanding of how temperature and substrate influence the chemical properties of charcoal.

**Keywords** Charcoal · Soil solution chemistry · Douglas-fir and ponderosa pine ecosystems

M. J. Gundale (✉) · T. H. DeLuca
Department of Ecosystem and Conservation Sciences
University of Montana,
32 Campus Drive,
Missoula, MT 59812, USA
e-mail: mjgundal@yahoo.com

T. H. DeLuca
e-mail: tom.deluca@cfc.umt.edu

## Introduction

It is well-established that fire alters N cycling in the ponderosa pine/Douglas-fir (*Pinus ponderosa*/*Psuedotsuga menziesii*) ecosystem (Neary et al. 1999; Hart et al. 2005). Nitrogen availability has been shown to increase immediately after fire (Covington and Sackett 1990, 1992; DeLuca and Zouhar 2000) and may remain elevated on the scale of months to years as a result of enhanced mineralization (Covington and Sackett 1990, 1992; Monleon et al. 1997; Kaye and Hart 1998; Gundale et al. 2005). Numerous processes that increase N mineralization after fire have been identified, including improved substrate quality (White 1991, 1994; Fernandez et al. 1997; Pietikainen et al. 2000a), death of roots and soil organisms resulting in a large labile organic N pool (DeBano et al. 1979; Dunn et al. 1979; Diaz-Ravina et al. 1996; Neary et al. 1999), and a reduction in C to N ratios due to preferential loss of C during combustion (Gundale et al. 2005). A potentially overlooked factor that may also enhance N cycling after fire is the addition of charcoal to soils.

Several recent studies have shown that charcoal has the potential to greatly enhance soil fertility. Amazonian forest soils amended centuries ago with charcoal and manure still maintain some of the highest biodiversity and productivity

of any soils within the Amazon basin (Glaser et al. 2001, 2002; Mann 2002). In boreal forest soils, charcoal was shown to enhance N cycling by ameliorating the inhibitory effects of litter extracts from late-successional species, which in turn promotes growth of early-successional species (Zackrisson et al. 1996; Wardle et al. 1998; DeLuca et al. 2002; Berglund et al. 2004). Recently, DeLuca et al. (2006) found that the addition of wildfire-formed charcoal to ponderosa pine forest soils increased nitrification rates.

Charcoal may enhance soil fertility through a variety of mechanisms. Increased N turnover may occur by charcoal sorption of high C:N organic molecules from the soil solution (Zackrisson et al. 1996; Wardle et al. 1998; Glaser et al. 2002), resulting in reduced microbial N immobilization and higher net mineralization and nitrification rates. In addition, charcoal may remove specific groups of organic molecules, including polyphenol or monoterpene compounds that are thought to inhibit nitrification (Rice and Pancholy 1972; Zackrisson et al. 1996; DeLuca et al. 2002; Berglund et al. 2004). Sorption of organic molecules, along with the gradual breakdown of charcoal, may initiate humus formation and, thus, enhance long-term soil fertility (Glaser et al. 2002). Charcoal may also enhance soil fertility by creating habitat for microbes within its porous structure (Pietikainen et al. 2000b).

Despite these potential roles that charcoal may have in increasing soil fertility, its ecological role in forest ecosystems, such as ponderosa pine/Douglas-fir, has received little attention. We conducted three separate experiments using low-temperature charcoal to investigate whether charcoal influences soil solution chemistry and growth of an early successional species. In our first experiment, our objective was to determine whether charcoal had an influence on soil solution chemistry after addition of the extracts of a late successional species, *Arctostapholos uvi-ursi*, via surface adsorption of phenolic compounds. We hypothesized that charcoal added to a ponderosa pine forest soil will effectively sorb the phenol fraction in litter extracts, which would correspond with enhanced N cycling.

In our second experiment, our objective was to compare the influence of charcoal made from the bark of two species, ponderosa pine and Douglas-fir, on growth of *Koeleria macrantha*, a perennial grass species that thrives after fire disturbance in western Montana ponderosa pine/Douglas-fir forests. Bark charring during low-intensity wildfire is a potentially significant source of charcoal in this system. Charred bark may gradually slough from trees after fire and become incorporated in the soils surrounding trees. It is recognized that ponderosa pine is a more fire-adapted species than Douglas-fir; thus, an intriguing hypothesis is that charred bark of the more fire-adapted species will have a stronger positive effect on N cycling processes and plant growth.

In our third experiment, our objective was to determine whether charcoal generated during a wildfire would have any effect on *K. macrantha* growth and to determine whether this relationship is dependent on soil charcoal concentration. We hypothesized that wildfire charcoal will positively influence *K. macrantha* and that this effect will increase as a function of soil charcoal concentration. Collectively, these three experiments address our central hypothesis that charcoal will alter solution chemistry by sorbing phenols and enhancing N cycling, which in turn will improve the growth of early successional species.

## Materials and methods

All three experiments utilized field-collected soil, which was collected from the subsurface horizon (20–30 cm, $B_w$ Horizon) of a forest soil associated with low elevation (1,100 m) ponderosa pine/Douglas-fir vegetation in western Montana, USA. The soil is a sandy-skeletal, mixed, frigid Typic Dystrustepts. This ecosystem is characterized by low annual rainfall (<350 mm annually) with approximately 50% falling as snow during the winter months. Soil was collected during the month of September, returned to the lab, upon which they were sieved (4 mm) and homogenized. We then added one part sand to three parts field moist soil (by mass) to decrease fertility and increase porosity and gas exchange, such that nitrification would not be limited by low $O_2$ availability. The sand fraction was purchased as filter grade silica sand (for pool filters) and was washed with 1 M HCl, followed by distilled water, before being homogenized with field collected soil. This sand-amended soil had a pH of 6.8, electrical conductance of 91.2 $\mu S\ m^{-1}$, and had a textural distribution of 71% sand, 21% silt, and 8% clay.

All experiments also included the addition of either laboratory-generated charcoal from Douglas-fir and ponderosa pine or charcoal collected in the field after a wildfire. Laboratory charcoal was generated by burying wood or bark from these species in silica sand and heating at 350°C for 2 h. Charcoal was then ground and sieved as specified for each experiment. Various physical and chemical properties of these charcoals were measured (Table 1). Charcoal pH was measured from a 4:1 slurry of deionized water to charcoal. Electrical conductance (EC) was measured from charcoal paste (2:1 distilled water and charcoal). Cation exchange capacity (CEC) was estimated on charcoal samples via $NH_4^+$ replacement where 1 g of charcoal was rinsed twice with 25 ml of 1 M ammonium acetate (pH 7) to saturate exchange sites. Excess saturating solution was removed from charcoal samples with three consecutive washes with 25 ml of 95% ethyl alcohol. Sorbed $NH_4^+$ was then extracted with 25 ml of 2 M KCl and analyzed on a segmented flow analyzer (Auto

**Table 1** Chemical properties of four charcoal types (*df* Douglas-fir, *pp* ponderosa pine, *wildfire* wildfire collected) used in the laboratory and greenhouse experiments

| | df Wood | df Bark | pp Bark | Wildfire |
|---|---|---|---|---|
| pH | 4.15 | 4.18 | 4.81 | 5.74 |
| EC ($\mu S\ g^{-1}$) | 20.5 | 24.2 | 111.6 | 141.5 |
| CEC ($cmol_c\ kg^{-1}$) | 20.66 | 19.42 | 34.48 | 29.35 |
| Density ($g\ cm^{-3}$) | 0.30 | 0.08 | 0.21 | 0.29 |
| Total C (%) | 72.9 | 72.4 | 71.5 | 82.3 |
| Soluble phenols ($\mu g\ g^{-1}$) | 34.9 | 30.7 | 43.4 | 48.2 |
| Total phenols ($\mu g\ g^{-1}$) | 441.7 | 148.1 | 810.4 | 393.8 |
| $PO_4^{-3}$ ($\mu g\ g^{-1}$) | 0.94 | 0.84 | 2.46 | 0.95 |
| $NH_4^+$ ($\mu g\ g^{-1}$) | 7.12 | 9.0 | 5.6 | 38.3 |
| $NO_3^-$ ($\mu g\ g^{-1}$) | 0.12 | 0.3 | 0.0 | 4.4 |

Analyzer III, Bran Luebbe, Chicago, IL) using the Berthelot reaction (Willis et al. 1993). Charcoal density was measured by measuring dry mass of intact charcoal pieces and measuring volume displacement in deionized water. Total C was measured via dry combustion on a Fissions Elemental Analyzer (Milano, Italy). Soluble and total phenols were measured by extracting 1 g of ground charcoal in 25 ml of deionized water and 50% methanol, respectively, and were analyzed using the Prussian Blue Method (Stern et al. 1996). Extractable $NH_4^+$ and $NO_3^-$ (Mulvaney 1996) were extracted by shaking 1 g of charcoal for 30 min in 25 ml of 2 M KCl, and then filtering through Whatman #2 filters. The extracts were analyzed for $NH_4^+$–N using the Berthelot reaction (Willis et al. 1993) and $NO_3^-$–N by the cadmium reduction method (Willis and Gentry 1987) on a segmented flow analyzer (Auto Analyzer III). Soluble $PO_4^{-3}$ was extracted by placing 1 g of charcoal in 25 ml of 0.01 M $CaCl_2$ for 30 min. Extracts were filtered through Whatman #42 filter paper and then analyzed on a segmented flow analyzer using the molybdate method as described by Kuo (1996).

## Experiment 1: charcoal sorption potential

We conducted a laboratory incubation study using the soil described above, where Douglas-fir charcoal and extract of *Arctostaphylos uva-ursi* were added in a factorial combination yielding four treatments (Charcoal/Extract, Charcoal/No extract, No Charcoal/Extract, and No Charcoal/No extract). Each treatment was replicated five times and consisted of 300 g of soil and placed into mason jars. The treatments receiving charcoal addition received a 2% charcoal amendment (20 g/kg). Charcoal was generated in a muffle furnace by submerging Douglas-fir wood in sand and heating it at 350°C for 2 h. Charcoal was ground and sieved through a 4.75-mm sieve. *A. uva-ursi* extract was made by extracting 100 g of *A. uva-ursi* leaves in 1 l of deionized water for 24 h and filtering this extract through Whatman #42 filters. The total phenol concentration of this extract was 267.5 mg/l. Extract treatments received 25 ml of this extract. No-extract treatments received an equivalent volume of deionized water. Soils were homogenized following this addition. This addition brought the soil in each mason jar to a water content of approximately 60% WHC. Mason jars were incubated in the dark for 14 days after which a portion of the soil was extracted and analyzed.

This entire experiment was repeated exactly as described above but with glycine added to all mason jars as a source of highly labile organic N to stimulate a more marked N response. Glycine, a simple amino acid that is readily mineralized to $NH_4^+$, was added to each mason jar at a rate of 75 mg/jar (250 mg/kg of soil). These two experiments will hereafter be referred to as the glycine and no-glycine trials.

## Experiment 2: effects of bark charcoal on plant growth

This greenhouse experiment consisted of three treatments (Douglas-fir charcoal, ponderosa pine charcoal, and a control) using the sand-amended soil described above to evaluate the influence of charcoal source on *K. macrantha*. Each treatment consisted of 20 replicate pots where each pot received 1.5 kg of soil, and charcoal treatments received a 2% (by mass) charcoal amendment. One percent of this charcoal was homogenized into the soil, while the other 1% was evenly distributed on the soil surface. We made charcoal from Douglas-fir and ponderosa pine in the laboratory by burying bark of each species in silica sand and heating to 350°C in a muffle furnace for 2 h. Charcoal was ground and sieved (<1 mm) using a Wiley mill. Organic horizons ($O_i$, $O_e$, and $O_a$) were added to the surface of each pot to add an additional and substantial mineralizable pool of plant essential nutrients, as well as to provide a source of bioavailable organic C that may influence soil nutrient transformations. This organic material was randomly collected (as described in Gundale et al. 2005) from a ponderosa pine/Douglas-fir forest that had not been exposed to fire for approximately 80 years and originated from numerous species, including understory and overstory species, but appeared to be primarily composed of undecomposed ponderosa pine and Douglas-fir litter. The organic material was homogenized and 100 g was added to the surface of each pot. A mixed bed ionic resin capsule (Unibest, Bozeman, MT) was placed in the center of each pot to sorb nutrients throughout the duration of the experiment.

*K. macrantha* was grown in these pots between October 2004 and March 2005 under ambient light conditions. An average greenhouse temperature of 21°C was maintained. *K. macrantha* seeds (Western Native Seeds, Coaldale, CO) were germinated in a separate soil medium, and a single seedling was transplanted into each pot. Pots were watered 3 days a week throughout the duration of the experiment. At

the end of the experiment, resin capsules were recovered, and soil was rinsed from roots. Plants were oven-dried at 65°C, and above- and belowground masses were measured.

### Experiment 3: effect of wildfire charcoal on plant growth

Charcoal collected from a wildfire site was added to the soil described above at a rate of 0, 0.5, 1, 2, 5, and 10%, and placed in greenhouse pots seeded with *K. macrantha* to determine whether an increase in soil charcoal content has any influence on the growth of *K. macrantha*. Each treatment ($n$=10) was established by adding 1.0 kg of charcoal-amended soil per pot. The charcoal used in this experiment differed from both previous experiments because it was collected after a wildfire rather than generated in the laboratory. Large particles (>5-cm diameter) of charcoal were collected in the spring of 2004 from the Black Mountain Fire (August 2003), Missoula, MT, (DeLuca et al. 2006). It was impossible to decipher the species origin of this charcoal, but it was likely primarily Douglas-fir and ponderosa pine wood and bark char. The charcoal particles were crushed, using a mallet, producing fragments ranging from a diameter of 2 cm to microscopic. No attempt was made to discriminate against any size class in an attempt to simulate the range of charcoal particle sizes likely incorporated into the soil under natural conditions. Organic horizon materials (50 g) were collected from a forest stand not exposed to fire for over 80 years and added to the surface of each pot as described earlier. All other experimental conditions were run identically to experiment 2.

### Laboratory analyses

At the end of experiment 1, 30 g of soil were extracted with 2 M KCl and analyzed for $NH_4^+$ and $NO_3^-$ as described above. Amino N was measured on these same extracts using the ninhydrin method (Moore 1968). Soluble phenols were extracted by shaking 30 g of soil for 1 h with 50 ml of deionized water followed by filtration. Sorbed phenols were extracted by shaking 30 g of soil with 50% methanol for 24 h followed by filtration. Phenols in these extracts were measured using the Prussian blue method (Stern et al. 1996). Respiration was measured at the end of the incubation by incubating 50 g dry weight equivalent soil in a sealed container with 20 ml 1 M NaOH traps for 3 days (Zibilske 1994).

Mixed bed ionic resin capsules (Unibest) were used in experiments 2 and 3 to determine solution $NH_4^+$, $NO_3^-$, and $PO_4^-$ throughout the duration of the experiments. Capsules were placed in the center of each pot, directly beneath each plant, and were removed and extracted in 10 ml of 2-M KCl three consecutive times. We analyzed $NH_4^+$, $NO_3^-$, and $PO_4^{-3}$ from these extracts as described previously.

### Statistical analyses

Data in experiment 1 meeting assumptions of normality and homoscedasticity were analyzed using two-factor analysis of variance (ANOVA), where extract and charcoal were entered as fixed factors under the general linear model. Variables not meeting these assumptions were analyzed using a Kruskal–Wallis test (K–W test). This analysis tests for differences among treatments but does not evaluate the significance of individual factors or interactions between factors.

Data in experiments 2 and 3 were analyzed using one-factor ANOVA followed by the Student–Newman–Keuls post hoc procedure. Different letters are used to display post hoc differences. Data not meeting assumption of normality and homoscedasticity were compared using K–W tests, which were not followed by post hoc procedures. All analyses were conducted using SPSS 12.0 software.

## Results and discussion

### Experiment 1: low temperature charcoal sorption potential

Both charcoal and litter extract significantly influenced numerous soil chemical variables (Fig. 1). In both glycine and no-glycine trials, litter extract negatively influenced extractable $NO_3^-$ concentrations. The negative influence of *A. uva-ursi* on extractable $NO_3^-$ reported here is consistent with our previous studies in ponderosa pine forest soils (DeLuca et al. 2006) and with studies that showed that litter from late-successional boreal species, such as the ericaceous shrub *Empetrum hermaphroditum*, diminishes net nitrification (DeLuca et al. 2002; Berglund et al. 2004). Charcoal had an unexpected negative effect on $NO_3^-$ in the no-glycine trial. In contrast, the addition of charcoal increased $NO_3^-$ concentrations in the glycine trial. These results may be a function of the charcoal we used in this study, which was generated at a low temperature (350°C). Charcoal contains a significant concentration of bioavailable C, specifically soluble phenols (Table 1) that may have caused net $NO_3^-$ immobilization (Schimel et al. 1996) in the no-glycine trial where low $NH_4^+$ concentrations existed (Rice and Tiedje 1989). The $NO_3^-$ immobilization effect did not occur in the glycine trial because $NH_4^+$ limitations were drastically reduced with glycine addition. In addition, higher rates of nitrification in the glycine trial likely occurred because this process was not limited by a lack of substrate availability (glycine additions resulted in high $NH_4^+$ concentrations).

The higher rate of nitrification associated with charcoal in the glycine trial is consistent with the finding reported by DeLuca et al. (2006), which suggests that charcoal may sorb compounds from litter extract and the soil solution that

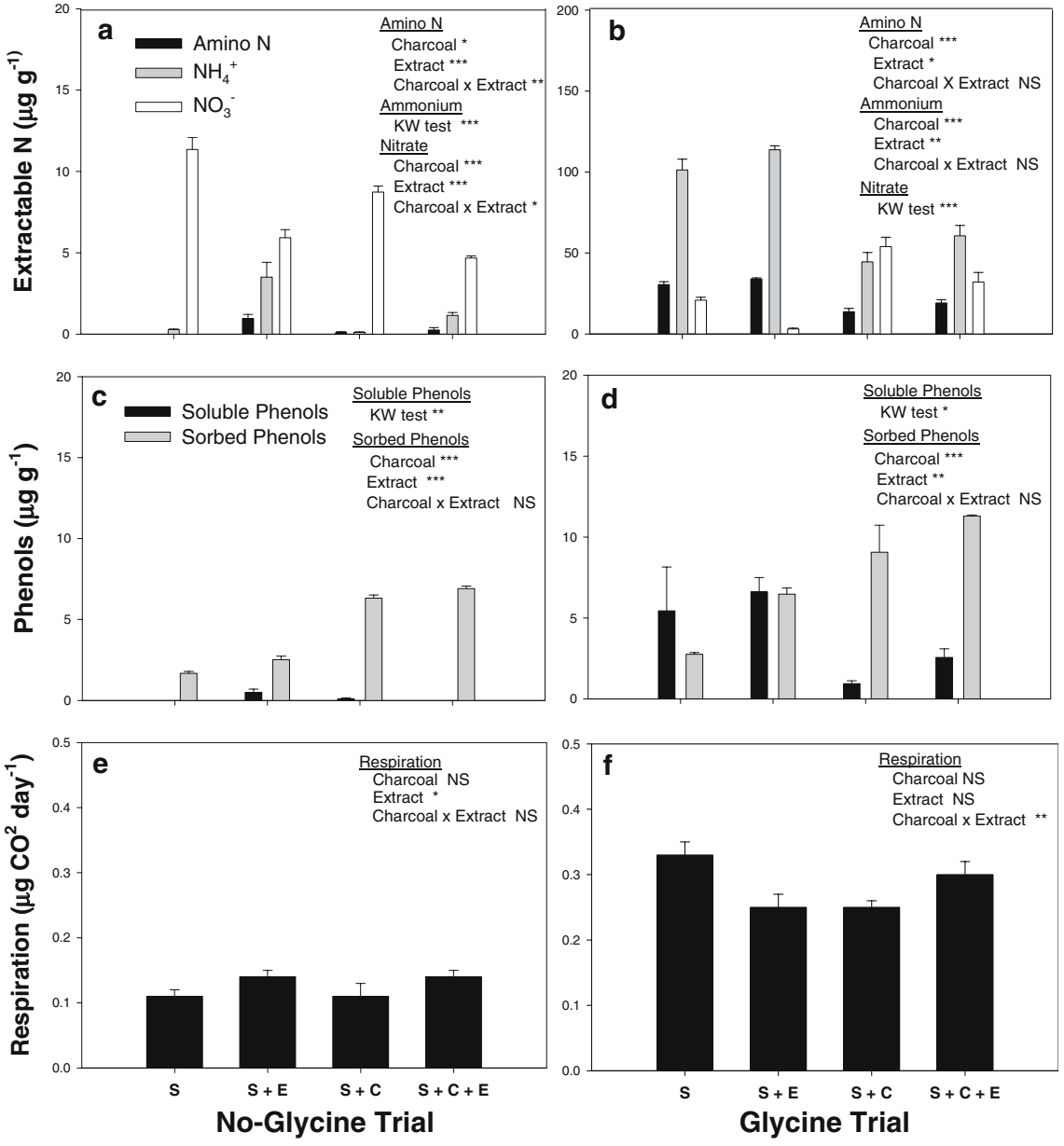


**Fig. 1** Extractable amino N, $NH_4^+$, and $NO_3^-$ [mean (SE)] without (**a**) and with (**b**) glycine addition; soluble (water extracted) and sorbed phenols (methanol extracted) [mean (SE)] without (**c**) and with (**d**) glycine addition; and basal soil respiration [mean (SE)] without (**e**) and with (**f**) glycine addition, from a 14-d soil incubation experiment where soils were amended with a factorial combination of charcoal and extracts from *Arctostaphylos uva-ursi* leaves (*S* soil only, *S*+*E* soil plus extract, *S*+*C* soil plus charcoal, *S*+*C*+*E* soil plus charcoal plus extract). Data were analyzed with a two-factor ANOVA where significance was tested for Charcoal, Extract, and Charcoal × Extract interaction. Data that did not meet parametric assumptions of normality or homoscedasticity were analyzed using a Kruskal–Wallis (*KW*) test. *Asterisks* represent statistical significance (*p* value, ns >0.1, *<0.05, **<0.01, ***<0.001)

are inhibitory to nitrifying bacteria, or sorb carbon-rich molecules that would otherwise stimulate microbial immobilization of N.

*A. uva-ursi* extract had a strong positive effect on $NH_4^+$ in both no-glycine and glycine trials because it likely contained some $NH_4^+$ and substrates that are rapidly mineralized to $NH_4^+$. Charcoal had a strong negative effect on $NH_4^+$ in both no-glycine and glycine trials. The mechanisms for this pattern may differ between the two trials. In the no-glycine trial, the most likely explanation for reduced $NH_4^+$ is that immobilization occurred as a function of N limitations in these soils. In the glycine trial, higher rates of nitrification associated with charcoal likely contributed to lower $NH_4^+$ concentrations.

Both charcoal and extract significantly influenced concentrations of amino N that represent a highly labile

**Table 2** Plant mass and resin sorbed nutrients (mean±SE, *n*=20) from a greenhouse experiment where soil was amended with 2% charcoal made from Douglas-fir (*df*) and ponderosa pine (*pp*) bark at 350°C

| | df Charcoal | pp Charcoal | no Charcoal | *p* value |
|---|---|---|---|---|
| Total mass (g) | **a**1.6 (0.2) | **a**1.9 (0.1) | **b**2.5 (1.0) | <0.001 |
| Root mass (g) | **a**0.8 (0.1) | **a**0.9 (0.1) | **b**1.2 (0.1) | <0.05 |
| Aboveground mass (g) | **a**0.7 (0.1) | **b**1.0 (0.1) | **c**1.3 (0.1) | <0.001 |
| Root to shoot ratio | **a**1.1 (0.1) | **b**0.9 (0.1) | **b**0.9 (0.1) | <0.01 |
| $NH_4^+$ (μg resin capsule$^{-1}$) | **a**4.6 (0.9) | **b**1.4 (0.6) | **b**2.2 (0.8) | <0.05 |
| $NO_3^-$ (μg resin capsule$^{-1}$) | **a**1,770.8 (286.2) | **b**935.8 (241.9) | **b**581.5 (211.8) | <0.01 |
| $PO_4^{-3}$ (μg resin capsule$^{-1}$) | **a**5.1 (1.4) | **a**5.6 (0.9) | **b**0.5 (1.2) | <0.05 |

Letters in bold indicate differences using the Student–Newman–Keuls post hoc procedure

fraction of organic N that can be rapidly mineralized. Glycine, which is a simple amino N molecule, stimulated rapid rates of N mineralization and resulted in increased amino N concentrations, which suggests that the added glycine was not completely utilized and that substrate limitations were eliminated during this trial. In glycine and no-glycine trials, the litter extract resulted in higher concentrations of amino N to soils. The effect of charcoal on amino N, however, differed in glycine and no-glycine trials. In the no-glycine trial, charcoal significantly increased amino N concentrations. This response may have occurred because charcoal sorbed phenolic molecules that otherwise would form insoluble complexes with amino N groups. In contrast, charcoal had a negative effect on amino N in the glycine trial, which is likely the result of charcoal enhancing microbial utilization of glycine.

As expected, *A. uva-ursi* extract significantly increased phenols (soluble and sorbed) in both trials. The addition of charcoal to soil significantly diminished the soluble phenol concentration while increasing the pool of sorbed phenol. This result is consistent with several studies in the boreal forest that have demonstrated a high capacity of charcoal to adsorb phenolic compounds (Zackrisson et al. 1996; Wardle et al. 1998; DeLuca et al. 2002; Berglund et al. 2004). Solubility of these fractions likely influences the degree to which they are bioavailable and, therefore, their ability to interfere with N transformations (Harborne 1997). It is interesting to note that total phenols (sorbed and soluble) was higher in the charcoal-only treatment of both trials than the control, demonstrating that charcoal itself adds a substantial amount of total phenol to the soil (Table 1). These phenols are likely derived from the components of wood, such as lignin that are degraded during charcoal formation. It is unclear what effect these phenols have on soil processes, but it is likely that they could be utilized as a food source by microbes, stimulating N immobilization.

Soil respiration showed little response to charcoal in glycine or no-glycine trials. In the no-glycine trial, the extract significantly increased soil respiration. Extract and charcoal had no individual effect on soil respiration in the glycine trial; however, the interaction between charcoal and extract showed a significant effect. We speculate that this response may reflect that amines and degradable carbon substrates were better utilized by microbes when phenolic molecules in the same extract were sorbed by charcoal.

These data demonstrate that low-temperature charcoal effectively sorbs soluble phenols from *A. uva-ursi* extracts, which in turn stimulates nitrification, provided nitrification is not substrate-limited. Our results are consistent with Berglund et al. 2004 and DeLuca et al. (2002), who showed that the effect of charcoal on nitrification only occurred when a labile N source was also present. These studies are also consistent with the Terra Preta phenomenon reported in the Amazonian basin where charcoal and manure (high labile N concentration) were historically incorporated into the soil (Glaser et al. 2001, 2002). Today, these soils maintain the highest fertility in the region, which may in part be a function of the interactive effect of charcoal and manure.

## Experiment 2: effects of bark charcoal on plant growth

In this experiment, we unexpectedly found that charcoal from both species diminished growth of *K. macrantha* relative to the control with reduced mass in both aboveground and belowground growth (Table 2). *K. macrantha* growing in pots with Douglas-fir charcoal had a significantly higher root to shoot ratio than the other treatments that appeared to be primarily driven by low aboveground biomass. This data suggests that there is likely no difference in the effect of ponderosa pine and Douglas-fir charcoal on plant species in this ecosystem.

We found that resin-sorbed $NH_4^+$ and $NO_3^-$ were significantly higher in the Douglas-fir charcoal treatment relative to the ponderosa pine charcoal treatment and the control. Resin-sorbed $PO_4^{-3}$ was significantly higher in both Douglas-fir and ponderosa pine charcoal treatments than the control. These results may be interpreted in several ways. First, they may indicate higher mineralization and nitrification rates in the presence of charcoal as suggested by experiment 1. If higher mineralization occurred in the presence of charcoal, it is unclear why a corresponding increase in plant growth did not occur. It is possible that some toxic substance was generated during charcoal formation that inhibited root growth of *K. macrantha*, despite a positive effect on nutrient availability (Fritze et al.

**Table 3** Plant mass and resin sorbed nutrients (mean±SE, $n$=10) from a greenhouse experiment where soil was amended with 0, 0.5, 1, 2, 5, and 10% charcoal collected from a wildfire

| | Percent charcoal | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0% | 0.5% | 1% | 2% | 5% | 10% | $p$ |
| Total mass (g) | **a**0.5 (0.2) | **ab**1.0 (0.3) | **ab**1.1 (0.2) | **ab**1.1 (0.2) | **b**1.3 (0.1) | **b**1.4 (0.1) | <0.05 |
| Root mass (g) | 0.3 (0.2) | 0.6 (0.2) | 0.7 (0.1) | 0.7 (0.1) | 0.8 (0.1) | 0.8 (0.1) | >0.05 |
| Aboveground mass (g) | **a**0.2 (0.1) | **ab**0.4 (0.1) | **ab**0.4 (0.1) | **ab**0.4 (0.1) | **b**0.5 (0.1) | **b**0.6 (0.1) | <0.01 |
| Root to shoot ratio | 1.5 (0.2) | 1.5 (0.3) | 1.8 (0.2) | 1.7 (0.3) | 1.6 (0.2) | 1.3 (0.1) | >0.05 |
| $NH_4^+$ (μg resin capsule$^{-1}$) | 55.6 (4.0) | 49.8 (4.6) | 36.9 (6.0) | 42.7 (2.2) | 43.0 (1.3) | 44.4 (2.9) | <0.05[a] |
| $NO_3^-$ (μg resin capsule$^{-1}$) | **a**1,539.8 (463.4) | **b**947.9 (128.4) | **bc**552.3 (116.3) | **bc**556.1 (93.4) | **bc**561.8 (278.7) | **c**248.6 (29.8) | <0.001 |
| $PO_4^{-3}$ (μg resin capsule$^{-1}$) | **a**10.1 (1.4) | **a**8.8 (2.1) | **ab**5.8 (1.2) | **ab**6.5 (1.7) | **bc**1.7 (1.0) | **c**0.0 (1.2) | <0.001 |

Letters in bold indicate differences using the Student–Newman–Keuls post hoc procedure
All $p$ values are for one-way ANOVA, unless otherwise noted
[a]Kruskal–Wallis test $p$ value

1998; Villar et al. 1998). These toxic substances are likely to be more abundant in low temperature charcoals, such as used in this experiment, and may be prone to volatilization at higher temperatures. An additional explanation is that charcoal may have enhanced soil macroporosity, allowing more soil solution to pass through capsules, resulting in misleading resin-sorbed nutrient concentrations.

### Experiment 3: effect of wildfire charcoal on plant growth

In support of our hypothesis, natural charcoal collected from a wildfire showed a positive effect on growth of *K. macrantha* (Table 3). Both total and aboveground masses were significantly higher in pots amended with 5 and 10% charcoal addition than the control. Pots with lower charcoal content (0.5–2%) showed an intermediate growth response. No significant shift in allocation to above- or belowground structures was detected across the charcoal gradient. As in experiment 2, resin-sorbed $NO_3^-$ and $PO_4^{-3}$ decreased as plant growth increased. These results suggest that these measurements do not reflect any direct effect charcoal may have on nutrient cycling, but are rather indicative of the solution nutrient concentration as influenced by plant uptake. No difference in resin sorbed $NH_4^+$ occurred across the charcoal gradient.

The different responses of *K. macrantha* to charcoal in experiments 2 and 3 suggest that charcoal produced in a laboratory may be greatly different from charcoal generated during wildfire. Differences in charring conditions may influence the chemical and structural nature of charcoal and may therefore change its influence on soil solution chemistry. One potentially important difference between laboratory- and wildfire-collected charcoal was the ratio of soluble phenols to $NH_4^+$ concentration extracted from the charcoals (Table 1). While all charcoal had relatively similar soluble phenol contents, which may stimulate microbial N immobilization, high $NH_4^+$ concentrations may have offset this immobilization effect when wildfire charcoal was used. Another potentially important difference is the different pH of laboratory charcoal and wildfire charcoal (Table 1). The low pH associated with the lab charcoals may have indirectly diminished P availability in these treatments. Another difference between the charcoal used in experiments 2 and 3 was the range of charcoal particle size used. Experiment 3 incorporated charcoal ranging from large (1–2 cm) to microscopic fractions. We noted substantial root penetration into large charcoal particles at the end of this greenhouse experiment, which suggests that some resource, such as water, is more available inside large charcoal particles. It is also possible that grinding charcoal to a smaller size class, in some way, eliminates its beneficial effects on soil fertility. For instance, grinding may enhance the availability of organic carbon because it is very immobile, whereas N ions are significantly more mobile; thus, nutrient immobilization may be more substantial when charcoal is ground.

## Conclusion

It is clear that charcoal has the potential to significantly alter soil solution chemistry and growth of *K. macrantha*. Charcoal did not appear to stimulate N cycling in a low-nutrient setting, but when glycine was added to soil, charcoal greatly enhanced N mineralization and nitrification. This result may indicate that low temperature charcoal contributes bioavailable carbon that causes N immobilization under low nutrient conditions. As hypothesized, charcoal effectively sorbed soluble phenols from litter extracts. This sorption may effectively reduce the inhibitory effect of litter extracts on soil microorganisms, plants, and biogeochemical processes. Low-temperature, laboratory-generated charcoal had a negative effect on growth of *K. macrantha*, possibly as a result of

a toxicity effect caused by some compound formed during low temperature charring or by N immobilization, as suggested by the no-glycine soil incubation. In contrast, charcoal created during a wildfire had a positive effect on the growth of *K. macrantha*, suggesting low-temperature, laboratory charcoal may not adequately represent field-collected charcoal. Field-collected charcoal may have been generated in a higher oxygen, higher temperature environment and may have been exposed to leaching by rainwater and occlusion by soil organic compounds before collection. Further investigation is required to evaluate how charcoal formation conditions alter its effect on soil processes and plant growth and how these processes manifest themselves in natural ecosystems.

**Acknowledgements** We thank V. Kurth, D. Mackenzie, and T. Burgoyne for their assistance in the laboratory and greenhouse. We also acknowledge funding from the NSF (NSF-DEB-03171108) and the USDA Joint Fire Sciences Program (FFS #107) for this research.

## References

Berglund LM, DeLuca TH, Zackrisson O (2004) Activated carbon amendments of soil alters nitrification rates in Scots pine forests. Soil Biol Biochem 36:2067–2073

Covington WW, Sackett SS (1990) Fire effects on ponderosa pine soils and their management implications. USDA Forest Service RM-GTR-191. Rocky Mountain Forest and Range Experiment Station, Flagstaff, AZ, pp 105–111

Covington WW, Sackett SS (1992) Soil mineral nitrogen changes following prescribed burning in ponderosa pine. For Ecol Manag 54:175–191

DeBano LF, Eberlein GE, PHD (1979) Effects of burning on chaparral Soils: I. Soil nitrogen. Soil Sci Soc Am J 43:504–509

DeLuca TH, Zouhar KL (2000) Effects of selection harvest and prescribed fire on the soil nitrogen status of ponderosa pine forests. For Ecol Manag 138:263–271

DeLuca TH, Nilsson M-C, Zackrisson O (2002) Nitrogen mineralization and phenol accumulation along a fire chronosequence in northern Sweden. Oecologia 133:206–214

DeLuca TH, MacKenzie MD, Gundale MJ, Holben WE (2006) Wildfire-produced charcoal directly influences nitrogen cycling in forest ecosystems. Soil Sci Soc Am J 70:448–453

Diaz-Ravina M, Prieto A, Baath E (1996) Bacterial activity in a forest soil after soil heating and organic amendments measured by the thymidine and leucine incorporation techniques. Soil Biol Biochem 28:419–426

Dunn PH, DeBano LF, Eberlein GE (1979) Effects of burning on chaparral soils: II. Soil microbes and nitrogen mineralization. Soil Sci Soc Am J 43:509–514

Fernandez I, Cabaneiro A, Carballas T (1997) Organic matter changes immediately after a wildfire in an Atlantic forest soil and comparison with laboratory soil heating. Soil Biol Biochem 29:1–11

Fritze H, Pennanen T, Kitunen V (1998) Characterization of dissolved organic carbon from burned humus and its effects on microbial activity and community structure. Soil Biol Biochem 30:687–693

Glaser B, Haumaier L, Guggenberger G, Zech W (2001) The 'Terra Preta' phenomenon: a model for sustainable agriculture in the humid tropics. Naturwissenschaften 88:37–41

Glaser B, Lehmann J, Zech W (2002) Amerliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—a review. Biol Fertil Soils 35:219–230

Gundale MJ, DeLuca TH, Fiedler CE, Ramsey PW, Harrington MG, Gannon JE (2005) Restoration management in a Montana ponderosa pine forest: effects on soil physical, chemical, and biological properties. For Ecol Manag 213:25–38

Harborne JB (1997) Role of phenolic secondary metabolites in plants and their degradation in nature. In: Cadisch G, Giller KE (eds) Driven by nature: plant litter quality and decomposition. CAB International, Oxon, UK

Hart SC, DeLuca TH, Newman GS, MacKenzie MD, Boyle SI (2005) Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. For Ecol Manag 220:166–184

Kaye JP, Hart SC (1998) Ecological restoration alters nitrogen transformations in a ponderosa pine-bunchgrass ecosystem. Ecol Appl 8:1052–1060

Kuo S (1996) Phosphorus. In: Sparks DL (ed) Methods of soil analysis. Part 3—chemical methods. SSSA Book Series 5. Soil Science Society of America, Madison, WI, pp 896–919

Mann CC (2002) The real dirt on rainforest fertility. Science 297:920–923

Monleon VJ, Cromack K, Landsberg JD (1997) Short-and long-term effects of prescribed underburning on nitrogen availability in ponderosa pine stands in central Oregon. Can J For Res 27:369–378

Moore S (1968) Amino acid analysis: aqueous dimethyl sulfide as a solvent for the ninhydrin reagent. J Biol Chem 243:6281–6283

Mulvaney RS (1996) Nitrogen—inorganic forms. In: Sparks DL (ed) Methods of soil analysis. Part 3—chemical methods. Soil Science Society of America, Madison, WI, pp 1123–1184

Neary DG, Klopatek CC, DeBano LF, Ffolliott PF (1999) Fire effects on belowground sustainability: a review and synthesis. For Ecol Manag 122:51–71

Pietikainen J, Hiukka R, Fritze H (2000a) Does short-term heating of forest humus change its properties as a substrate for microbes? Soil Biol Biochem 32:277–288

Pietikainen J, Kiikkila O, Fritze H (2000b) Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Oikos 89:231–242

Rice EL, Pancholy SK (1972) Inhibition of nitrification by climax ecosystems. Am J Bot 59:1033–1040

Rice C, Tiedje J (1989) Regulation of nitrate assimilation by ammonium in soils and in isolated soil microorganisms. Soil Biol Biochem 21:597–602

Schimel JP, VanCleve K, Cates RG, Clausen TP, Reichardt PB (1996) Effects of balsam poplar (Populus balsamifera) tannins and low molecular weight phenolics on microbial activity in taiga floodplain soil: implications for changes in N cycling during succession. Can J Bot 74:84–90

Stern JL, Hagerman AE, Steinberg PD, Winter FC, Estes JA (1996) A new assay for quantifying brown algal phlorotannins and comparisons to previous methods. J Chem Ecol 22:1273–1293

Villar MC, González-Prieto SJ, Carballas T (1998) Evaluation of three organic wastes for reclaiming burnt soils: improvement in the recovery of vegetation cover and soil fertility in pot experiments. Biol Fertil Soils 26:122–129

Wardle DA, Zackrisson O, Nilsson M-C (1998) The charcoal effect in boreal forests: mechanisms and ecological consequences. Oecologia 115:419–426

White CS (1991) The role of monoterpenes in soil in soil nitrogen cycling processes in ponderosa pine: results from laboratory bioassays and field studies. Biogeochemistry 12:43–68

White CS (1994) Monoterpenes: their effects on ecosystem nutrient cycling. J Chem Ecol 20:1381–1406

Willis RB, Gentry CE (1987) Automated method for determining nitrate and nitrite in water and soil extracts. Commun Soil Sci Plant Anal 18:625–636

Willis RB, Schwab GJ, Gentry CE (1993) Elimination of interferences in the colormetric analysis of ammonium in water and soil extracts. Commun Soil Sci Plant Anal 24:1009–1019

Zackrisson O, Nilsson M-C, Wardle DA (1996) Key ecological function of charcoal from wildfire in the Boreal forest. Oikos 77:10–19

Zibilske LM (1994) Carbon mineralization. In: Weaver RW, Angle S, Bottomly P (eds) Methods of soil analysis. Part 2: microbiological and biochemical properties. Soil Science Society America, Madison, WI, pp 835–863