Effect of Charcoal Quantity on Microbial Biomass and Activity in Temperate Soils

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Wildfire-produced charcoal is a common component of soils, affecting a range of important abiotic and biotic soil processes. Our ability to predict the effects of charcoal addition to soil is currently limited, however, by our understanding of how charcoal affects the soil microbial community mediating many of these processes. This study sought to improve our understanding of the relationship between charcoal addition and soil microbial biomass and activity among temperate soils. Charcoal was added to four distinct temperate soils, a Mollisol, an Alfisol, an Entisol, and a Spodosol, at five application levels ranging from 0 to 0.1 kg charcoal kg^{-1} soil, and incubated at 25°C with measurements at approximately 0, 1.5, and 3 mo. We hypothesized that microbial biomass and activity would increase with increasing charcoal application in all soils, but the relative magnitude of the response would depend on the texture and fertility of each soil. As hypothesized, microbial biomass and activity and Bray P increased significantly with increasing charcoal application, while extractable N decreased. The coniferous forest soil provided a notable exception to the general patterns of N availability, having the highest total extractable N at the highest charcoal application level. Our results suggest that charcoal additions affected microbial biomass, microbial activity, and nutrient availability in relatively similar ways in all four soils that we studied, suggesting considerable predictability in response to charcoal application. Differences in the magnitude of the microbial response, however, appeared dependent on differences in nutrient availability among soils.

Abbreviations: BR, basal respiration; DOC, dissolved organic carbon; MQ, metabolic quotient; SIR, substrate-induced respiration.

Charcoal is a common soil component in ecosystems prone to periodic wildfires (Zackrisson et al., 1996; Schmidt and Noack, 2000; Skjemstad et al., 2002; Brown et al., 2005), representing up to 45% of the organic C in a survey of German soils (Schmidt et al., 1999). Despite the abundance of charcoal in soils, we have a rudimentary understanding of the specific responses that charcoal generates within soil ecosystems, and the mechanisms by which charcoal initiates these responses. Recent research suggests that charcoal influences a range of important soil characteristics and processes (Schmidt and Noack, 2000; Glaser et al., 2002). For example, charcoal content affects both soil water and nutrient retention and availability (Glaser et al., 2002; Steiner et al., 2007), and appears to play an important role in ecosystem response to wildfire by enhancing post-fire soil nitrification rates (Zackrisson et al., 1996; DeLuca et al., 2006). Charcoal's recalcitrant nature facilitates the accrual and retention of soil organic matter (SOM) in the humid tropics, where SOM decay is generally rapid (Glaser et al., 2002; Steiner et al., 2007), and charcoal contributes significantly to soil C pools in fire-prone temperate ecosystems (DeLuca and Aplet, 2008). Recent evidence also suggests, however, that charcoal addition may simultaneously enhance the loss of preexisting SOM in boreal systems (Wardle et al., 2008).

Based on the range of responses to charcoal additions observed in natural ecosystems, charcoal may serve as an important soil amendment for managed systems (Marris, 2006). Charcoal's polycyclic, aromatic structure makes it relatively stable in soil, and thus charcoal-induced changes to soil processes will probably persist for years following charcoal addition (Schmidt et al., 1999; Schmidt and Noack, 2000; Glaser et al., 2002; Liang et al., 2006; DeLuca and Aplet, 2008). Wildfire-produced charcoal in boreal forests increased N uptake by seedlings for up to 100 yr following its formation (Zackrisson et al., 1996; Wardle et al., 1998). Soils amended with charcoal many centuries ago by pre-Colombian peoples in the Amazon Basin still retain higher organic matter, pH, and plant-available nutrients than adjacent, unamended soils (Glaser et al., 2000, 2001). Cowpea [*Vigna unguiculata* (L.) Walp.] and rice (*Oryza sativa* L.) production remains 38 to 45% higher in these charcoal-amended soils than in unamended soils from the same region (Lehmann et al., 2003).

Our understanding of the mechanisms by which charcoal influences soil processes is limited primarily by a lack of information on how charcoal affects microbial biomass and activity (Zackrisson et al., 1996; Wardle et al., 1998; Glaser et al., 2002). Based on what we know about charcoal-induced changes in nutrient availability and plant production, charcoal amendment is likely to significantly impact the soil microbial

Soil Sci. Soc. Am. J. 73:1173-1181
doi:10.2136/sssaj2008.0232
Received 10 July 2008.
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community. In addition, charcoal itself provides a recalcitrant food source for microbes (Pietikäinen et al., 2000), and charcoal's high porosity may provide favorable habitats for soil microflora, altering predation rates by soil microfauna (Zackrisson et al., 1996, Warnock et al., 2007).

Our understanding of soil–charcoal–microbe interactions is further limited by the fact that most charcoal amendment studies have used soils from northern forests (Zackrisson et al., 1996; Wardle et al., 1998; Pietikäinen et al., 2000) and tropical ecosystems (Tryon, 1948; Chidumayo, 1994; Mbagwu and Piccolo, 1997; Lehmann et al., 2003; Steiner et al., 2007), with few studies within temperate systems (see Glaser et al., 2002). Differences in charcoal properties, soil types, application levels, study durations, and land use histories among published studies complicate generalizations about the effects of charcoal in soil (Schmidt and Noack, 2000). Furthermore, there is no currently accepted range of effective application levels, which is not surprising given that the effects of charcoal application will probably depend on how the microbial community interacts with both soil and charcoal properties (Glaser et al., 2002).

Our objective was to evaluate the microbial response to a range of charcoal application levels across a survey of temperate soils varying in their properties and management histories. Specifically, we sought to elucidate relationships between charcoal application, soil properties, and microbial biomass and activity. We attempted to avoid problems caused by charcoal variability by using a charcoal produced under controlled conditions from the pyrolysis of a manure-based feedstock. We hypothesized that microbial biomass and activity would increase with increasing charcoal application in all soils, but the relative magnitude of the response would depend on the specific properties of each soil, primarily soil texture and nutrient availability. To our knowledge, this study provides the most extensive evaluation of microbial response to charcoal application in soils from managed temperate systems.

MATERIALS AND METHODS

In June 2006, we collected four managed soils representing a range of soil properties and land use histories from across Wisconsin (Table 1). The Kewaunee soil series (a fine, mixed, active, mesic Typic Hapludalf formed in calcareous clayey till) was collected from an actively cropped section of the University of Wisconsin–Green Bay campus (44.5° N, 87.9° W). This area was historically mesic hardwood forest and was logged approximately 150 yr ago. The sample field has been under tillage for at least 65 yr, although probably much longer, and is currently in a wheat (*Triticum aestivum* L.)–corn (*Zea mays* L.) rotation. The Pence soil series (a sandy, isotic, frigid Typic Haplorthod formed in loamy alluvium or eolian deposits over sandy stratified outwash) was collected from the Nicolet National Forest (45.4° N, 88.4° W). The sample area was historically coniferous forest that was logged approximately 100 to 150 yr ago, then probably relogged and planted to red pine (*Pinus resinosa* Aiton) approximately 70 yr ago. The Plainfield soil series (a mixed, mesic Typic Udipsamment formed in sandy deposits in glacial lake or outwash plains) was collected from an actively cropped area at the University of Wisconsin Hancock Agricultural Research Station (44.1° N, 89.5° W) in the Central Sands area of Wisconsin. The sample field has been irrigated for nearly 40 yr and was cultivated for at least 80 yr. Recent crop rotation included potato (*Solanum tuberosum* L.) and clover (*Trifolium pratense* L.). Historically, the area was largely oak savannah and pine barrens. The Plano soil series (a fine-silty, mixed, superactive, mesic Typic Argiudoll formed in loess over loamy outwash or till) was collected from an actively cropped area at the University of Wisconsin Arlington Agricultural Research Station (43.3° N, 89.4° W). Historically, this area was largely prairie and oak savannah. The sample field has been under tillage for at least 100 yr and probably much longer. Soil series are as mapped by the NRCS (from websoilsurvey.nrcs.usda.gov/app/; verified 22 Mar. 2009). We refer to these four soils as Alfisol, Spodosol, Entisol, and Mollisol, respectively.

Table 1. Soil properties of the four Wisconsin soils used for charcoal application.

Soil order	Textural class	Sand	Clay	Soil pH	Total C
		%			%
Alfisol	clay loam	28.5	34.7	6.8	1.8
Spodosol	sandy loam	53.3	12.2	5.1	2.6
Entisol	loamy sand	82.9	11.0	6.4	0.9
Mollisol	silt loam	11.2	24.8	6.8	3.1

The majority of Wisconsin, including three of the four soil collection sites, was last glaciated approximately 11,000 yr ago (Dott and Attig, 2004), and thus all our study soils are relatively young. Wisconsin has a continental climate with average annual temperatures at the soil collection sites ranging from 5.3 to 7.7°C, and average annual precipitation ranging from 74.1 to 83.3 cm (from www.wcc.nrcs.usda.gov/climate/; verified 22 Mar. 2009). In all cases, soil was collected from the top 10 cm of the mineral soil profile from areas with a 0 to 2% slope, sieved through a 1-cm mesh screen to remove gravel and large organic debris, and stored in the field-moist condition until needed.

The charcoal used in the experiment was generated by pyrolyzing a feedstock mixture containing two parts bull manure, one part dairy manure (both *Bos taurus*), and one part pine (*Pinus* spp.) shavings by weight at 500°C (provided by BEST Energies, Inc., Madison, WI). By weight, 16.5% of the charcoal particles were >2 mm, 81.4% were between 0.05 and 2 mm, and 2.1% were <0.05 mm in diameter, and no particles were >2 cm in diameter. The charcoal had an initial pH of 9.4. Charcoal total C and N analyses were conducted by the Iowa State University Soil and Plant Analysis Laboratory, Ames, with the remaining elemental analyses performed by the University of Wisconsin Soil and Plant Analysis Laboratory, Madison (Table 2).

To investigate the effect of charcoal addition on soil microbial biomass and activity, soil microcosms were constructed using 0.47-L canning jars. Each jar received 100 g of 105°C dry-weight-equivalent soil, a charcoal amendment ranging from 0 to 10% by weight, and deionized water to bring the moisture content to approximately 60% water-holding capacity of the mixture. Charcoal amendments were 0, 1.0, 2.5, 5.0, and 10% by weight, which spans the range of soil–charcoal concentrations reported from other studies (Glaser et al., 2002). Following charcoal amendment, the jars were sealed and incubated in the dark at 25°C, and opened frequently to exchange air and readjust moisture levels. Three replicates of each charcoal–soil mixture were destructively sampled at the beginning of the experiment and at approximately 1.5 and 3 mo.

Active microbial biomass was measured at each sampling time by the substrate-induced respiration (SIR) method (Anderson and Domsch, 1978; Lin and Brookes, 1999). Briefly, SIR was assessed by adding a 1:4 glucose/talcum mixture to treatment soils at a concentration of 12.0 g glucose kg^{-1} soil (Anderson and Domsch, 1978; Lin and

Brookes, 1999), adjusting to 60% water-holding capacity, sealing in 65-mL vials flushed free of CO_2, and incubating for 2 h. The accumulated headspace CO_2 was measured using a Quibit S151 CO_2 infrared gas analyzer (Quibit Systems, Kingston, ON, Canada). Basal respiration (BR) was measured identically, but without the addition of the glucose/talcum mixture. Respiration rates are reported as milliliters CO_2 per kilogram soil per hour. As a method of assessing the efficiency of the microbial biomass, the metabolic quotient (BR/SIR) was also calculated (Anderson and Domsch, 1978; Insam and Haselwandter, 1989).

Dissolved organic C (DOC) and extractable N were obtained by 1 h of mechanical agitation of 10 g 105°C dry-weight-equivalent soil mixture in 100 mL of 2 mol L^{-1} KCl. All extracts were filtered using Whatman no. 42 filter paper before analysis. Dissolved organic C and N samples were acidified to ≤2 pH, stored at 4°C, and analyzed within 48 h. Dissolved organic C was measured using a Shimadzu DOC-V analyzer, and N was measured simultaneously with a Shimadzu TNM-1 (Shimadzu Corp., Kyoto, Japan). Bray P was obtained by a 5-min mechanical agitation of 2 g 105°C dry-weight-equivalent soil mixture in 50 mL of Bray's extraction solution and analyzed on a Latchet QuikChem 8500 Flow-Injection Autoanalyzer (Hach Co., Loveland, CO).

Statistical analysis was conducted using SAS 9.1 (SAS Institute, Cary, NC). The effects of soil type, incubation time, and charcoal application level were analyzed using a three-way ANOVA (α = 0.05). Nonsignificant interaction terms ($P > 0.05$) were removed and combined into the residual error. All responses were logarithmically transformed before analysis to meet the assumption of equal variance.

RESULTS

Microbial Biomass and Activity

Substrate-induced respiration and BR were both significantly affected by charcoal application, soil type, and incubation duration (Table 3). In addition, all possible interactions were also significant ($P \leq 0.05$), with the exception of a nonsignificant SIR × soil × time interaction ($P = 0.4$). The complete models accounted for 93 and 95% of the observed variation in SIR and BR, respectively. Charcoal application level accounted for 77% of the model-explained variation for BR, but only 25% of the model-explained variation for SIR. In contrast, soil type most strongly influenced SIR, accounting for 56% of the model-explained variation, but only 4% of the model-explained variation for BR. Incubation duration accounted for 2% of the model-explained variation in both SIR and BR. The three remaining interaction terms accounted for 16% of the model-explained variation in SIR, and the four remaining interaction terms accounted for a total of 17% of the model-explained variation in BR. Thus, despite significant interaction effects, the differences in SIR and BR were primarily driven by differences in soil type and charcoal application levels, respectively.

As hypothesized, both SIR and BR increased significantly with increasing charcoal application in all soil types (Fig. 1); however, the strong influence of soil type on SIR, as indicated by our ANOVA (Table 3), is highlighted by the fact that, in general, the larger the level of SIR in the unamended soil, the larger the level of SIR at the highest charcoal application levels (Fig. 1). The notable exception to this pattern is the larger relative increase in SIR with increasing charcoal application in the Entisol, which had a fairly low SIR value in the unamended soil (Fig. 2). The large relative increases in BR in the Entisol on the first and second sampling dates (Fig. 2) were driven primarily by very low BR rates in the unamended soil (Fig. 3). These patterns highlight the positive effect that charcoal addition had on both SIR and BR in all of the soils and the strong effect that soil type in particular had on SIR.

Basal respiration increased more than SIR with increasing charcoal application in all soils, resulting in a steady increase in the metabolic quotient (MQ) with increasing charcoal application (Fig. 1). The increases were not equal among soils (Fig. 1), however, and in general the MQ increased more in the lower organic C soils (the Alfisol and Entisol; Table 1), than in the higher organic C soils (the Spodosol and Mollisol; Table 1). In fact, there was virtually no respiratory increase from glucose addition at the highest charcoal application levels in either the Alfisol or the Entisol on the third sampling date (Fig. 1).

Basal respiration generally increased with incubation duration in the higher charcoal-amended treatments but remained relatively constant throughout the experiment in the unamended treatments (Fig. 3). Substrate-induced respiration increased with incubation duration in the 10% charcoal-amended Mollisol and Alfisol, increased marginally in the 10%

Table 2. Chemical composition of the charcoal used in this experiment.

Element	Total quantity
	%
C	73.3
N	1.2
K	1.9
Ca	1.0
Mg	0.4
P	0.3
Na	0.2
	mg kg^{-1}
S	847
Fe	378
Mn	190
Al	181
Cu	159
Zn	147
B	22.1
Ni	3.2
Pb	3.1

Table 3. Analysis of variance results examining the effects of the charcoal application level (charcoal), soil order (soil), and incubation duration (time) on substrate-induced respiration (SIR), basal respiration (BR), 1 mol L^{-1} KCl extractable N, extractable Bray P, and dissolved organic C (DOC).

Model	*F*				
	SIR	BR	N	P	DOC
Charcoal	125.1***	402.9***	42.7***	123.7***	66.4***
Soil	366.6***	29.3***	283.6***	363.7***	479.7***
Time	22.0***	19.0***	180.3***	21.4***	24.7***
Charcoal × soil	13.4***	15.2***	12.2***	13.4***	4.4***
Charcoal × time	12.9***	4.7***	10.1***	12.7***	6.4***
Soil × time	7.6***	4.0**	12.0***	7.5***	6.3***
Charcoal × soil × time	NS	4.4***	4.4***	NS	1.6*

* Significant at $P < 0.05$; NS is not significant at $P > 0.05$.

** Significant at $P < 0.01$.

*** Significant at $P < 0.001$.

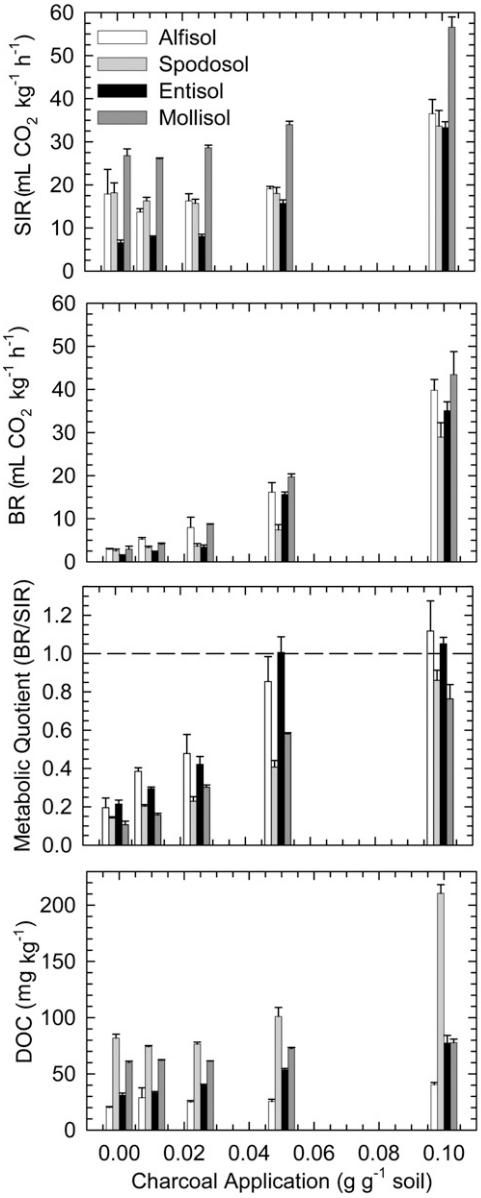

Fig. 1. Effect of charcoal application rate on substrate-induced respiration (SIR), basal respiration (BR), the metabolic quotient (BR/SIR), and dissolved organic C (DOC) in four temperate soils collected in Wisconsin. Values represent treatment means after 96 d of incubation at 25°C and 60% water-holding capacity. Error bars represent one standard error of the mean.

charcoal-amended Entisol, but remained relatively constant in the 10% charcoal-amended Spodosol (Fig. 3). In contrast, SIR generally declined throughout the experiment in the unamended and lower amendment treatments in all four soils (Fig. 3). The greater temporal increase in BR than in SIR in the charcoal-amended treatments led to a steady increase in the MQ throughout the experiment. The MQ also increased slightly in all unamended soils.

Extractable Nitrogen, Bray Phosphorus, Dissolved Organic Carbon, and Soil pH

Extractable N, Bray P, and DOC were all significantly affected by charcoal application level, soil type, incubation duration, and all possible interactions, with the exception of a nonsignificant three-way interaction for P (Table 3). The final models accounted for 94% of the observed variation in both N and DOC, and 93% of the variation in P (Table 3). Nitrogen, P, and DOC were most strongly influenced by soil type, which accounted for 47, 56, and 73% of the model-explained variability, respectively. Charcoal quantity accounted for 9, 25, and 14% of the model-explained variation in N, P, and DOC, respectively. Incubation duration most strongly affected N, accounting for 20% of the model-explained variability. Incubation duration accounted for 2 and 3% of the model-explained variation in P and DOC, respectively. All remaining interactions accounted for 22, 16, and 9% of the model-explained variation in N, P, and DOC, respectively. Thus, while most interactions were significant, differences in N, P, and DOC were driven primarily by differences in soil type, followed by the effects of charcoal application level and incubation duration.

Charcoal application did not significantly affect extractable N on the first sampling date, but N declined with increasing charcoal application after the first sampling time (Fig. 4). The Spodosol provided the only exception to this pattern, with N generally increasing with increasing charcoal application on the second and third sampling dates (Fig. 4). Nitrogen also tended to increase with increasing incubation duration in all soils (Fig. 4). As stated above, however, the magnitude of this increase decreased with increasing charcoal application, to the point that at the highest charcoal application level there were no appreciable temporal increases in N (Fig. 4). The Spodosol again provided an exception to this pattern, as N was notably higher by the end of the experiment, even at the highest charcoal application rate (Fig. 4). Thus, in general, extractable N increased with increasing incubation duration in all soils, but this effect decreased with increasing charcoal application in all soils but the Spodosol, in which N remained high.

Bray P increased with increasing charcoal application and incubation duration in all four soils (Fig. 4). In general, the higher the initial P, the higher the absolute increase in P with increasing charcoal application and incubation dura-

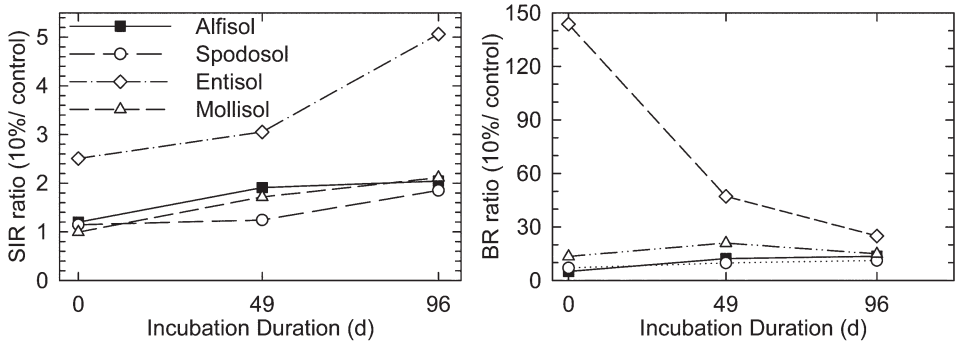

Fig. 2. Temporal changes in the ratio of substrate-induced respiration (SIR) and basal respiration (BR) of soil amended with 0.10 kg charcoal kg^{-1} dry weight to that of unamended soil incubated at 25°C and 60% water-holding capacity for four different Wisconsin soils. High BR response ratios in the Entisol on the first and second sampling dates were driven by extremely low BR rates in the unamended soil.

tion. Charcoal application appeared to increase P slightly more in the Entisol than the other soils (Fig. 4), while increasing incubation time appeared to increase P slightly more in the Mollisol (Fig. 4). These two differences may account for the significant soil × application and soil × time interaction terms, respectively, in our ANOVA (Table 3). In general, while there were slight differences in the response of Bray P to charcoal addition among soils and with time, these differences were minimal, and responses appear quite predictable among all the soils we examined. For emphasis, soil type and application level alone, independent of interactions, accounted for 81% of the model-explained variation in Bray P in our ANOVA.

Dissolved organic C increased with increasing charcoal application in the Entisol and Spodosol, but remained relatively unchanged in the Alfisol and Mollisol (Fig. 1). Increases in DOC in the Spodosol were significantly larger in magnitude than seen in the other soils (Fig. 1). For example, DOC in the highest charcoal-application treatment was nearly triple the DOC present in the unamended treatment (Fig. 1). The notably higher DOC in the unamended Spodosol and Mollisol than in the unamended Entisol and Alfisol (Fig. 1) reflects the significant effect of soil type on DOC reported in our ANOVA (Table 3). There were minimal temporal changes to DOC in any of the soils, with the sole exception of the 10% charcoal-amended Spodosol (data not shown). In this treatment, the DOC measured on the final sampling date was nearly double that measured on the first day of incubation (data not shown), suggesting that the highest charcoal application significantly increased extractable DOC in the Spodosol.

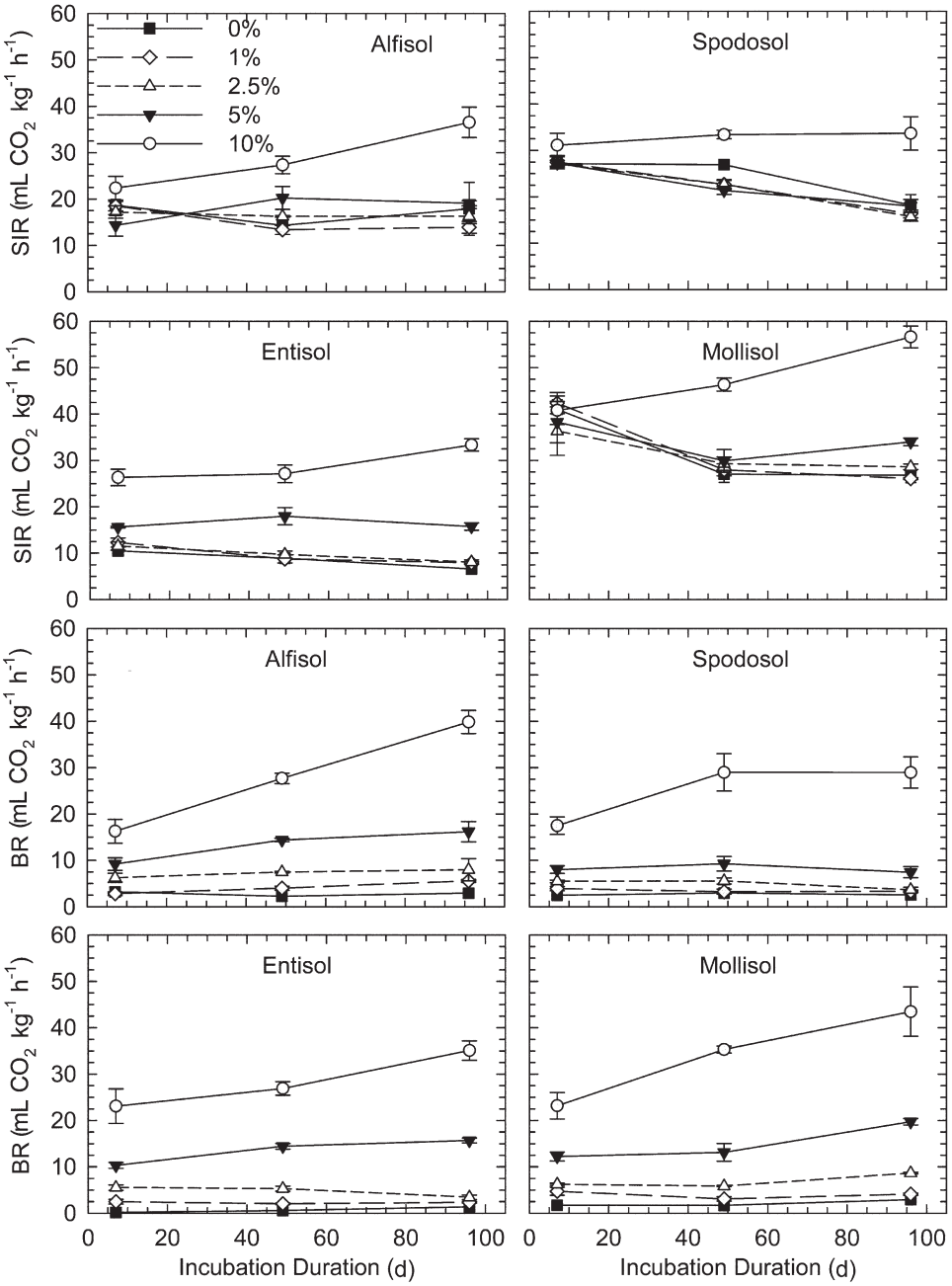

Fig. 3. Effect of charcoal application rate on basal respiration (BR) and substrate-induced respiration (SIR) throughout the incubation. Data points represent treatment means incubated at 25°C and 60% water-holding capacity. Error bars represent one standard error of the mean.

Soil pH initially ranged from 5.1 to 6.8 in the unamended soils (Table 1), and increased in all soils with increasing charcoal application (data not shown). The most marked increase was in the Entisol, which after 96 d ranged from 6.3 in the unamended treatment to 8.9 in the 10% charcoal-amended treatment. Soil pH also increased slightly with time in the other three soils at the 10% application level, but generally increasing by no more than 0.4 pH units. In all other treatments, the pH remained constant or slightly declined with time (data not shown). Thus, the most notable effect of charcoal application on soil pH occurred in the Entisol at the highest charcoal application level.

DISCUSSION

As hypothesized, charcoal application increased microbial biomass (SIR) and activity (BR) in all soils (Fig. 1). In addition, both microbial biomass and activity increased with increasing charcoal application, with no asymptote apparent within the charcoal application range we utilized (0–0.10 kg kg^{-1} soil). Differences among soils in the response of microbial biomass and activity to charcoal application were restricted to differences in the magnitude and relative increase of the responses rather than to the general direction of the responses, with the notable exception of N in the Spodosol; however, soil type was

the single best explanatory variable for SIR, N, P, and DOC. Thus, while all soils generally responded in a similar manner to charcoal application, the relative responses of these variables to charcoal addition were influenced by the properties of our soils.

Microbial biomass in the unamended soils was largest in the Mollisol, and this difference became notably larger with increasing charcoal application rate and incubation time (Fig. 3). This response may have resulted from greater nutrient availability within the Mollisol, as this soil initially had approximately twice the extractable N of the Alfisol or Entisol and two to eight times the P of the other soils (Fig. 4). The response of the microbial biomass to charcoal application within the Mollisol may also have been enhanced by a priming effect resulting from the larger preexisting soil microbial biomass (Fig. 1) and soil organic C pool of the Mollisol (Table 1) (Kuzyakov et al., 2000; Cheng et al., 2003; Malosso et al., 2003; Fontaine et al., 2004, 2007; Carney et al., 2007). In agreement, the Mollisol had consistently higher BR rates at high charcoal applications (Fig. 1), reflecting higher microbial activity. This explanation also agrees with the findings of Pietikäinen et al. (2000) and Wardle et al. (2008), who also reported increased microbial activity following charcoal additions. In agreement, charcoal application was the single best predictor of BR, accounting for 77% of the model-explained variance in this variable (Table 3).

Greater overall microbial activity would result in greater gross mineralization rates and more available N and P to support microbial growth (Mary et al., 1993; Hart et al., 1994; Zaman et al., 1999; Barrett and Burke, 2000; Perakis and Hedin, 2001; Vance and Chapin, 2001). Nutrient limitations on microbial biomass in this study appear supported by the consistent decline in N with increasing charcoal application (Fig. 4), although the Spodosol provides a notable exception to this trend that we discuss below (Fig. 4). Thus, we suggest that the large response by microbial biomass and activity following charcoal addition in the Mollisol is best explained by a combination of the larger preexisting microbial biomass and the greater potential nutrient availability in this soil relative to the other soils. Similar results have been reported following the addition of other C-rich substrates to agricultural soils (Mary et al., 1993; Recous et al., 1995; Zaman et al., 1999), suggesting that the short-term response of soil microbial biomass and activity to charcoal addition may not be fundamentally different than microbial responses to the addition of other C-rich substrates to soils.

While the largest absolute increase in microbial biomass and activity resulted from charcoal application to the Mollisol, the response in the Entisol was nearly as high (Fig. 1), and the Entisol supported the greatest increase in microbial biomass and activity relative to levels in the unamended soil (Fig. 2). The Entisol was the sandiest soil, had the lowest soil C content (Table 1), and had low extractable N (Fig. 4), and thus it was inherently infertile relative to the Mollisol and Alfisol. In this less

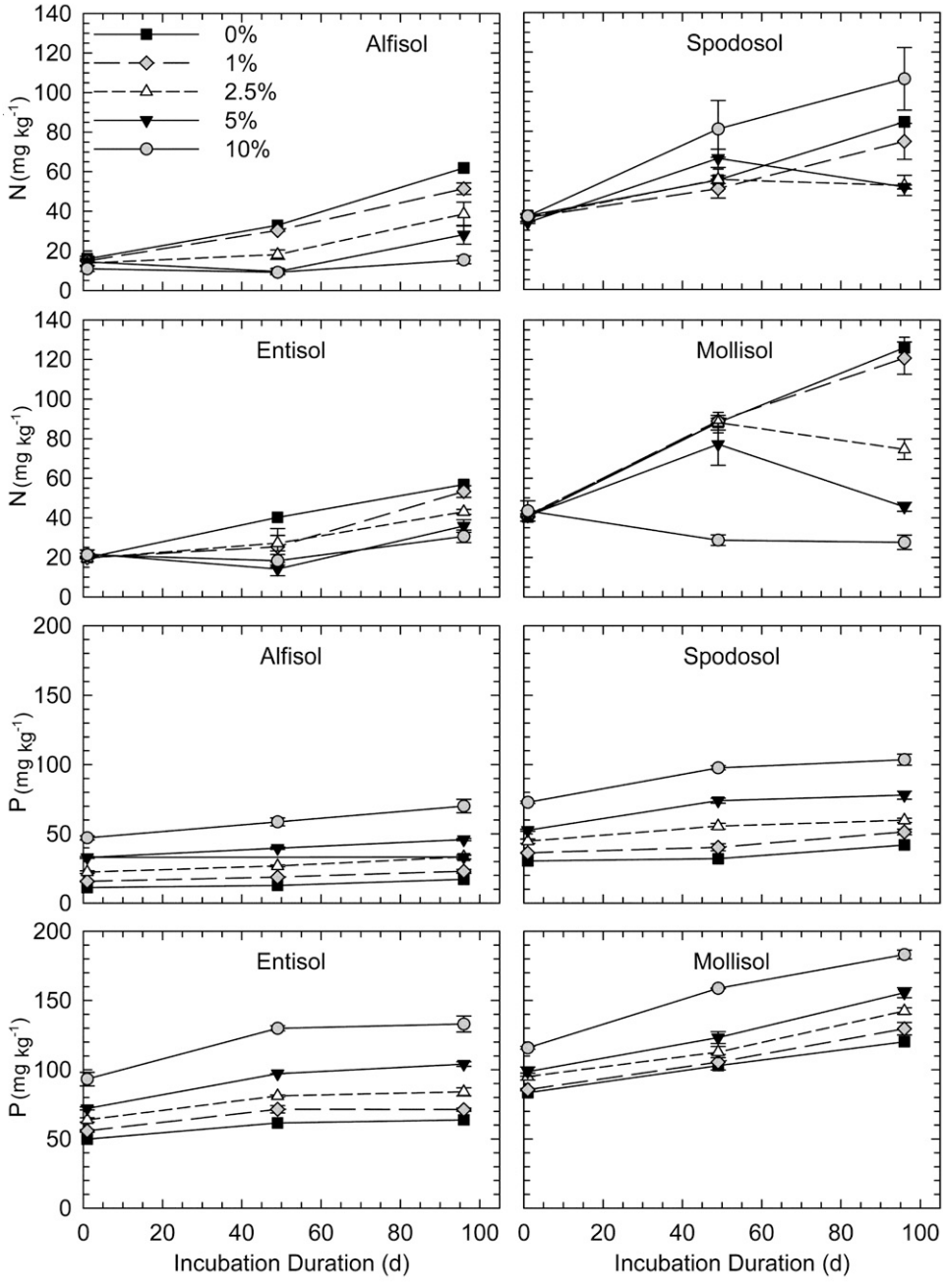

Fig. 4. Effect of charcoal application rate on total extractable N and Bray P throughout the incubation. Data points represent treatment means incubated at 25°C and 60% water-holding capacity. Error bars represent one standard error of the mean.

fertile, coarser-textured soil, microbial biomass was probably limited by both habitat (Elliott et al., 1980; Whitford, 1989; Heijnen and van Veen, 1991; Griffiths and Young, 1994) and C availability (Zak et al., 1994). The addition of charcoal to the Entisol would have increased the soil surface area (Chun et al., 2004; Liang et al., 2006) and C substrate availability. The latter is highlighted by increases in both SIR and BR with increasing charcoal application in all soils (Fig. 1). Relative to the finer-textured soils with higher preexisting habitat and C availability (Table 1), however, charcoal application appears to have most dramatically increased microbial biomass and activity in the Entisol (Fig. 2). We attribute the pronounced increase in microbial biomass and activity in the Entisol to the relatively larger increase in both habitat and substrate availability following charcoal addition.

Wardle (1993) discussed MQ as an indicator of stress in soil ecosystems where C substrate is not limiting, and therefore C availability becomes secondary to other factors, such as nutrient availability. We found that BR increased more than SIR with increasing charcoal application, resulting in an increase in MQ in all four soils (Fig. 1). In fact, at the highest charcoal application, there was minimal respiratory response to the addition of glucose, which is a readily available C source (Fig. 1). Based on the observed MQs, the soils appeared to fall into two distinct groups at higher charcoal application levels (Fig. 1). The Mollisol and the Spodosol, which had higher N (Fig. 4) and more soil organic C (Table 1), had distinctly lower MQs than the Alfisol and Entisol, which had lower N (Fig. 4) and less soil organic C (Table 1). This pattern further supports our interpretation that nutrient limitation played a significant role in shaping the differences among soils in the response of the microbial biomass to charcoal application.

The charcoal used in this study was understandably high in P, since it was the solid remnant of manure pyrolysis. The addition of elemental P at the highest application level equated to adding 275 mg P kg^{-1} soil. In agreement, a substantial increase in soil P was observed as a result of charcoal application. At the highest charcoal application level, the amount of available P immediately released ranged from 12 to 16% of the P content in the charcoal. After approximately 3 mo, the total amount of available P released ranged from 18 to 25%, suggesting significant P release both immediately following charcoal application and from a slower mineralization of charcoal P through time. As stated above, however, these amounts were relatively stable and predictable across soil types.

The response of N and DOC in the Spodosol to increasing charcoal application suggests that charcoal addition altered the Spodosol in a manner different than in the other three soils. Our Spodosol is a northern coniferous forest soil, and microbial biomass and activity within northern forest soils are often hindered by the presence of inhibitory organic compounds, such as phenolics (Zackrisson et al., 1996; Fierer et al., 2001; DeLuca et al., 2006). The generally low BR of the Spodosol (Fig. 1), despite having the second highest organic C content (Table 1) and the highest N (Fig. 4) and DOC (Fig. 1), appears to support this position. Previous work has shown that charcoal addition to northern and temperate forest soils enhances microbial activity by absorbing inhibitory organic compounds (Zackrisson et al., 1996; Wardle et al., 1998, DeLuca et al., 2006), resulting in the accumulation of NO_3^- (DeLuca et al., 2002, 2006; Berglund et al., 2004). Following this line of reasoning, it seems relevant that the Spodosol was the only soil examined that had a net increase in N with increasing charcoal application (Fig. 4), and that the Spodosol had the largest increase in DOC with increasing charcoal application (Fig. 1). Our N results are similar to the findings of DeLuca et al. (2006), who reported no effect of charcoal on the nitrification potential of grassland Mollisols, but found positive effects on the nitrification potential of pine forest Inceptisols from Montana. We suggest that before charcoal application, the presence of inhibitory organic compounds limited substrate mineralization in the Spodosol, and that adsorption of these inhibitory compounds by the added charcoal, coupled with a charcoal-induced increase in activity, enabled the microbial community to increase mineralization processes.

The focus of this study was to examine how soil microbes respond to charcoal additions across a range of soil types, and for this reason we utilized a charcoal generated from known feedstock under controlled combustion conditions. It is well documented, however, that charcoal properties and their effect on the microbial community vary significantly among feedstocks and combustion conditions (Pietikäinen et al., 2000; Glaser et al., 2002; Chun et al., 2004; Lehmann 2007). For example, the charcoal we utilized was produced at temperatures identified as ideal for generating charcoal with high cation exchange capacity and surface area (Lehmann 2007). In addition, animal manure was the principle component of our feedstock, so our charcoal probably contained elevated levels of certain plant nutrients relative to other charcoals (Table 2). Thus, it seems reasonable to expect that our study documents microbial response to a higher quality charcoal, and that the addition of charcoals produced from different substrates or under different conditions might have produced altered responses. This criticism will pertain to all future charcoal addition studies until we have a better understanding of the direct relationship between soil microbial responses and the specific properties of added charcoal. For this reason it is also noteworthy, however, that despite the potential effects of feedstock and production conditions, our results shared many similarities with previous studies. For example, the N dynamics that we report from our grassland (Mollisol) and coniferous forest soils (Spodosol) are very similar to those reported by DeLuca et al. (2006) from western U.S. grassland (Mollisols) and pine forest soils (Inceptisols). Likewise, the enhanced microbial activity we report from all of our soils is very similar to the results of Wardle et al. (2008) following charcoal additions to boreal forest soils. Our study did not address the important question of charcoal quality effects on soil microbial activity, but rather provides a solid examination of variation in microbial responses to the addition of one controlled charcoal type across four distinct soils.

CONCLUSIONS

Both SIR and BR increased with increasing charcoal application in a relatively similar manner in the four temperate soils that we studied. We found no asymptotic response to increasing charcoal application within the application range examined in this study, suggesting that higher application levels may provide further enhancement of microbial biomass and activity. It

remains unclear to what degree the enhancement of microbial biomass and activity following charcoal addition resulted from the direct microbial utilization of charcoal for substrate, from enhanced degradation of preexisting soil organic matter, as recently suggested by Wardle et al. (2008), or as a result of altered soil properties benefiting microbial biomass and activity. In general, the response of microbial biomass appeared limited by nutrient availability at higher charcoal application rates, suggesting that the greatest absolute response by the microbial biomass will occur when charcoal is added to fertile soils. This was most evident from patterns of MQ among our soils and in the response of the microbial biomass within the fertile Mollisol relative to the other soils. The Entisol, our sandiest, lowest organic matter soil, had the largest increase in microbial biomass and activity relative to levels in the unamended soil. We argue that this resulted from a relatively greater increase in microbial habitat and available C in the charcoal-amended Entisol relative to the other, finer textured, higher organic matter soils. The increase in N and DOC in our Spodosol supports earlier findings that charcoal addition leads to significant increases in mineralization rates in coniferous forest soils (DeLuca et al., 2002, 2006; Berglund et al., 2004). Previous work has attributed this response to declines in inhibitory phenolic compounds or sorption of available C (DeLuca et al., 2002, 2006; Berglund et al., 2004). This property may make anthropogenic charcoal application a valuable management tool for controlling invasive species, whose success is often attributed to their production of allelochemicals (Hierro and Callaway, 2003; Callaway et al., 2005; Stinson et al., 2006, Rudrappa et al., 2007). Finally, because charcoal additions increased microbial biomass and activity in a relatively similar and seemingly predictable manner among the four distinct soil types we studied, our results lend further support for the potential use of charcoal additions for soil management applications.

ACKNOWLEDGMENTS

We thank J. Katers (Univ. of Wisconsin-Green Bay) and BEST Energies, Inc. (Madison, WI) for initiating our involvement in this study and for supplying the charcoal used in our incubation. We also thank D. Isted, K. Drews, J. Werner, M. Kolb, C. Sorden, and J. Raich for their valuable contributions to this project. Funds and equipment were provided by Univ. of Wisconsin-Green Bay Research Council and the Dep. of Natural and Applied Sciences.

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