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Nitrogen dynamics following field application of biochar in a temperate North American maize-based production system

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Abstract

Background and aims Biochar additions to tropical soils have been shown to reduce N leaching and increase N use efficiency. No studies exist verifying reduced N leaching in field experiments on temperate agricultural soils or identifying the mechanism for N retention.

Methods Biochar derived from maize stover was applied to a maize cropping system in central New York State at rates of 0, 1, 3, 12, and 30 tha^{-1} in 2007. Secondary N fertilizer was added at 100, 90, 70, and 50 % of the recommended rate (108 kg N ha^{-1}). Nitrogen fertilizer enriched with ^{15}N was applied in 2009 to the 0 and 12 tha^{-1} of biochar at 100 and 50 % secondary N application.

Results Maize yield and plant N uptake did not change with biochar additions ($p > 0.05$; $n = 3$). Less N (by 82 %; $p < 0.05$) was lost after biochar application through leaching only at 100 %N fertilization. The reason for an observed 140 % greater retention of applied ^{15}N in the topsoil may have been the incorporation of added ^{15}N into microbial biomass which increased approximately three-fold which warrants further research. The low leaching of applied fertilizer ^{15}N (0.42 % of applied N; $p < 0.05$) and comparatively high recovery of applied ^{15}N in the soil (39 %) after biochar additions after one cropping season may also indicate greater overall N retention through lower gaseous or erosion N losses with biochar.

Conclusions Addition of biochar to fertile soil in a temperate climate did not improve crop growth or N use efficiency, but increased retention of fertilizer N in the topsoil.

Keywords Activated carbon · Biochar · Charcoal · Leaching · Maize · Nitrogen

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Introduction

Agriculture is a major contributor to terrestrial anthropogenic nitrogen (N) pollution and has resulted in profound ecological changes (Vitousek et al. 1997). Nitrogen losses to the environment from intensive agricultural production have consequently led to both direct and indirect negative feedbacks to environmental and human health. These losses occur from agricultural production systems in developed and developing countries and in tropical and temperate ecosystems (Townsend et al. 2003).

Nitrogen loading in waterways through leaching of nitrate (NO_3^-) from agricultural fields contributes to eutrophication of rivers, lakes, and oceans (Burkholder 1998; Mitsch et al. 2001). In addition, pervasive groundwater NO_3^- contamination poses a threat to human health and has been correlated to fertilizer use in both developed and developing countries (Agrawal et al. 1999; Mitsch et al. 2001; Oenema et al. 1998; Randall et al. 1997). Leaching losses of NO_3^- have been found to be highest for maize-based cropping systems (Owens 1990; van Es et al. 2006) and can represent small (Sogbedji et al. 2000) to large (Cahn et al. 1993) losses of applied fertilizer N. These losses of N represent inherent inefficiencies in current nutrient management and result not only in environmental pollution but additional economic cost to farmers and land managers.

Various methods have been proposed to improve fertilizer N use efficiencies and limit N losses to the environment. These methods include limiting N fertilizer usage (Francis 1992), switching to perennial-based agricultural systems (Drinkwater et al. 1998), or applying nitrification inhibiting chemicals to reduce the amount of mobile NO_3^- in the soil (Walters and Malzer 1990). Limited work has been done to improve fertilizer N use efficiency through greater retention of N in the soil. Applications of biochar (BC) could be one mechanism to improve N retention and reduce N leaching (Lehmann 2007).

Several mechanisms may control N retention in biochar. Biochar may retain ammonium through increases in soil cation exchange capacity (CEC) (Liang et al. 2006) and changes in soil pH (Chan et al. 2008; Matsubara et al. 2002; Novak et al. 2009). Indeed, ammonium retention has been shown to occur after biochar additions to a Brazilian Oxisol (Lehmann et al. 2003) and both ammonium and nitrate retention after biochar additions to an Australian Sodosol (Dempster et al. 2012a) in greenhouse experiments. Biochar could also alter soil water percolation through changes in pore-size distribution, soil solution residence times, and flow paths (Major et al. 2009) for which experimental evidence is still lacking. Changes in soil microbial community composition have been found in biochar-rich soils (O'Neill et al. 2009; Grossman et al. 2010); these changes could alter microbial mediated N dynamics including nitrification (DeLuca et al. 2006). Steiner et al. (2008) found significantly greater residual fertilizer N in

the soil following application of biochar. The authors attributed the difference to increased N recycling through the above-ground biomass, and possibly reduced leaching and gaseous losses, immobilization of N by microbial biomass, or retention of ammonium (NH_4^+) on the cation exchange sites as possible explanations. The retention of other cations as well as improvements in soil fertility in general, may have increased N uptake (Lehmann et al. 2003; Major et al. 2010; Haefele et al. 2011) and hence reduced N leaching.

While biochar studies on soil fertility and agronomic effects have increased in recent years most of the work has been done in tropical cropping systems (Chan et al. 2007; Steiner et al. 2007; Steiner et al. 2008; Kimetu et al. 2008; Hidetoshi et al. 2009; Gaskin et al. 2010; Major et al. 2010; Van Zwieten et al. 2010a; Haefele et al. 2011; Zhang et al. 2012a, b). Very few studies exist documenting the soil fertility (Novak et al. 2009; Laird et al. 2010a; Jones et al. 2012) and yield effects (Vaccari et al. 2011) of biochar in temperate cropping systems and only one published study for a tropical agroecosystem could be found that quantified N leaching losses in the field (Major et al. 2012). The increased yields commonly reported in highly weathered and acid tropical soils have frequently been attributed to increases in pH, CEC and nutrient retention (Lehmann et al. 2003; Van Zwieten et al. 2010a). However, in many soils currently under production in temperate climates, CEC and pH are typically not limiting crop productivity. The management problem in temperate cropping systems is rather an excess of applied nutrients, the opposite problem of agricultural systems in the tropics. Some studies have found lower N leaching after biochar additions in both greenhouse and field experiments (Lehmann et al. 2003; Laird et al. 2010b; Major et al. 2012). Other studies in tropical soils lead to the hypothesis that these leaching reductions may result in improved plant N use (Chan et al. 2007; Steiner et al. 2008). Therefore, the potential may exist to increase soil N retention, reduce N leakages and maintain yields even with lower N applications by improving N use efficiency (greater N uptake per unit N applied) in temperate soils.

A long-term experiment was established in a temperate maize cropping system in central New York State to evaluate the effect of biochar applications on crop yields, N leaching, and fertilizer N use efficiency using ^{15}N as a

tracer. The specific objectives of the experiment were to: (1) evaluate the effect of increasing rates of biochar application on maize grain yield; (2) determine the efficacy of biochar applications to maintain maize grain yield with reductions in N fertilizer applications; (3) quantify the effects of biochar additions on in-situ leaching losses of fertilizer N over 1 year.

Methods

Field site

The field experiment was established at the Cornell University Musgrave Research Farm in Aurora, NY (42°43'48.64"N, 76°39'16.03"W). The climate is humid continental, with a mean annual rainfall of 940 mm, and a mean maximum temperature of 14 °C and a mean minimum temperature of 4 °C. The mean growing degree days are 2400 (GDD, 86-50° system). The soils are classified as a Kendaia silt loam (2–5 % slopes, fine-loamy, mixed, semiactive, nonacid, mesic Aeric Endoaquepts) and Lima loam (2–6 % slopes, fine-loamy, mixed, semiactive, mesic Oxyaquic Hapludalfs). The studied soil has a pH of 7.36 in 1 M KCl (ratio of 1:2 w/v; control plots of this study), a bulk density of 1.29 $g cm^{-3}$, CEC of 97.6 $mmol_c kg^{-1}$, particle size distribution of 27 % clay, 31 % silt and 42 % sand, total C content of 16.2 $g kg^{-1}$, total N of 1.62 $g kg^{-1}$, and Mehlich-3 extractable P of 35.8 mg kg^{-1}, K of 84.1 mg kg^{-1}, Ca of 3739 mg kg^{-1}, Mg of 483 mg kg^{-1} and Na of 75 mg kg^{-1} (Rajkovich et al. 2012).

Biochar

Maize stover from a commercial farm in New South Wales, Australia, was oven dried to approximately 10 % moisture before pyrolysis. Biochar was produced at approximately 600 °C using slow pyrolysis in a continuous system with an average residence time of about 30 min with relatively high purge (Pyrochar 300; BEST Energies, Somersby, Australia). The biochar had the following properties: pH (KCl) 10.02; ash 64 %, volatiles 26 %, fixed carbon 10 % (all w/w); total C 290 mg g^{-1}; C/N ratio 96; total P 0.41 mg g^{-1} (additional data in supplementary online material). Ash, volatile, and fixed carbon was measured according to ASTM D1762-84 Chemical Analysis of Wood Charcoal. The biochar was stored moist for two

months at 8.6 % moisture before application and was applied on a dry-weight basis. Biochar was characterized in the state it was applied.

Experimental setup

Prior to the experiment the field had been planted to continuous maize for over 30 years. The research area was split into 33 plots each with a size of 4.5 by 7.5 m (33.75 m^2 per plot). Two meter buffer zones were established between plots on all sides. In April 2007, biochar was applied once at rates of 0, 3, 12, and 30 $t ha^{-1}$ (Table 1). An additional treatment consisted of annual applications of 1 $t ha^{-1}$. This biochar applied annually was from the same batch as that of the other treatments. It was stored moist until application. This treatment was applied for the 2007, 2008, and 2010 growing seasons, but not in 2009. All biochar applications were incorporated by hand rake and shovel to a depth of approximately 50 mm which was then followed by mechanical tillage to about 0.13 m uniformly for all treatments.

Each year, fields were chisel plowed, followed by disc plowing before planting. Maize (Dyna-Gro Yieldgard hybrid seed, Crop Production Services, Loveland, CO) was planted at a rate of 79,040 seeds ha^{-1}, with 0.4 m distance within rows that were 0.76 m apart, between May 11 and 21, depending on weather conditions in each year. At planting, atrazine was sprayed at 1 $L ha^{-1}$ and a mixture of S-Metolachlor, atrazine and mesotrione (Lumax®, Syngenta, Basel, Switzerland) at 5 $L ha^{-1}$. In 2008, in addition post-emergent herbicides ricosulfuron/rimsulfuron (Steadfast®, DuPont, Wilmington, DE) and diglycolamine (Banvel®, DuPont, Wilmington, DE)

Table 1 Experimental design

Biochar (t ha⁻¹)	Fertilization (% of full recommended fertilization)			
	50	70	90	100
0	X	X	X	X
3			X	
12	X	X	X	X
30			X	
1 (annually)			X	

All treatment combinations were established in three replicates. (n=3)

were applied at rates of 52.5 g ha^{-1} and 140 g ha^{-1}, respectively.

A 10-20-20 granular fertilizer was applied as a mixture of urea, muriate of potash, and triple super phosphate at the rate of 123.5 kg ha^{-1} (12.35 kg N ha^{-1}; 5.43 kg P ha^{-1}; 20.51 kg K ha^{-1}) at planting for all plots for all planting years. Secondary N fertilizer was applied approximately 6 weeks after planting in the form of ammonium nitrate. The standard recommended secondary N fertilizer application rate for the area is 107.61 kg N ha^{-1} (Ketterings et al. 2001). Plots at each application rate of biochar (including the control without biochar) received 90 % (96.85 kg N ha^{-1}) of the recommended secondary N fertilizer application rate in order to investigate the effect of biochar application rate on grain yield. For the 0 and 12 t BC ha^{-1} application rates, additional treatments with varying amounts of secondary N fertilizer application rates at 50 % (53.81 kg N ha^{-1}), 70 % (75.33 kg N ha^{-1}), and 100 % (107.61 kg N ha^{-1}) of the recommended rate were included. All treatments were replicated three times in a completely randomized design.

Lysimeters

In the spring of 2009, before the field was tilled or planted, free-draining lysimeters were installed in each of three replicate plots that received 50 or 100 % secondary N fertilizer application rates for both 0 and 12 t biochar ha^{-1} application rates. One lysimeter was installed per plot. Rectangular lysimeters were manufactured from stainless steel and filled with acid-washed quartz sand. The dimensions of the lysimeters that interfaced with the soil surface were 101.6 mm by 304.8 mm, with a depth of 101.6 mm. In April 2009 vertical holes were dug in the inter-row spaces bordering the aforementioned plots. Lateral holes were dug into the soil beneath the targeted plots and the lysimeters were installed with each uppermost surface being approximately 0.6 m below the soil surface (Supplementary Fig. S1), which captures the majority of root nutrient uptake due to an underlying restrictive layer typical for the studied soils (Timlin et al. 2001). The lysimeters were connected to a glass collection bottle placed in the bottom of the vertical pit via PVC tubing (VWR Signature Tubing, VWR, Batavia, IL). Two PVC evacuation tubes were also installed into the collection bottles that connected to the soil surface to allow the leachate to be collected via vacuum. After the instillation was completed the entire pit was back-filled with soil, leaving the evacuation tubes exposed. When not in use, evacuation tubes were sealed shut to prevent contamination.

Isotopic labeling

In July of 2009, secondary N fertilizer application in plots that received 50 or 100 % fertilization at 0 and 12 t biochar ha^{-1} was combined with an application of ^{15}N isotope enriched NH$_4$NO$_3$ at 10 atom% ^{15}N with the isotopic label on both the NH$_4$-N and NO$_3$-N. The ^{15}N was applied to sub-plots of 6.02 by 2.78 m (16.74 m^2) within the chosen treatment combinations. ^{15}N with the labeled fertilizer was applied at the rate of 1 kg ^{15}N ha^{-1} or total N of 10 kg ha^{-1}, which replaced the equivalent amount of non-labeled N to maintain uniformity in the total amount of N applied within treatments. The isotopically labeled fertilizer was mixed with the normal NH$_4$NO$_3$ fertilizer in individual containers for each plot. The fertilizer was completely dissolved in water and applied to the moist soil by hand pipettes.

Lysimeter sampling and analysis

All lysimeter collection bottles were completely evacuated into acid-washed glass bottles following each significant rain event greater than 10 mm for the entire 2009 growing season from 4 June to 17 October, in weekly intervals. After evacuation, 10 mL of toluene was injected back into the buried collection bottles to minimize microbial transformation of N. The samples were immediately transported to the laboratory at Cornell University and total leachate volume was determined. Two subsamples were collected for each lysimeter from each leaching event and immediately placed in refrigeration. One set of subsamples was analyzed for NH$_4^+$, NO$_2^-$, and NO$_3^-$ colorimetrically using a continuous flow analyzer (Bran and Luebbe Autoanalyzer, SPX, Charlotte, NC). A second 20-mL subsample was freeze-dried (Dura-Dry^{tm} μP, FTS Systems Inc., Stone Ridge, NY) and analyzed for total ^{15}N by isotope ratio mass spectrometry (PDZ Europa ANCA-GSL elemental analyzer, PDZ Europa 20-20 isotope ratio mass spectrometer, Sercon Ltd., Cheshire, UK).

Soil sampling, pH and N analyses

Representative soil samples were taken in 0.1-m increments from the surface to a depth of 0.6 m from all plots that received ^{15}N. Three replicate soil samples were taken at random from each plot and homogenized. Soil samples were taken from the field in the Spring of 2009 prior to planting and ^{15}N application as well as just after harvest in the Fall of 2009. Soil, plant, and leachate samples taken prior to ^{15}N application were used as the reference natural abundance values for ^{15}N analysis. Exchangeable NO_3^- and NH_4^+ were extracted from 10 g oven-dry soil taken in Fall with 100 mL 2 M KCl for 1 h (Mulvaney 1996). Nitrate, NO_2^-, and NH_4^+ in all soil extracts were quantified colorimetrically using a continuous flow analyzer (Bran and Luebbe Autoanalyzer, SPX, Charlotte, NC). The soil samples were air-dried and passed through a 2-mm sieve. A sub-sample of the sieved soil was finely ground for total ^{15}N. Total ^{15}N was determined by isotope ratio mass spectrometry (PDZ Europa ANCA-GSL elemental analyzer, PDZ Europa 20-20 isotope ratio mass spectrometer, Sercon Ltd., Cheshire, UK). pH was measured in 1 M KCl using a ratio of 1:2 (w/v) and a glass electrode (Thermo Fisher Scientific Inc. Beverly, MA).

Nitrogen mineralization

Nitrogen mineralization potential was quantified from a sub-sample of air-dried and sieved soil taken after harvest. Nitrogen mineralization potential was determined following Campbell et al. (1993) with the following modifications. Buchner funnels were used in place of leaching tubes, which received a glass fiber filter over the funnel plate, followed by glass wool, the sand/soil mixture, and a final portion of glass wool over the soil to allow leaching of accumulated N with minimal disturbance of the soil. The funnels were covered with two layers of parafilm to prevent desiccation of the soil. The soil was incubated at 30 °C between extractions with 100 mL of 0.01 M $CaCl_2$ and addition of 25 mL of a non-N nutrient solution (0.002 M $CaSO_4$, 0.002 M $MgSO_4$, 0.005 M $Ca(H_2PO_4)_2$, and 0.0025 M K_2SO_4) at day 2, 5, 10, 20, and 30.

Bulk density was determined with 100-cm^3 rings using three measurements per plot at the center of each depth increment from the soil surface to 0.6 m in 0.1-m increments.

Adsorption correction factor

A DON stock solution for the correction factor was prepared by shaking 400 g of soil taken from the topsoil (0–0.1 m) adjacent to the experimental area in October 2009 with 1000 mL of deionized water overnight. The dissolved total N concentration of the extract was determined by a total DOC/DON analyzer (Shimadzu TOC-5000a Autoanalyzer, Columbia, MD, USA). Mineral N values for these samples were determined by a continuous flow analyzer (Bran and Luebbe Autoanalyzer, SPX, Charlotte, NC). Dissolved organic N was then determined by subtracting the mineral N values from the total dissolved N values. Fourty milliliters of DON stock solution was added at six concentrations (20, 30, 40, 60, 80 and 100 μg ml^{-1}) to 10 g (oven-dry weight) soil from each treatment using the three field replicates and shaken for 12 h. The mixture was then centrifuged at 10,000 x g. The supernatant was extracted and analyzed with a TON/TOC analyzer (Shimadzu TOC-5000A Autoanalyzer). These values were used to evaluate the differential adsorption of DON to soils with and without biochar and estimate a relative correction factor for the quantification of microbial biomass N.

Microbial biomass

Microbial biomass was determined from a sub-sample of air-dried soil passed through a 2-mm sieve. The chloroform fumigation method was used to determine microbial biomass N (Witt et al. 2000) under homogeneous conditions following a 12-hour incubation at room temperature wetted to field capacity. Total microbial biomass N and ^{15}N was determined in dried extracts (modified after Bruulsema and Duxbury 1996) by isotope mass spectrometry (PDZ Europa ANCA-GSL elemental analyzer, PDZ Europa 20-20 isotope ratio mass spectrometer, Sercon Ltd., Cheshire, UK). Final microbial biomass N was adjusted to normalize for differential soil and biochar adsorption of lysed cells using a correction factor for possible preferential DON adsorption to biochar (described above) following the method by Jin (2010). This correction recognizes the stronger adsorption of dissolved organic matter to biochar than soil (Liang et al. 2010; Lehmann et al. 2011). The correction for adsorption was made by determining the slope of a linear correlation between equilibrium solution concentration and adsorption (Supplementary Fig. S2),

Table 2 Maize grain yield on a New York Alfisol amended with biochar either once in April 2007 or annually. Secondary fertilizer N application is maintained at 90 % of the recommended rate for all treatments

Biochar (t ha⁻¹)	Year			
	2007	2008	2009	2010
0	4.74	9.26	8.50	8.69
3	4.11	7.80	8.51	8.60
12	4.19	8.14	7.93	8.62
30	4.02	7.66	6.59	7.81
1 (annually)	4.05	7.48	8.56	9.08
p (biochar effect)	0.54	0.33	0.66	0.49

Different letters indicate significant differences between biochar application rates within single years (Students t-test; $p < 0.05$; $n = 3$). Letters are not shown when differences are not significant

recognizing that corrections with DON soil extracts will only give a relative measure of adsorption between soils.

Harvest and plant sampling

In the Fall, maize grain and stover yields were determined from the same 16.74-m² sub-plots that were used for ¹⁵N application. Total number of cobs and total wet biomass and grain weight for the sub-plot was determined in the field. Five plants and ten cobs were randomly selected from each of the subplots and were dried to constant weight at 60 °C to determine moisture content. The dried grain was removed from the cob and used to determine grain yield. A composited sub-sample of all plant parts from the five plants and ten cobs was finely ground to determine total above ground N recovery. Total N and ¹⁵N was determined by isotope

ratio mass spectrometry (PDZ Europa ANCA-GSL elemental analyzer, PDZ Europa 20-20 isotope ratio mass spectrometer, Sercon Ltd., Cheshire, UK).

Calculation of fertilizer derived nitrogen

Fertilizer derived N was determined using Eq. 1:

$$\left([^{15}N_f] - [^{15}N_r] \right) / \left(^{15}N_i \times N_t \right) \tag{1}$$

whereby $^{15}N_f$ is the ^{15}N content from ^{15}N–fertilized treatments, $^{15}N_r$ is the ^{15}N content of the reference material (determined from samples taken before application of the isotopically enriched N), $^{15}N_i$ is the initial ^{15}N application, N_t is the total N content of soil or plant biomass.

Statistical analyses

Statistical analyses were performed with JMP software (SAS Institute 2007). All procedures were performed at $p < 0.05$, unless otherwise indicated. Significant treatment effects were determined using the Students t-test.

Results

Maize yields and nitrogen uptake

At the 90 % secondary N fertilizer application rate, increasing the biochar application rate did not significantly ($p > 0.05$) change maize grain yield in any year (Table 2). Maize grain yields also did not change with biochar application of 12 tha⁻¹ ($p > 0.05$) at lower or higher secondary N fertilizer application (Table 3). Similarly,

Table 3 Maize grain yield (t ha⁻¹) with varying N fertilization following biochar soil application in April 2007

N application rate	Year								*p* (biochar effect)
	2007		2008		2009		2010		
(%)	0 tha⁻¹	12 tha⁻¹	0 tha⁻¹	12 tha⁻¹	0 tha⁻¹	12 tha⁻¹	0 tha⁻¹	12 tha⁻¹	
50	5.66	3.38	6.99	6.83b	6.50	6.64b	7.29b	7.41	0.3221
70	4.27	3.38	7.37	8.28ab	8.01	7.75ab	8.42ab	8.23	0.9948
90	4.74	4.20	9.26	8.14ab	8.50	7.89ab	8.69a	8.62	0.5364
100	4.50	4.66	10.41	11.38a	8.59	10.91a	8.94a	8.94	0.4064
p (N effect)	0.2307	0.1986	0.1667	0.0134	0.0984	0.0131	0.0126	0.1010	

Different letters indicate significant differences between treatment means within single years (Students t-test; $p < 0.05$; $n = 3$). Letters not shown when differences are not significant

Table 4 Tissue N concentration and total above-ground maize N uptake following biochar soil application in April 2007. Different letters indicate significant differences between treatment means within single years (Students t-test; $p < 0.05$; $n = 3$)

Year	Secondary N fertilizer (% of recommended fertilizer application)	Tissue N concentrations (mg g^{-1})			Total N uptake (kg total N ha^{-1})		
		0 tha^{-1}	12 tha^{-1}	p (biochar effect)	0 tha^{-1}	12 tha^{-1}	p (biochar effect)
2007	50	6.63	6.41	0.7903	55.69	46.61	0.5996
	100	8.05	6.80	0.0646	68.77	58.68	0.2596
2008	50	7.21	7.55	0.5851	78.26	80.44	0.8683
	100	8.45	8.47	0.9605	116.22	116.28	0.9958
2009	50	7.55	7.84	0.3816	72.88	69.55	0.7416
	100	9.43	9.02	0.2401	112.28	121.58	0.4565
2010	50	8.62	8.01	0.8257	97.50	87.82	0.7899
	100	9.04	8.86	0.7720	113.01	116.23	0.7294
p (N effect)		0.0044	0.0438		0.0117	0.0048	

Letters not shown when differences are not significant

biochar did not affect ($p > 0.05$) tissue N concentrations, total N uptake or N uptake from applied fertilizer within any year (Tables 4 and 5). With the exception of 2007, maize yields increased with greater fertilizer N additions. In addition, aggregated across all years, a positive trend was seen in greater N tissue concentrations and N with 100 % fertilizer application in comparison to 50 % fertilizer addition (Table 5; N concentration with biochar only at $p < 0.1$).

Soil pH and nitrogen

Biochar applications did not significantly change soil pH (Table 6). There were no consistent trends after biochar additions in measured soil mineral N contents with depth (Fig. 1). In contrast, the $\delta^{15}N$ values and the N derived from fertilizer of total N were significantly ($p < 0.05$) greater in the topsoil (0–0.2 m) with biochar application where 100 % of recommended fertilizer was applied (Fig. 2; no differences with

Table 5 Above-ground maize biomass recovery of isotopically labeled N and N derived from fertilizer (year=2009); microbial biomass after incubation and microbial biomass recovery of isotopically labeled N and N derived from fertilizer from soils taken in October 2009; nitrogen mineralization potential of soils taken in October 2009; DON adsorption constants for the Freundlich isotherms

	Secondary N fertilizer (% of recommended fertilizer application)					
	50			100		
	0 tha^{-1}	12 tha^{-1}	p (biochar effect)	0 tha^{-1}	12 tha^{-1}	p (biochar effect)
Maize biomass $\delta^{15}N$ (‰)	1115.5	1344.2	0.434	1143.1	1126.6	0.919
Maize biomass N derived from fertilizer (kg total N ha^{-1})	19.1	22.3	0.459	54.9	60.4	0.474
Total microbial biomass N (mg kg^{-1} soil)	5.73	6.58	0.843	1.24	2.06	0.161
Microbial biomass $\delta^{15}N$ (‰)	84.1	133.1	0.670	85.5	204.4	0.125
Microbial biomass N derived from fertilizer (kg total N ha^{-1})	0.03	0.06	0.638	0.03	0.10	0.091
Nitrogen mineralization (kg N ha^{-1} day^{-1})	1.30	1.09	0.392	1.16	0.94	0.958

Students t-test ($p < 0.05$, $n = 3$)

Table 6 Soil pH 2 years after biochar application (measured in 2009)

Biochar (t ha^{-1})	Secondary N fertilizer application rate (%)			
	50	70	90[a]	100
0	7.42	7.29	7.26	7.46
3	–	–	7.48	–
12	7.32	7.30	7.22	7.50
30	–	–	7.36	–
1 (annually)	–	–	7.26	–
p (biochar effect)	0.64	0.61	0.21	0.59

pH was determined using 1 M KCl at a ratio of 1:2 w/v ([a] Tukey's HSD, all others Students t-test; $p > 0.05$; $n = 3$)

50 % fertilization). In the subsoil, ^{15}N enrichment from applied fertilizer tended to be greater without biochar additions (significant only at 0.3–0.4 m).

Nitrogen in microbial biomass was not significantly different at any fertilizer application rate ($p > 0.05$). However, three times more fertilizer N was recovered with biochar in the microbial biomass than without biochar albeit with high variability ($p = 0.091$). Nitrogen mineralization potential did not change irrespective of biochar additions or fertilizer application rates (Table 5), but DON adsorption was 50 % greater ($p < 0.05$) with than without biochar application (difference in the intercept in Supplementary Fig. S2).

Nitrogen leaching

There were no significant differences in any leaching metric between the control and biochar treatments at 50 % of recommended N fertilization ($p > 0.05$) (Table 7). However, at 100 % of recommended fertilization, total and mineral N flux, flow-weighted average
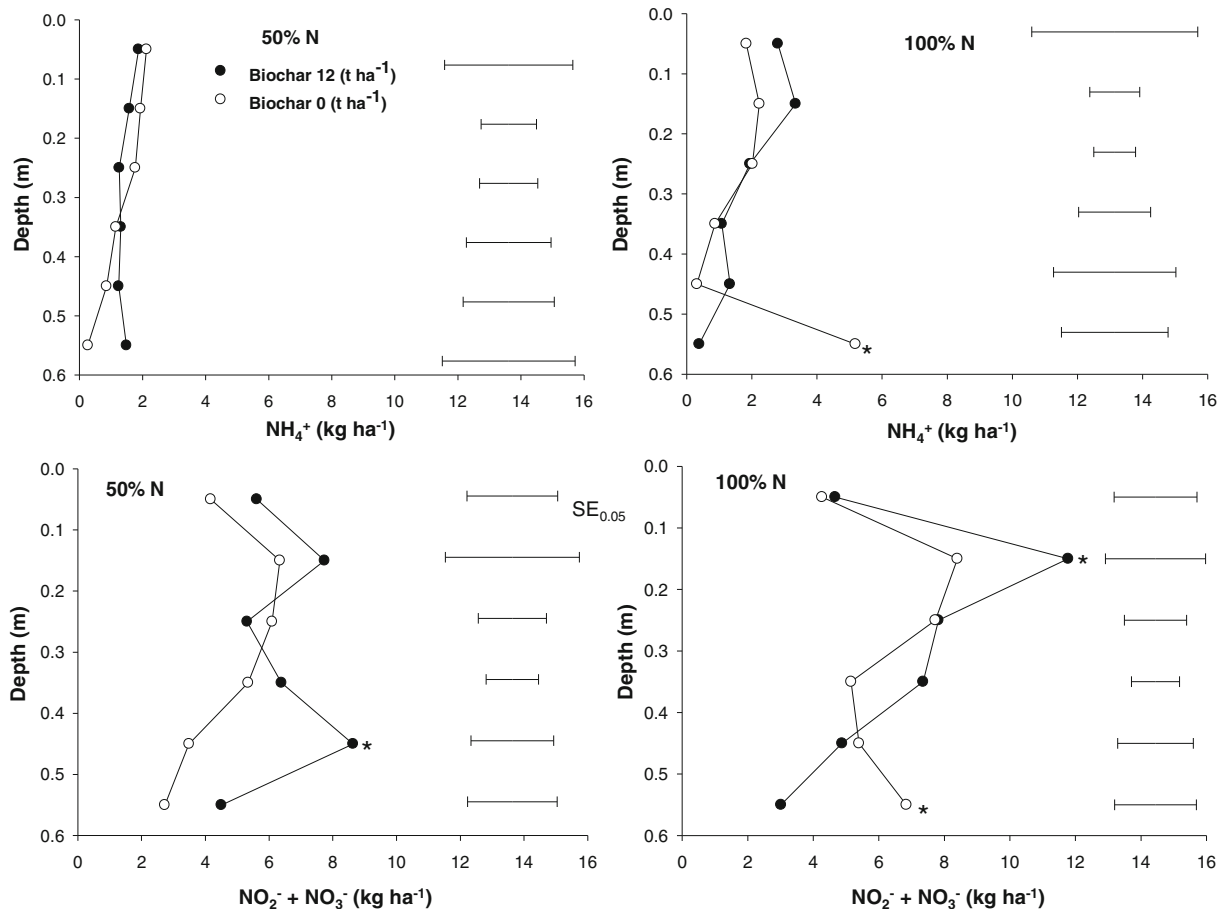

Fig. 1 Soil profile mineral N at 50 and 100 % secondary fertilizer N application rate and 0 and 12 t ha^{-1} biochar application rates after harvest in October 2009. * indicates significant differences ($p < 0.05$; $n = 3$) within an individual depth and between treatments
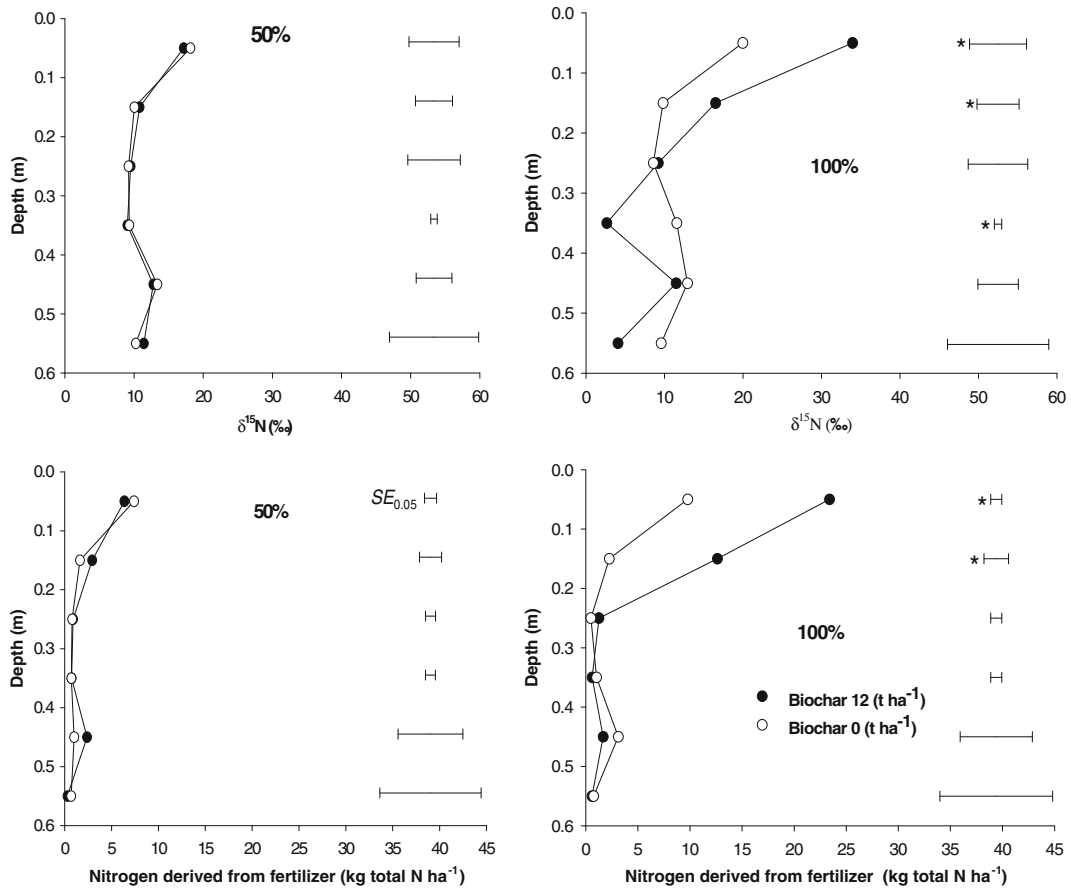
Fig. 2 Soil profile ^{15}N concentration and recovery of N derived from fertilizer at 50 and 100 % secondary fertilizer N application rates and 0 and 12 t ha^{-1} biochar application rates after harvest in October 2009. * indicates significant differences ($p<$ 0.05; $n=3$) within an individual depth and between treatments

NH_4^+ and NO_3^- concentrations and their fluxes, N flux and flow-weighted average N derived from fertilizer, and total water drainage were greater in the control than with biochar. Flow and concentrations of organic N were not significantly different between any treatment.

The largest single rainfall event for the 2009 season occurred at the end of June (Fig. 3). This event corresponded to a major leaching loss of nitrate but was not reflected in $\delta^{15}N$ values or the leaching losses of N derived from fertilizer. Discharge and N losses were only different for few individual sampling dates.

Fertilizer nitrogen recovery

At 100 % fertilization, the proportion of total soil N was greater ($p=0.05$) with than without biochar additions (not significantly different at 50 % fertilization) (Fig. 4). Conversely, the proportion of fertilizer N leaching losses were greater in the control than with biochar. The proportion of total fertilizer N recovery in plant, soil, and microbial biomass N was not significantly different in fields that received biochar and the un-amended control at 50 % of recommended fertilization, but tended to be greater ($p<0.1$) at 100 % fertilization (Fig. 4).

Discussion

Crop yield, nitrogen uptake and leaching

In contrast to our study, yield increases in maize following biochar applications have been widely reported from field trials with biochar manufactured under various production conditions and from diverse feedstocks (Yamato et al. 2006; Kimetu et al. 2008;

Table 7 Nitrogen forms in leachate collected from free-draining lysimeters and N leaching as a result of biochar additions to soil with high and low secondary N fertilizer application rates during 2009

	Secondary N fertilizer application rate (%)					
	50			100		
	Biochar application rate (t ha^{-1})					
	0	12	p (biochar effect)	0	12	p (biochar effect)
Total N flux (kg ha^{-1})	52.40	67.46	0.198	150.68	27.48	0.039
Flow-weighted average total N (mg L^{-1})	4.80	9.85	0.322	8.42	5.23	0.321
Total mineral N flux (kg ha^{-1})	32.42	63.69	0.267	121.59	17.24	0.007
NH$_4^+$ flux (kg ha^{-1})	0.67	1.05	0.396	11.42	0.95	0.024
Flow-weighted average NH$_4^+$ (mg L^{-1})	0.07	0.14	0.253	0.90	0.16	0.047
NO$_3^-$ and NO$_2^-$ flux (kg ha^{-1})	31.75	62.64	0.266	110.17	16.29	0.006
Flow-weighted average NO$_3^-$ and NO$_2^-$ (mg L^{-1})	3.70	8.09	0.208	8.67	2.94	0.043
Organic N flux (kg ha^{-1})	8.73	1.57	0.286	23.64	10.24	0.545
Flow-weighted average organic N (mg L^{-1})	1.03	1.62	0.438	3.34	2.19	0.407
Δ^{15}N (‰) of total N	391.99	53.85	0.231	702.03	242.20	0.068
δ^{15}N (‰) of flow weighted average	14.93	8.76	0.547	33.53	12.34	0.258
Total N flux derived from fertilizer (kg ha^{-1})	0.007	0.05	0.407	0.42	0.05	0.024
Flow-weighted average N derived from fertilizer (mg L^{-1})	0.0003	0.002	0.405	0.01	0.003	0.035
Total water flow (mm)	898	676	0.513	1282	611	0.026

(Students t-test; $p<0.05$; $n=3$). 521.5 mm total measured rainfall during the sampling period

Major et al. 2010; Van Zwieten et al. 2010a; Zhang et al. 2012). Most of this work has been done in tropical cropping systems where biochar may alleviate low pH, Al toxicity, and improve CEC (Lehmann et al. 2003; Van Zwieten et al. 2010a). However, the soils studied here have sufficiently high native fertility, adequate CEC, neutral pH, and yields with full fertilization are within the range of current average yields for North America (Martin et al. 2006). The crop received appropriate amounts of pesticides and therefore any benefits of decreasing severity of plant diseases as observed after biochar additions by Elad et al. (2010) are not expected. This also points at the need for identifying specific soil productivity constraints if biochar is to be applied to improve crop yields as these may largely depend on the soil-biochar combination (Van Zwieten et al. 2010a). The low yields observed in 2007 may have been a result of 2 weeks of hot and dry weather immediately following the secondary N fertilization.

While the biochar applications did not alter crop yield, biochar significantly reduced cumulative N losses due to leaching, but only at the high N fertilization rate. The fact that both total leached N as well as N leaching from applied ^{15}N showed proportionally similar reductions after biochar additions lends additional credence to the interpretation that biochar significantly reduced leaching of applied fertilizer, but evidence from individual sampling dates is weak and variability was high. In addition the δ^{15}N value of the total N recovered in the leachate was 290 % greater without biochar than with biochar at the high fertilization rate ($p=0.0675$). Other leaching studies with biochar applications have also reported net reductions in leaching of N and other nutrients in lysimeter studies with (Lehmann et al. 2003) and without plants (Novak et al. 2009; Laird et al. 2010b; Dempster et al. 2012a) and in the field with a maize crop (Major et al. 2012). It is interesting that there were no measured differences in leaching losses with 50 % fertilization in our study. The reason may be a trend towards overall greater fertilizer N retention ($p<0.1$) in soil at the low N application rate (36-37 % of applied N without and with biochar, respectively) than with the high N application rate (15–33 %) irrespective of biochar additions.
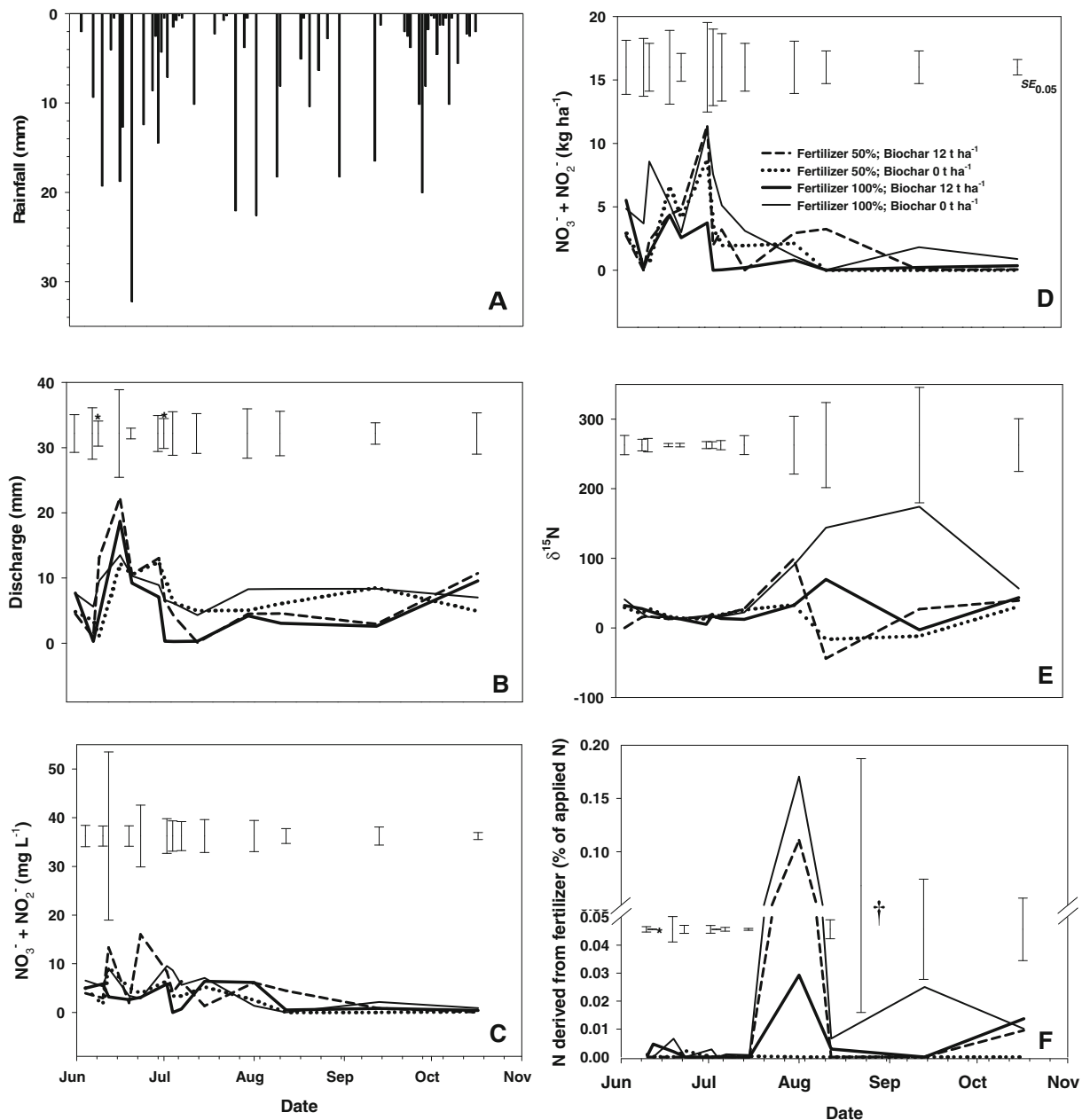
Fig. 3 Leaching data as a function of time for the 2009 growing season. Data include rainfall **A**, discharge **B**, NO_3^- and NO_2^- concentrations **C**, NO_3^- and NO_2^- fluxes **D**, $\delta^{15}N$ **E**, and N derived from fertilizer **F**. * indicates significant differences ($p<0.05$; $n=3$). Note scale change in the Y-axis in graph **D**. Error bar marked with † in graph **F** is the error bar for the August 1 data points. Arrows in graph **C** indicate initial and secondary fertilization events, respectively

In N-limited soils from Japan and Colombia, biochar was demonstrated to increase N availability and plant N uptake (Yamato et al. 2006; Major et al. 2010), however, in N-limited soils in Kenya, applications of biochar had beneficial effects on plant growth without increasing plant N uptake (Kimetu et al. 2008). In an

Oxisol from Brazil, N uptake even decreased likely due to N immobilization with biochar (Lehmann et al. 2003). This was similar to the decrease observed with a non-fixing bean isoline grown on an Oxisol from Colombia (Rondon et al. 2007). In the present experiment biochar did not affect aboveground maize N
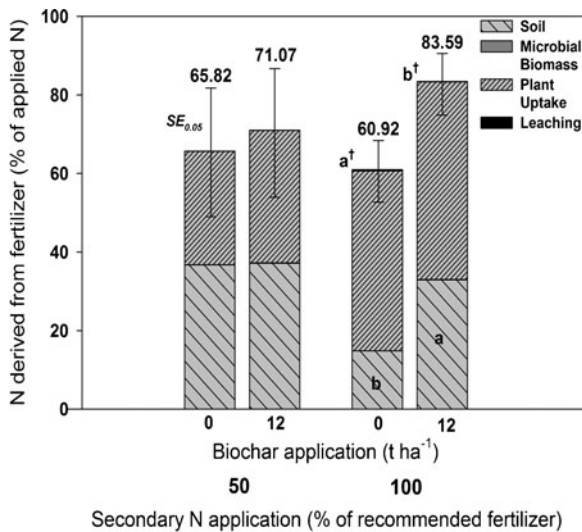
Fig. 4 Proportion of N recovery in soil, microbial biomass, plant, and leachate derived from fertilizer in control soil and biochar-amended soil with high and low secondary N fertilizer application rates in 2009 (means and standard errors). Values above bars show total N recovery. Different letters indicate significant differences between biochar application rates within secondary N fertilizer application rates and N pools (Students t-test; $p=0.05$; $n=3$). Letters not shown when differences are not significant. †N recovered in leachate

uptake. Therefore, a reduction in leaching with biochar can be unambiguously interpreted as being due to a greater retention of N in soil, and not a result of greater plant N uptake. To our knowledge this is the first time retention of N by biochar in soil is shown for a field experiment including plants that can not also be explained by increased N uptake.

Mechanism of nitrogen retention

The classic mechanism of nutrient retention on biochar is the greater sorptive capacity of biochar added to the soil through increases in CEC (Liang et al. 2006). The observed reductions in NH_4^+ leaching may be explained by adsorption, similar to the observations made by Lehmann et al. (2003) with applied ammonium sulfate in a short-term lysimeter study. However, NO_3^- was the dominant N species responsible for N leaching losses in our study, being about one order of magnitude greater than NH_4^+. While fresh biochar may decrease NO_3^- leaching (Dempster et al. 2012a) and may have some anion exchange capacity (AEC), at the pH of this soil and after several years in soil the AEC would be negligible (Cheng et al. 2008).

Therefore electrostatic adsorption of NO_3^- by biochar is not a likely mechanism to explain greater N retention in the soil with added biochar. This is also confirmed by the lack of a difference in exchangeable NO_3^- in the topsoil with biochar additions.

Nonetheless, N leaching mainly in the form of NO_3^- was reduced by biochar additions in the present experiment without an increase in exchangeable NO_3^- and more total N from fertilizer was found in the soil (35 % of the applied N with biochar, 15 % without biochar in the total soil at $p=0.0498$). Therefore, the remaining N must be held in the organic pool (35 % of the applied N with biochar, 15 % without biochar in total soil), with 0.08–0.09 % of applied ^{15}N being recovered in the microbial biomass pool with additions of biochar and 0.02–0.05 % recovered without biochar. Increases in microbial biomass after biochar additions have also been documented in other studies (Steiner et al. 2004; Kolb et al. 2009; Kuzyakov et al. 2009; Jones et al. 2012) and retention of fertilizer N by microbial cycling has been suggested by Steiner et al. (2008). Our experiment may indicate that one mechanism for N retention and leaching reduction is indeed the incorporation into microbial biomass and cycling into the organic N pool and possibly subsequent adsorption of organic N to biochar and minerals. Whether these observations can be generalized to other locations would need to be verified. The increased incorporation of N into microbial biomass may either be a result of mineralization of a comparatively labile fraction of biochar with a high C/N ratio (Lehmann et al. 2003; Rondon et al. 2007; Laird et al. 2010b) or a long-term increase of the microbial biomass as found in Terra Preta soils (Liang et al. 2010) and through substrate-induced respiration 10 years after biochar addition to an organic horizon (Wardle et al. 2008). The fact that this effect persisted more than 2 years after biochar additions suggests that the latter explanation may have played a role, as well. However, the enrichment of N in the microbial biomass pool did not result in measurably lower inorganic N contents in soil, changes in N mineralization or N uptake and crop yields in 2009. The reason may be that any relevant net N immobilization had already taken place in the preceding 2 years, but not to an extent to affect plant N uptake. Similar to our study, Jones et al. (2012) did not find lower total soil N mineralization, and also Dempster et al. (2012b) found no relevant changes in the mineralization of added aminoacids added to two soils.

In addition to microbial processes, biochar is known to have a high sorption affinity for organic C compounds, both of percolating dissolved organic C (Pietikäinen et al. 2000; Chun et al. 2004) and organic pollutants (Smernik 2009). Consequently, Jin (2010) found significantly greater adsorption of dissolved organic C (DOC) to soil amended with biochar. Even though we did not find a statistically significant reduction in DON leaching, adsorption of DON was greater in the presence of biochar. It is conceivable that surface oxidation of biochars over time leads to decreased adsorption of non-polar organic compounds as shown by Cheng and Lehmann (2009) and adsorption is a transient phenomenon. However, the greater microbial adsorptivity several hundred years after biochar deposition (Liang et al. 2010) may indicate that the effect may persist.

The findings of greater N recovery provides direct evidence for improved N use efficiency through retention of N in microbial biomass and organic N in soil. Such greater N retention in soil microbial biomass may also explain the findings of several other studies who reported greater N use efficiency in a range of soils (Chan et al. 2007; Steiner et al. 2008; Van Zwieten et al. 2010b).

Applications of biochar derived from maize stover reduced subsurface leakage of water-transported N while not affecting yields or N uptake over the first 4 years after application. In this part of the North Eastern United States, groundwater pollution with N is a major environmental burden from agriculture (Howarth et al. 1996; Matson et al. 1997; Carpenter et al. 1998). Based on the results from this study, applying biochar to the soil may reduce N leaching losses while not adversely affecting agricultural productivity.

Gaseous nitrogen losses

Between 17 % and 39 % of the applied fertilizer was not accounted for by leaching, plant uptake and soil retention with or without biochar additions, respectively. Erosion is unlikely to have played a major role, as the site is tile-drained and is not sloping. Some of the unaccounted losses may have occurred in gaseous form, and would appear to be lower after biochar additions. Taghizadeh-Toosi et al. (2011a) found lower nitrous oxide emissions from pasture soils that received biochar, which may be indicative of lower gaseous N losses by denitrification. However, not all available studies showed a reduction in nitrous oxide

emissions (Scheer et al. 2011) and no published study investigated total N losses by denitrification including N_2. Also reduced losses by ammonia volatilization is a possibility as shown by Taghizadeh-Toosi et al. (2011b), but was not directly quantified in our study.

Conclusion

Based on this study, applications of biochar up to 30 t ha^{-1} do not adversely affect agricultural productivity in temperate soil that has little soil quality constraints. Expectations of biochar to increase crop yields in such fertile temperate soils may not be expected. It is the norm rather than the exception for maize yields in the region to consistently achieve genotypic and phenotypic yield potentials. The greater N recovery in the topsoil after one season may suggest that N retention at full fertilization can be increased with one-time biochar additions even to fertile soils, but did not result in greater N uptake here. This result would need to be verified across a range of biochars to determine if the microbial N accumulation is a product of this particular feedstock or production procedure.

This study provides some indication that the accumulation of applied fertilizer N in the topsoil may be linked to N cycling through the microbial biomass and retention of organic N during the first few years after application. Less clear is the micro-location of the microbial biomass N, the form of the retained N and what processes and properties of biochar were responsible for an enhanced cycling of N through the microbial biomass. Future research should investigate N cycling with different biochars and soil types, and how N accrual in soil, availability and leaching changes over decadal time scales and what the mechanism is that leads to incorporation of applied N in microbial biomass following biochar additions.

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