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Comparing Biochar Application Methods for Switchgrass Yield and C Sequestration on Contrasting Marginal Lands in Pennsylvania, USA

Roger T. Koide[1] · Binh Thanh Nguyen[2] · R. Howard Skinner[3] · Curtis J. Dell[3] · Paul R. Adler[3] · Patrick J. Drohan[4] · Megan Licht[1] · Monica Boyer Matthews[1] · Rachel Nettles[1] · Kevin Ricks[1] · John Watkins[1]

Abstract

To avoid competition with food crops, biofuel feedstocks may need to be produced on economically marginal lands where yields are limited and replacement of existing vegetation will reduce soil C, foregoing some CO_2 emission savings. Therefore, our first goal was to determine whether biochar application to marginal lands could improve switchgrass yield while sequestering sufficient soil C to eliminate the negative impact of cultivation. Because it may be difficult to obtain large quantities of biochar, our second goal was to compare small, incremental and large, all-at-once biochar applications. Our third goal was to determine whether biochar had any negative effects on earthworms, mycorrhizal fungi, soil bacteria, soil fungi, and soil enzyme activity. We grew switchgrass at two sites with poorly drained soils and two sites with excessively drained soils. Irrespective of site, biochar significantly increased yield when we rototilled in the entire amount before planting but not when we applied it incrementally between crop rows using a chisel plow. Biochar increased soil C stocks, in some cases increasing it beyond that found in soils of intact marginal land vegetation. Nevertheless, mixing biochar with soil had little or no impact on earthworm activity, mycorrhizal colonization, soil bacterial and fungal communities, and soil enzyme activities. We conclude that biochar may be part of an effective strategy for producing switchgrass on marginal lands, but the choice of application method depends on the relative importance of several considerations including biochar availability, switchgrass yield, C sequestration, soil erosion, and ease of application.

Keywords Crop yield · Soil C · Mycorrhizal fungi · Root growth · Soil enzymes · Soil microbes

Introduction

The renewable fuel standard of the US Environmental Protection Agency requires the production of 36 billion gallons of renewable fuel by 2022, including 15 billion gallons of grain-based ethanol and 21 billion gallons of fuel from cellulosic or other non-grain feedstocks. In order to avoid competition with food production, much of the biofuel feedstock production is projected to occur on land that is, for various reasons, economically marginal for food crop production [1]. There are 51 to 67 million hectares of land considered to be economically marginal in the USA, including abandoned croplands and pastures, some of which are currently enrolled in the USDA Conservation Reserve Program to protect highly erodible or otherwise environmentally sensitive lands [2–4]. Switchgrass (*Panicum virgatum* L.), a perennial prairie grass native to North America, has been identified as a model biofuel crop for much of the USA. [2, 3]. It does not require high fertility [5] so it may be more productive than food crops on lands that are economically marginal due to poor fertility.

One of the goals of biofuel use is to reduce net CO_2 emissions when compared with fossil fuel use. But savings in net CO_2 emissions for biofuel crops grown on marginal lands may be limited for two major reasons. First, biofuel crop

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✉ Roger T. Koide
rogerkoide@byu.edu

[1] Department of Biology, Brigham Young University, Provo, UT 84602, USA

[2] Institute of Environmental Science, Engineering and Management, Industrial University of Ho Chi Minh City, Ho Chi Minh City, Vietnam

[3] Pasture Systems and Watershed Management Research Unit, USDA-ARS, University Park, PA 16802, USA

[4] Department of Ecosystem Science and Management, The Pennsylvania State University, University Park, PA 16802, USA

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productivity may be low on marginal lands. Second, while perennial biofuel crops such as switchgrass may be superior to annual crops in terms of soil C sequestration [6], the soil disturbance associated with their cultivation may still render them inferior to existing vegetation on idled lands [3]. However, low productivity and reductions in soil C stocks may be at least partially overcome by incorporating biochar into the soil. Biochar is a byproduct of the pyrolysis biofuel pathway [7].

A great deal of enthusiasm for using biochar as a soil amendment to improve the sustainability of agriculture [8, 9] has stemmed from the discoveries of relatively fertile soils containing unusual concentrations of very old charred organic materials in the Amazon [10] and elsewhere [11]. While it is not surprising that the addition of significant quantities of naturally alkaline biochar to highly weathered and acidic tropical soils results in increased plant growth [12], it is less certain whether the addition of biochar to less acidic soils in temperate regions, at practical rates of field application, will generally result in crop yield increases comparable with those demonstrated in the *terra preta* and *terra mulata* soils of the Amazon [10, 13, 14]. However, even in less acidic soils, biochar may serve to retain nutrients, making them available to plants over longer periods of time [12] as a consequence of its high anion [15] and cation [8, 12, 16, 17] exchange capacities, which may result in increased fertilizer use efficiency [18, 19] and reduced N_2O emissions [20, 21]. By virtue of its low density, biochar may increase aeration of heavy, excessively wet soils [9, 22–25] and, by virtue of its ability to retain water [24–27], improve water availability in excessively drained soils. Therefore, biochar may have the capacity to substantially improve the soils of lands that are marginal because of poor fertility, excessive drainage or poor drainage. Moreover, because biochar resists decomposition for long periods of time, it stably sequesters carbon in the soil [8, 12, 28, 29]. Therefore, amending soils with biochar could reduce net CO_2 emission, rendering any biofuel production system closer to CO_2 neutral than it otherwise could be [30].

While many researchers have enthusiastically embraced the concept of applying biochar to soils, very little consideration has been given to the practical problem of incorporating significant quantities in the context of current agricultural practices [9]. If simply applied on the soil surface, substantial quantities of biochar would be lost to the wind or in surface water runoff [31, 32]. In order to remain onsite and to produce its beneficial effects, biochar must be placed *into* the soil. Perhaps the simplest approach to do so would be to apply the biochar to the surface of the soil before tillage. The main drawbacks to this approach include the need for the desired quantity of biochar prior to the establishment of the perennial crop, the potentially large CO_2 efflux associated with SOM decomposition due to the tillage [33], and the soil erosion risk created by the tillage. If there is a desire to avoid tilling the entire field for these reasons, and especially if the entire amount of biochar is not available prior to establishment of the crop, another approach must be used. Recent development of technology for low disturbance, sub-surface placement of dairy manure, poultry litter, and compost [34, 35] suggests an alternative approach for biochar incorporation. Using that approach, smaller quantities of biochar could be added to the soil on an incremental basis between crop rows. However, this would result in the concentrated placement of biochar rather than uniform mixing with the soil, and any benefit accruing from mixing with soil, such as improvements in aeration of heavy soils [9, 22–25, 36], or improvements in plant water availability in light-textured soils [24–27, 36–38] would not be realized.

In this study, we had three goals. Our first goal was to determine whether biochar could be used to improve switchgrass yield on marginal lands while sequestering sufficient C to eliminate the negative impact of cultivation on soil C stocks. Because large quantities of biochar may be difficult for growers to obtain, our second goal was to compare incremental and all-at-once applications of biochar in terms of yield and C sequestration. The incremental application was a one quarter dose applied in each of 4 years with a chisel plow between crop rows. This resulted in a concentration of biochar between crop rows. The all-at-once application consisted of a full dose of biochar applied uniformly to the plots and rototilling it in prior to crop establishment. Because biochar influences various chemical (i.e., pH) and physical (i.e., aeration, moisture content, color, and therefore temperature) soil properties [26, 39, 40], it has the potential to substantially influence the activity of soil dwellers of all kinds [41]. Therefore, our third objective was to document how it influences other important components of the agroecosystem including (1) earthworms, which influence a variety of soil physical, biochemical and microbiological properties including soil hydrology, soil structure, organic matter decomposition, and nutrient cycling [42], (2) colonization of roots by mycorrhizal fungi, which are responsible for much of plant phosphorus uptake [43] and significantly improve soil structure [44], (3) soil fungal and bacterial communities, and (4) the enzymes they produce that are responsible for nutrient cycling and litter decomposition [45].

Materials and Methods

Study Sites

We performed our research at four sites in Pennsylvania, USA. Two of the sites were considered marginal due to poor drainage. The other two were considered marginal due to excessive drainage and low water holding capacity. The four sites occurred across the Appalachian Plateau and the Ridge and Valley physiographic provinces in Pennsylvania, USA, that possessed soils varying significantly in texture (Table 1).

Table 1 Mean (SEM) of some characteristics of the soils sampled in July 2011 at 15 cm at the four sites of this study ($n = 4$)

Site, soil series	Sand (%)	Silt (%)	Clay (%)	pH[a]
Toftrees, Morrison sandy loam (Ultic Hapludalf)	61 (0.2)	27 (0.2)	12 (0.1)	5.9 (0.02)
Duff, Weikert loam (Lithic Dystrudept)	44 (0.7)	40 (0.6)	16 (0.3)	6.0 (0.03)
Krasinski, Wharton silty clay loam (Aquic Hapludult)	15 (0.3)	58 (0.3)	27 (0.1)	5.4 (0.03)
Gibboney, Edom silty clay loam (Typic Hapludalf)	6 (0.1)	62 (0.5)	32 (0.3)	6.0 (0.08)

[a] In water

The Toftrees site (77° 53′ 55.5″ W, 40° 49′ 34.062″ N) is located in State College, PA at an elevation of 365 m. The Morrison sandy loam of this site is excessively drained. Since 1998, this site was primarily in a corn-soybean rotation with occasional minor variations (wheat in 1 year and fallow in 2 others) prior to the establishment of switchgrass.

The Duff site (77° 53′ 38.94″ W, 40° 39′ 8.106″ N) is located 17 km SSW of State College, PA at an elevation of 315 m. The Weikert loam of this site is excessively drained. The site had been planted as a timothy (*Phleum pretense* L.) hay field in 2010. Other known crops since 2002 prior to switchgrass establishment included wheat, oats, corn (2 years), and grass hay (4 years). At the time of switchgrass establishment, the existing vegetation was timothy (45%), green foxtail (*Setaria viridis* L. P. Beuv., 35%), yellow toadflax (*Linaria vulgaris* Mill., 12%), and other annual and perennial weeds.

The Krasinski site (78° 9′ 41.034″ W, 41°1′ 55.388″ N) is the only one located on the Allegheny Plateau, 34 km NW of State College, PA at an elevation of 440 m. The Wharton silty clay loam at this site frequently experiences extended periods of excessive wetness. The vegetation at this site prior to the establishment of switchgrass was a mixture of cool-season grasses planted in August 2008 and dominated by meadow fescue, *Festuca pratensis* Huds., (74% of cover), and tall fescue, *Festuca arundinacea* Schreb., (9% of cover). The remaining 17% were a combination of 13 species of forage grasses, forage legumes, and weeds.

The Gibboney site (77° 54′ 32.848″ W, 40° 36′ 56.727″ N) is located 21 km SSW of State College, PA at an elevation of 275 m. The Edom silty clay loam of this site frequently experiences extended periods of excessive wetness. There is no known history of previous agricultural activity as this site is located near the edge of an agricultural field in a low spot that is too wet to cultivate. The existing vegetation prior to switchgrass establishment was a mixture of annual and perennial weeds. The species comprising > 10% of total cover included goldenrod, *Solidago* sp. L. (36%), hemp dogbane, *Apocynum cannibinum* L. (16%), and yellow nutsedge, *Cyperus esculentus* L. (14%).

Rainfall and air temperatures were monitored at each site with weather stations mounted on tripod bases containing Campbell Scientific (Logan, UT, USA) CR1000 dataloggers and instruments. Precipitation was measured with a Texas Electronics (Dallas, TX, USA) tipping bucket RG Model TE-525. Air temperature was measured with a Campbell Scientific HMP45 temperature probe. Data were logged hourly.

Biochar

Biochar was produced from shoots of switchgrass (*Panicum virgatum* var. Cave-In-Rock) by the torrefaction facility at North Carolina State University (Raleigh, NC, USA). The torrefaction chamber had a low oxygen environment and was held between 375 and 475 °C. Details outlining the procedures for biochar production can be found in Koide et al. (2014) [26]. The ash, S, C, H, O, and N concentrations (% of dry weight) of three biochar samples were determined using ASTM method D3176 by Hazen Research, Inc. (Golden, Colorado, USA). The mean (SEM) biochar bulk density was 0.083 (0.004) g cm^{-3}, the mean ash concentration was 13.5% (0.1), the mean sulfur concentration was 0.082% (0.003), the mean C concentration was 65.8% (0.1), the mean H concentration was 3.45% (0.02), the mean N concentration was 1.01% (0.01), and the mean pH in water was 9.5 (0.1). Only 3% of the biochar (by weight) was > 2.0 mm in diameter, 93% was in the 0.05–2.0-mm fraction, and 4% was < 0.05 mm in diameter.

Plot Layouts

Plots were laid out at each site in May 2011. Each plot was 3.7 m (12 ft) wide and 6.1 m (20 ft) long, and each plot type was replicated four times in a randomized complete block design at each of the four sites. There were five plot types at each site: the control vegetation (ConVeg), and four plot types containing switchgrass: biochar incorporated by rototilling (BC-RT), rototilling without biochar (RT), biochar incorporated between crop rows via chisel plowing (BC-CP), and chisel plowing between crop rows without biochar (CP). There were, therefore, 20 plots at each site (4 blocks × 5 plot types). Complete descriptions of the RT, BC-RT, CP and BC-CP treatments are given below. At Krasinski, Duff and Gibboney, the vegetation in the control plots consisted of the vegetation naturally established at the site and described above (see study sites). At Toftrees, the vegetation in the control plots each year was corn (Pioneer 0970 AMX), planted using an Alamaco 2-row no-till corn planter on 30-in. rows,

four rows per plot. The dates of corn planting are given in Table S1. All plots (except the ConVeg plots at Gibboney, Duff, and Krasinski) were sprayed with a combination of glyphosate (*N*-(phosphonomethyl)glycine) and 2,4-dichlorphenoxyacetic acid (2,4-d) in the summer or fall of 2011 and, again, in the spring of 2012, to prepare for establishment of switchgrass (and corn in the ConVeg plots at Toftrees).

Pre-planting Soil pH and Texture

In July 2011, prior to biochar incorporation or switchgrass establishment, soil samples were taken from each plot using 2 cm diameter soil corers to a depth of 15 cm. One core was taken from each plot, and all cores from a single block were composited. Soils were air dried and sieved to pass 2 mm. Soil pH was measured with a standard glass electrode using a 1:1 soil:distilled water (*w*:*v*) ratio following a 30-min equilibration. Soil textural characterization was carried out using the pipette method with organic matter removal pre-treatment with hydrogen peroxide and soil dispersion with sodium hexametaphosphate [46].

Biochar Incorporation

Biochar was incorporated into the soil using two methods. The dates of biochar incorporation are given in Table S2. One treatment (BC-RT) involved rototilling in the entire amount of biochar, 10 t ha^{-1}, on a single occasion prior to switchgrass establishment. This involved spreading the appropriate amount of biochar uniformly across the entire surface of the plot followed by tilling to a depth of approximately 12 cm using a rototiller mounted on a small tractor. The control for that treatment consisted of rototilling the soil in the same manner but without the addition of biochar (RT). In the other method (BC-CP), we applied 2.5 t ha^{-1} biochar to plots on four occasions (Table S2) by creating a narrow furrow between each row of switchgrass to a depth of approximately 12 cm, adding biochar to the open furrows and closing them manually with spades. The furrows were created using a single-tine chisel plow mounted on a small tractor. The control for that treatment consisted of creating furrows between each row of switchgrass to the same depth and then closing them using spades without the addition of biochar.

Switchgrass Establishment

Typically, switchgrass is directly seeded in the field, but this crop can be difficult to establish from seed due to strong weed competition [47]. Therefore, seeds of the Kanlow variety of switchgrass were sown in a greenhouse in starter flats containing Redi-Earth seedling/plug mix (sun gro Horticulture, Agawam, MA, USA) on 13 Feb 2012. On 5 March 2012, seedlings were transplanted into seedling trays (model 50ST, Landmark Plastic Co., Akron, OH, USA), also maintained in the greenhouse. Each cell was 11.5 cm deep and held a volume of 144 cm^3. The greenhouse was maintained at 27 °C/18 °C (day/night). Plants in the seedling trays were simultaneously watered and fertilized using a siphoning system with Jack's 10-30-20 Bloom Buster (JR Peters, Inc., Allentown, PA, USA) at a rate of 30 ppm P. When seedlings were approximately 65–70 cm tall (May 2012), they were planted at each of the field sites (Table S1). Within each of the switchgrass plots, there were ten planting rows across the width of each plot spaced 35.6 cm (14 in.) apart. Dibble holes were made within rows every 30.5 cm (12 in.) and seedlings were transplanted into the holes for a total of 200 plants per plot, a plant density of 9.0 plants m^{-2}.

Field Plot Management

In May 2012, shortly after switchgrass planting, and again in the spring of 2013, we applied the pre-emergent herbicide pendimethalin (3,4-dimethyl-2,6-dinitro-*N*-pentan-3-yl-aniline) at 1.7 kg ai ha^{-1}) to all switchgrass plots. Over the course of the four growing seasons of this study, 2,4-d and 3,6-dichloro-2-methoxybenzoic acid (dicamba) were applied as needed to switchgrass plots in order to remove unwanted broadleaf weeds. Plots containing switchgrass were fertilized with ammonium sulfate in May or June of each year at the rate of 30 kg N ha^{-1} in 2012 and at 60 kg N ha^{-1} in subsequent years. The ConVeg (corn) plots at Toftrees were also fertilized with ammonium sulfate each year just after planting at the rate of 150–200 kg N ha^{-1} $year^{-1}$. ConVeg plots at all other sites received no fertilizer.

Switchgrass Harvest

Switchgrass was harvested in late October or early November in each year (2012–2015, Table S3) from the center five rows to a height of 10 cm using a sickle-bar forage harvester. Following the harvest of the five rows for yield determination in each year, the remaining rows were removed using the same harvester.

Switchgrass Shoot Nutrient Concentration

The most recently fully expanded leaf was taken from four to five plants in the center two to three rows of the RT and BC-RT plots at each site in mid-June of 2013, 2014, and 2015. Leaves were rinsed in distilled water, dried, ground, and digested in concentrated sulfuric acid and hydrogen peroxide at 400 °C, and the digestate was analyzed colorimetrically for N and P concentrations by the Nessler [48] and phosphomolybdate [49] methods, respectively.

Soil Fertility

In November of 2015, each plot of every replicate at each of the four sites was sampled to a depth of 15 cm. Thirty 2-cm diameter soil cores were sampled from each plot. As the switchgrass was harvested from the inner rows, soils were sampled from the outer rows to avoid impact on measured crop yield. On each side of each plot, three randomly positioned transects (five cores each) were taken from center to center of the two outer rows of switchgrass in order to sample the heterogeneous furrow-applied biochar plots in a representative fashion, and we used the same sampling scheme in the plots of all treatments and all sites. Soils were air dried and ground through a 2-mm sieve prior to analysis. The entire 15 cm of the 30 soil cores per plot were composited by plot. We analyzed, therefore, 4 treatments (BC-RT, RT, BC-CP, CP) and 16 samples per treatment (4 replicates × 4 sites). The samples were analyzed by the Agricultural Analytical Services Laboratory of the Pennsylvania State University for Mehlich 3 extractable P, K, Mg, Ca, Zn, Cu, and S via inductively coupled plasma spectroscopy. Cation exchange capacity (CEC) was calculated by summation of K, Mg, Ca, and acidity.

Soil Total C and N and pH

Thirty 2-cm diameter soil cores (to 15 cm depth) were sampled from each plot in November of each year (2012–2015) as indicated above for soil fertility testing. Each 5 cm depth increment (0–5, 5–10, and 10–15 cm) was composited separately from the 30 cores from each plot. Soils were air dried and ground through a 2-mm sieve. Total C and N were determined by combustion using an Elementar Vario-Max. Total C was assumed to be equivalent to organic C at the pH values of these soils at the beginning of the experiment (Table 1). Subsequent soil pH was measured with a standard glass electrode using a 1:1 soil:distilled water (*w*:*v*) ratio following a 30-min equilibration on samples collected 2012–2014.

Soil C and N Sequestration

By 2015, we had added a total of 10 t biochar ha^{-1} in both the BC-RT and BC-CP plots. This biochar had a C concentration of 67.5%, resulting in an expected sequestration of 6.75 t C ha^{-1} assuming 100% of the biochar C remained in the soil. We calculated the actual C sequestration due to biochar by subtracting for each replicate at each site the top 15 cm soil total C concentration in control plots (RT or CP) from the respective biochar addition plots (BC-RT or BC-CP).

The biochar had a N concentration of 0.982%, resulting in an expected sequestration of 0.0982 t N ha^{-1} based on the total addition of 10 t biochar ha^{-1} assuming 100% of the biochar N remained in the soil. We calculated the actual N sequestration due to biochar by subtracting for each replicate at each site the top 15 cm soil total N concentration in control plots (RT or CP) from the respective biochar addition plots (BC-RT or BC-CP).

Soil Color

Among other things, soil color can affect soil surface temperature, which can affect seed germination and soil moisture [50]. Bulk soil samples were collected using a spade from random locations in the BC-RT and RT plots at each site to a depth of approximately 5 cm in mid-May 2014. Samples were air dried and sieved to collect the 2-mm or less fraction. Color value and chroma were determined on two analytical replicates per true replicate for each of the two treatments, for both dry and wet (sprayed with water to field capacity) samples using a Chroma meter (model CR-200, Konica Minolta Corp., NJ, USA).

Colonization of Roots by Mycorrhizal Fungi

Root samples were collected from RT and BC-RT plots in early October 2012 and again in mid-June 2015. In both instances, six 2-cm diameter soil cores (to 15 cm depth) were taken randomly from the outer rows of each plot. The soil was washed from the roots and roots were pooled by plot. Root samples were stored in 50% ethanol until they were cleared, stained and scored according to Koide and Mooney [51].

Root Growth into Biochar

Between 13 and 20 May 2014, we bored six 7.6 cm (3 in.) diameter holes to a depth of 15 cm using a soil auger between plants within the second to the outermost row of switchgrass in each of the four BC-CP plots at each of the four sites. We used a row position because in these plots each year biochar was placed in the inter-row. Into three of the randomly selected holes, we replaced the soil that had been removed. Into the remaining three, we placed pure switchgrass biochar (described above). PVC-coated fiberglass window screen was secured over the biochar by nails driven into the surrounding soil to keep it in place. On 1 July, a 2.0-cm diameter core was taken to a depth of 15 cm from the center of the soil and biochar patches. Switchgrass roots collected from the cores were washed in water on soil sieves. Their lengths were determined using a Delta-T (Cambridge, UK) scanning and image analysis system.

Earthworm Middens

Anecic earthworms, such as *Lumbricus terrestris*, make middens (piles of casts and plant material) around the entrances to their vertical burrows. Thus, middens are indicators of

burrows, and the density of middens is an indication of earthworm population density [52]. Between 10 and 17 May 2013, we counted the number of earthworm middens, identified according to Brown [53] and Brown et al. [42] within the eight center rows of switchgrass plots at each site.

Soil Enzyme Analyses

Soils were collected in May 2013, July 2014, and June 2015 from RT and BC-RT plots using a 2-cm soil corer to a depth of 10 cm. One core was taken from each plot corner to avoid disturbing the eight central harvest rows, approximately 25 cm toward the center of the plot from the corner. Two additional cores were taken from an inter-row location in the center of the plot. A portion of each of the six individual cores from each plot was frozen at − 20 °C for later DNA analysis (see below). The remainders of the individual cores from each plot were homogenized by hand, sieved to 4 mm, and then immediately used to determine 1,4-ß-cellobiohydrolase (CBase), acid phosphatase (Pase), and ß-*N*-acetylglucosaminidase (NAGase) activities as in Peoples and Koide [54]. CBase, Pase, and NAGase activities are indicative of decomposition and mineralization of organic P and N compounds [55].

Univariate Data Analyses

The factors in the several fixed effects analysis of variance models were year, site, biochar treatment (yes vs. no), and tillage treatment (RT vs. CP). Depth was an additional factor in the analysis of soil C and N concentrations but not the soil fertility analyses (P, K, Ca, Mg, Zn, Cu, S). Block (replicate) was nested within site. Site was considered a fixed variable because we specifically chose sites that differed in soil texture; two were fine-textured and two were coarse-textured, and one of our explicit goals was to determine whether the biochar effect differed among these particular sites. We also considered year a fixed variable, and thus we had no repeated measures. This was because in the chisel-plow biochar treatment biochar was added sequentially so the biochar concentration increased with each year, and we were only interested in the effect of year during those years when biochar was added. For the variables we analyzed, we used different analysis of variance models because some variables were measured in all years while others were measured in only a subset of years, and some variables were measured in all treatments while others were measured in only a subset of treatments. The exact combination of factors in each model is clear from the analysis of variance tables in the supplementary materials.

Soil Microbial Community Analyses

Small (0.25 g), previously frozen subsamples of individual cores were used for DNA analysis for determination of bacterial and fungal community structure. DNA was extracted using PowerSoil DNA isolation kits (Mo Bio Laboratories, Carlsbad, CA, USA). Extraction tubes were vortexed for a total of 15 min. All other steps were completed as outlined in the PowerSoil protocol. Extracted genomic DNA was then stored at − 20 °C until amplification by PCR. To amplify the bacterial and fungal genomic DNA, Sigma Jumpstart Taq (Sigma–Aldrich, St. Louis, MO, USA) was used. Fungal DNA was amplified using ITS1F[Hex] and ITS4 primers [56]. Bacterial DNA was amplified using 1406F[FAM] and 23SR primers [57]. The thermal cycling program used to amplify fungal DNA was: 30 cycles of 95 °C for 40 s, 49 °C for 30 s, and 72 °C for 48 s with a final elongation at 72 °C for 7 min. The thermal cycling program used to amplify bacterial DNA was: 30 cycles of 95 °C for 40 s, 55 °C for 30 s, and 72 °C for 2 min with a final elongation at 72 °C for 7 min. Hex-labeled and FAM-labeled PCR products were separated on an ABI 3730xl genetic analyzer using MapMarker ROX-1000xl ladder (BioVentures, Inc., Murfreesboro, TN). The ROX-1000xl ladder was used because the vast majority of PCR products were less than 1000 bases long. GeneMapper Software (Applied Biosystems, Grand Island, NY, USA) was used to analyze fungal and bacterial ribosomal intergenic spacers (ARISA). The raw ARISA peaks were binned into operational taxonomic units (OTUs) with a bin width of 5 bp. Bins created by spurious peaks (usually ladder bleed-through) were manually eliminated prior to analysis. Peak areas were used in subsequent multivariate analyses. Bacterial and fungal communities were analyzed separately. Peak areas from the automated ribosomal intergenic spacer analysis (ARISA) were Hellinger-transformed prior to these analyses in order to relativize the peak areas for each sample [58]. Significant differences in community structure for each site × year combination were determined using permutational multivariate analysis of variance (PerMANOVA) in the R statistical environment [59] with the Vegan package [60]. Bray-Curtis distances were used. PerMANOVA is a non-parametric method for using multivariate data, such as the frequencies of occurrence of multiple microbial species, to compare groups, such as soil samples from different treatments.

Results

Site Characterization

The seasonal rainfall totals were similar across years and sites, but the Duff site had a slightly higher rainfall than the other sites (Table S4). Mean air temperatures were also similar across years and sites, but the Krasinski site was slightly cooler than the other sites (Table S4), consistent with its higher elevation on the Allegheny Plateau.

The soils at the four sites differed considerably in texture (Table 1). The soils at the Duff and Toftrees sites had high concentrations of sand relative to soils at the Krasinski and Gibboney sites. In addition to the sand, at the Duff site, 30% of the weight of the samples was gravel and did not pass the 2-mm sieve. Clay concentrations were relatively high at the Gibboney and Krasinski sites and lower at the Duff and Toftrees sites. At the beginning of the experiment, soil pH was approximately 6.0 at all sites with the exception of Krasinski, which had a pH of 5.4.

Switchgrass Yield

The grand mean of annual biomass yield over the 4 years of production was 12,800 kg ha^{-1}. Biochar application had a significant, positive effect on crop yield, but there was a significant interaction between biochar addition and tillage such that the four-year average biomass yield was increased by 8.3% with biochar in rototilled plots, while biochar had no significant impact on average yield when applied between crop rows in chisel-plowed plots (Table S5; Fig. 1a). Site, tillage, and year also significantly influenced crop yield (Table S5). Although there was a significant interaction between year and site, yield increased with time at all sites (Fig. 1b). The average yield was 8620 kg ha^{-1} in 2012, and this increased to an average of 15,100 kg ha^{-1} in the final year (2015).

Switchgrass Nutrient Concentration

Leaf nutrient concentrations were only determined in the rototilled plots. Biochar addition significantly (Table S6a) but only slightly increased switchgrass leaf P concentration (BC-RT, 2.64 μg g^{-1}; RT, 2.48 μg g^{-1}; SEM = 0.03). Moreover, there was a significant interaction between Site and Year such that switchgrass P concentrations at Gibboney, Toftrees, and Krasinski were highest in 2014 and lowest in 2013, but at Duff, they were highest in 2015 and lowest in 2013.

Biochar addition did not significantly increase switchgrass leaf N concentration (Table S6b). However, there was a significant interaction between site and year such that the highest N concentrations occurred in different years at the various sites. Leaf N concentration varied from 21.7 μg g^{-1} at Toftrees in 2013 to 26.0 μg g^{-1} at Krasinski in 2014.

Soil Fertility

The analyses of variance for extractable P, K, Mg, Ca, Zn Cu, S, and cation exchange capacity (CEC) in November 2015 are given in Table S7a–h. We focus here on the main effect of biochar addition because in the vast majority of cases biochar addition did not interact with other treatments including tillage or site. Biochar addition had no significant effect on P or Cu availability, but significantly increased K, Mg, Ca, and Zn availability, while it significantly decreased S availability (Table 2).

Soil Total C Concentration and C Sequestration

Soil total C concentration was significantly influenced by treatment, site, year, and depth, and there were a number of 2- and 3-factor interactions among these factors (Table S8). As expected, soils amended with biochar contained higher C concentrations than those not given biochar; soils in the BC-CP treatment contained higher C concentrations than those in the corresponding control (CP) treatment, and soils in the BC-RT treatment contained higher C concentrations than those in the corresponding RT treatment (Fig. 2). Year was expected to be a significant factor and to interact with treatment because biochar was added yearly on four occasions in the BC-CP treatment, but biochar was added only in year 1 in the BC-RT treatment, and biochar was not at any time added to the CP, RT, or ConVeg treatments. Indeed, there was a significant interaction between year and treatment (Table S8; Fig. 2a). Year also interacted significantly with site and with depth (Table S8). However, because year was not a large contributor to soil total C concentration variability (see sums of squares, Table S8); hereafter, we report on the data from 2015, collected after completion of biochar additions to the BC-CP plots.

In 2015, treatment, site, and depth were significant main effects, and there were significant two-factor interactions involving each of these (Table S9). In general, soils amended with biochar contained higher C concentrations than the corresponding unamended soils. The significant interaction between treatment and site (Table S9), however, can be appreciated from the fact that biochar addition in rototilled and chisel-plowed plots significantly increased soil C concentrations at all sites except Krasinski, where biochar addition increased soil C concentration in the chisel-plowed plots but not in the rototilled plots (Fig. 2b). Site accounted for more than a third of the total variation in soil total C (see sums of squares, Table S17). Irrespective of treatment, Krasinski and Gibboney soils contained the highest concentrations of soil C, and Duff and Toftrees the lowest (Fig. 2b.) Site interacted significantly with depth and with treatment, although these two-factor interactions contributed in only a minor way to the overall variability in soil total C (see sums of squares, Table S9). Although depth interacted significantly with treatment and site, these interactions contributed only slightly to overall variability in comparison with the depth main effect (see sums of squares, Table S9) and, in nearly every case, the order of soil total C by depth was 0–5 cm > 5–10 cm > 10–15 cm, irrespective of treatment within a site.

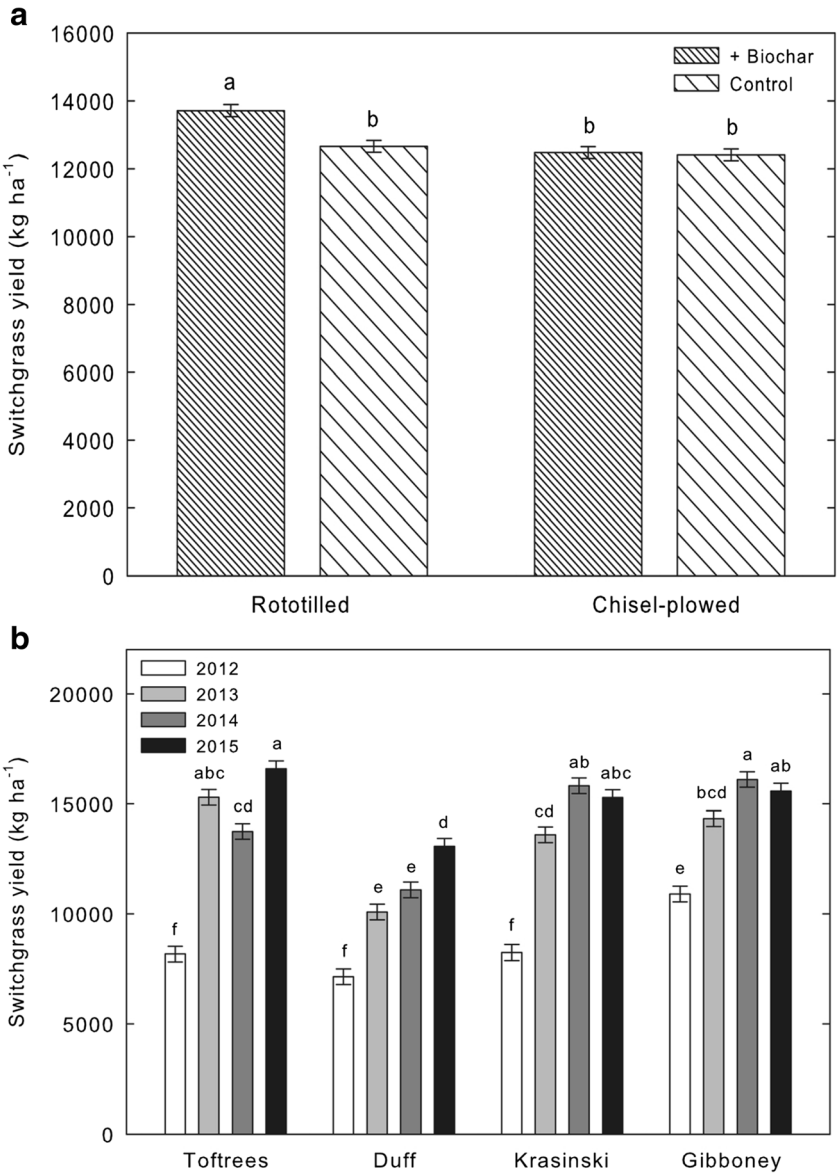

Fig. 1 Mean (± SEM) yield of switchgrass. Mean separations based on Tukey method. **a** Yield with and without biochar addition and in either rototilled or chisel-plowed plots averaged over 2012–2015 and all sites ($n = 64$). **b** Yield at the four sites in 2012–2015 averaged over both biochar treatments and both tillage treatments ($n = 16$)

Actual C sequestration due to biochar at Toftrees, Duff,m and Gibboney was not significantly different from expected, either in the chisel-plowed plots (Fig. 3a) or the rototilled plots (Fig. 3b). In other words, the actual amount of C in the top 15 cm of those soils was no different from the expected value based on the C concentration of the biochar, the amount of biochar actually applied, and the assumption that 100% of the C in the biochar remained in the soil. For Krasinski, however, C sequestration was significantly greater than expected in the chisel-plowed plots (Fig. 3a), but not in the rototilled plots (Fig. 3b).

Table 2 Mean (SEM) of Mehlich 3-extractable P, K, Mg, Ca, Zn, Cu, and S concentrations from soil samples taken November 2015 averaged across tillage treatments and sites

	P	K	Mg	Ca	Zn	Cu	S	CEC
Biochar	40.3 (1.1) a	84.7 (1.9) a	72.5 (1.4) a	1430 (31) a	1.23 (0.03) a	1.41 (0.03) a	15.7 (0.2) b	13.5 (0.2) a
Control	38.4 (1.1) a	77.3 (1.9) b	64.5 (1.4) b	1390 (31) b	1.14 (0.03) b	1.40 (0.03) a	16.7 (0.2) a	13.7 (0.1) a

The units for CEC are meq 100 g^{-1} soil. All other units are in micrograms per gram. Analysis of variance results are given in Tables S8, S9, S10, S11, S12, S13, S14, and S15 ($n = 16$)

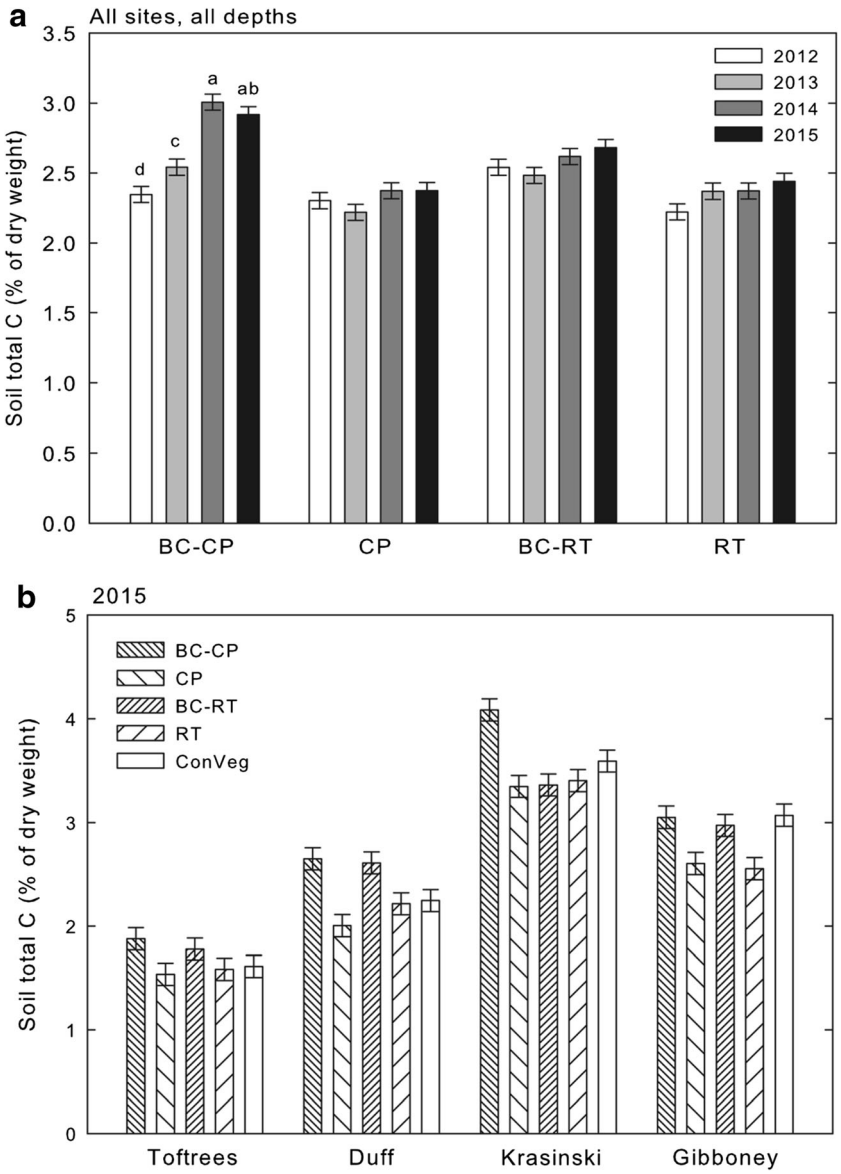

Fig. 2 Mean (± SEM) soil total C concentrations. **a** Soil C concentration for the four treatments (BC-CP, biochar chiseled plots; CP, control chiseled plots; BC-RT, biochar rototilled plots; RT, control rototilled plots) averaged across the three sampling depths (0–5, 5–10, and 10–15 cm) at each of the four sites in years 2012–2015. Mean separations within a treatment are based on the Tukey method. For treatments where no mean separations are given, years are not significantly different (n = 48). **b** Soil C concentrations for the five treatments (BC-CP, biochar chiseled plots; CP, control chiseled plots; BC-RT, biochar rototilled plots; RT, control rototilled plots; ConVeg, control vegetation plots) averaged across the three sampling depths (0–5, 5–10, and 10–15 cm) at each of the four sites in 2015 (n = 12)

Soil Total N Concentration and N Sequestration

Soil total N was significantly influenced by Treatment, Site, Year and Depth, and there were a number of significant 2-factor and 3-factor interactions (Table S10). Treatment, together with significant interactions involving treatment, contributed a total of less than 3%, which indicates that biochar addition did not have a large effect on soil N concentration. In contrast, site contributed about 56% of the total variability in soil total N concentration while depth contributed about 21%. Year and interactions involving year contributed to less than 4% of the variability. In order to examine soil N concentrations that are comparable with the soil C concentrations, above, hereafter, we report on the data from 2015, collected after completion of biochar additions to the BC-CP plots.

In 2015, treatment, site, and depth were significant main effects, and there were significant two-factor interactions involving each of these factors (Table S11). Site accounted for about 60% of the total variation in soil total N (see sums of squares, Table S11). Irrespective of treatment, the order of soil total N concentration was Gibboney > Duff > Krasinski > Toftrees (Fig. 4). Although depth interacted significantly with treatment and with site, these interactions contributed only slightly to the overall variability in soil N concentration compared with the depth main effect (which contributed to 21% of the variability, see sums of squares, Table S11) and, in nearly every case, the order of soil total N by depth was 0–5 cm > 5–10 cm > 10–15 cm, irrespective of treatment within a given site.

Actual N sequestration due to biochar amendment was significantly lower than expected at all sites both in the chisel-

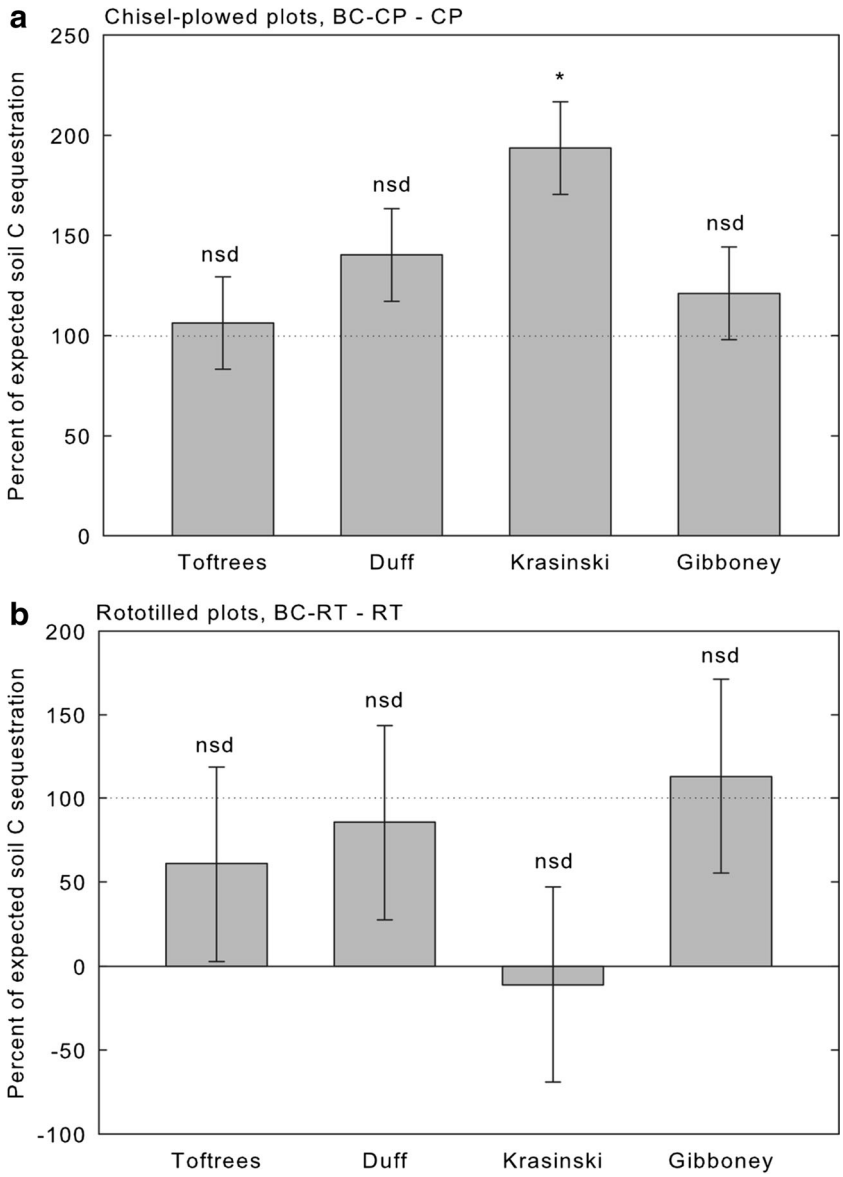

Fig. 3 Mean (± SEM) percentage of expected soil C sequestration. nsd, not significantly different from expected (100%); *significantly different from expected (100%) based on 95% confidence interval ($n = 4$). **a** Expected soil C sequestration based on the calculated contribution of biochar addition to soil C and the difference in soil total C content (top 15 cm) between BC-CP and CP plots in 2015. nsd, not significantly different from expected (100%). **b** Expected soil C sequestration based on the calculated contribution of biochar addition to soil C and the difference in soil total C content (top 15 cm) between BC-RT and RT plots in 2015

plowed plots (Fig. 5a) and in the rototilled plots, with the exception of the rototilled plots at Gibboney (Fig. 5b). In other words, the actual amount of N in the top 15 cm of those soils was significantly less than expected based on the biochar N concentration, the amount of biochar actually applied, and the assumption that 100% of the biochar N remained in the soil, except in the rototilled plots of Gibboney.

Soil pH

Soil pH was increased by biochar application, but the magnitude of the increase varied by site, with the largest increase at Gibboney, and no significant increase at Toftrees (Table S12; Fig. 6). Although there was a significant site × year interaction (Table S12), the pH was generally highest at Toftrees and Gibboney, intermediate at Duff, and lowest at Krasinski, irrespective of year (Fig. 6).

Soil Color, 2014

Because these values differed significantly between wet and dry samples, we analyzed wet and dry samples separately. The hue for all soil samples was yellow. The value (lightness) and chroma (color purity) differed according to site and, indeed, most of the variability was attributable to site. In addition, biochar addition was also a significant factor. For both dry and wet soils, biochar application significantly decreased color value (decreased lightness) (Table S13a, b; Fig. 7a) and significantly decreased chroma in both dry and wet soils (Table S14a, b; Fig. 7b).

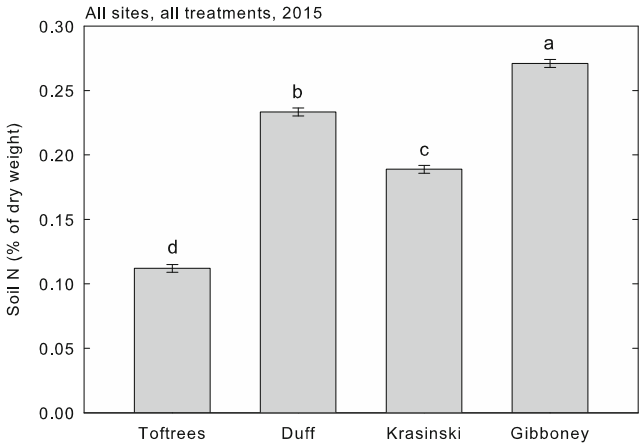

Fig. 4 Mean (± SEM) soil total N concentrations at each of the four sites in 2015 averaged for the five treatments (BC-CP, CP, BC-RT, RT, and ConVeg) and the three sampling depths, 0–5, 5–10, 10–15 cm. Mean separations based on Tukey method ($n = 180$)

Mycorrhizal Colonization

In 2012, there was no significant effect of biochar addition (RT vs. BC-RT) on mycorrhizal colonization of switchgrass roots (Table S15a). Colonization was significantly lower at Duff (49% of root length) compared with the other sites (Krasinski, 75%; Gibboney, 74%; Toftrees, 70%). In 2015, there was similarly no significant effect of biochar addition (RT vs. BC-RT) on mycorrhizal colonization (Table S15b) but, again, colonization was significantly lower at Duff (33%) compared with Gibboney (58%), while colonization at the other sites was intermediate (Krasinski, 47%, Toftrees, 45%).

Root Growth into 100% Biochar

At all sites, the growth of switchgrass roots was inhibited by 100% biochar (Fig. 8; Table S16). Root growth was also significantly affected by Site with the largest growth at Duff and smallest growth at Toftrees (Fig. 8; Table S16).

Earthworm Middens

There was no significant effect of biochar addition (comparing BC-RT to RT plots) on the number of earthworm middens (Table S17). In eight rows (160 linear feet), there were 47.1 middens in BC-RT plots and 47.0 middens in RT plots. There was a significant effect of site on earthworm middens (Table S17), with significantly more at Toftrees (72.6) and Gibboney (53.8) than at Krasinski (31.9) or Duff (30.1).

Soil Microbial Communities

Biochar treatment significantly affected the structure of microbial communities at the Gibboney site in 2014 and 2015 (for fungi) and in 2014 (for bacteria) (Table 3). Biochar also significantly impacted the structure of fungal communities at the Toftrees site in 2014. At the other sites, biochar had no significant impact on either fungal or bacterial communities.

Soil Enzyme Activities

Soil phosphatase activity was not significantly influenced by biochar in any year (Table S18a–c), but it was significantly influenced by site in all 3 years (Table S18a–c; Fig. 9a). However, the ranking of sites depended somewhat on the year. For example, Gibboney had the highest or next to the highest phosphatase activity in each of the years, while Krasinski had the lowest phosphatase activity in 2014, but the highest in 2015.

Soil *N*-acetylglucosaminidase activity was significantly influenced by biochar in 2013 but not in 2014 or 2015 (Table S19a–c). In 2013, biochar depressed *N*-acetylglucosaminidase activity; the RT treatment had a significantly greater (1.668 nmol min^{-1} g^{-1}, SEM = 0.067) activity than the BC-RT treatment (1.315, SEM = 0.067). Soil *N*-acetylglucosaminidase activity was also significantly influenced by site in 2013 and 2014 but not 2015 (Table S19a–c; Fig. 9b). Again, Gibboney had the highest activities in 2013 and 2014, but there was no significant effect of site in 2015 (Fig. 9b).

Soil cellobiohydrolase activity was not significantly affected by biochar in any year (Table S20a–c). Soil cellobiohydrolase activity was significantly influenced by site in 2013 and 2014, but not in 2015 (Table S20a–c; Fig. 9c). Again, Gibboney had the highest activities in 2013 and 2014, but there was no significant effect of Site in 2015 (Fig. 9c).

Discussion

We set out to determine whether biochar could increase the yield of switchgrass while increasing net C sequestration at four marginal sites in Pennsylvania, USA, differing markedly in soil texture. We also compared the effects of two methods for applying biochar to soils, either uniformly rototilling in the full 10 t ha^{-1} prior to crop establishment or by adding a total of 10 t ha^{-1} over the course of 4 years between crop rows using a chisel plow. Finally, we determined the effect of biochar on earthworms, on the colonization of roots by mycorrhizal fungi, on soil fungal and bacterial communities, and on some of the enzymes they produce.

Yield

We found that switchgrass yield was significantly increased by biochar amendment in the rototilled plots (8.3% overall). Meta-analyses suggest that there is large variability in the

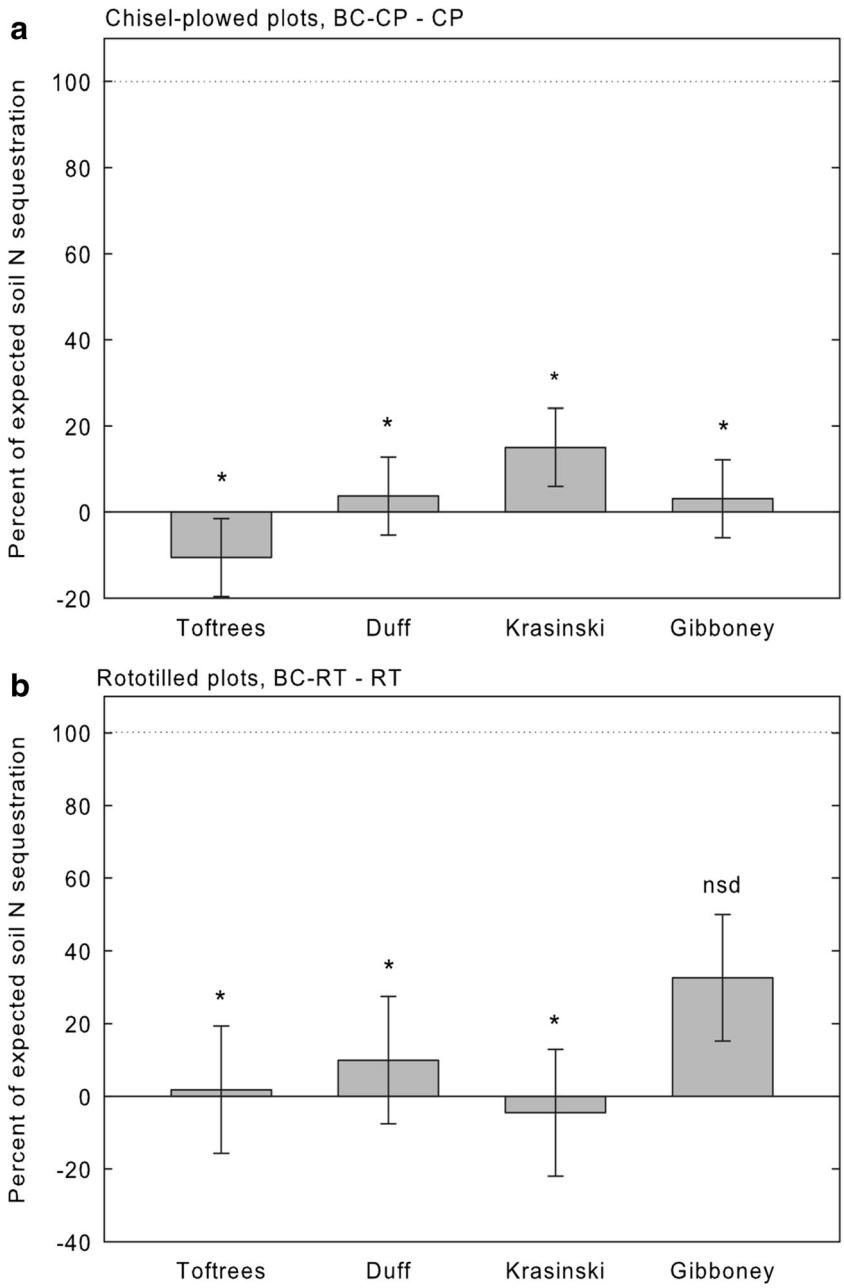

Fig. 5 Mean (± SEM) percentage of expected soil N sequestration. nsd, not significantly different from expected (100%); *significantly different from expected (100%) based on 95% confidence interval ($n = 4$). **a** Expected soil N sequestration based on the calculated contribution of biochar addition to soil N and the difference in soil total N content (top 15 cm) between BC-CP and CP plots in 2015. **b** Expected soil N sequestration based on the calculated contribution of biochar addition to soil N and the difference in soil total N content (top 15 cm) between BC-RT and RT plots in 2015

effect of biochar on crop yield. For example, Jeffery et al. [61] showed that yield effects of biochar ranged from a 28% *decrease* to a 39% *increase*. They found, however, that in field studies, the average effect on yield was a 5% improvement. In another meta-analysis, Liu et al. [62] found that the average effect on yield in field studies was an increase of 8–9%. These values compare well with the yield increase in our rototilled plots.

We demonstrated in this study that 10 t ha^{-1} of switchgrass biochar significantly increased switchgrass yield at our four field sites varying markedly in soil texture, which suggests that significant benefit can be derived from relatively small concentrations under fairly diverse conditions. In other studies, the effect of biochar concentration on plant growth is quite variable. In one meta-analysis, Jeffery et al. [61] found that the greatest improvement in yield occurred with 100 t ha^{-1} biochar, a concentration that may be impractical in most field settings. In another meta-analysis, Liu et al. [62] found that concentrations over 40 t ha^{-1} produced less benefit than lower concentrations. In yet a third meta-analysis, Biederman and Harpole [40] found no effect of biochar concentration on yield. Some of this variation may have to do with the confounding of biochar concentration with biochar type, experiment location (field vs. greenhouse), and soil type.

The increased yield due to biochar in our rototilled plots may have involved improved soil physical properties. The

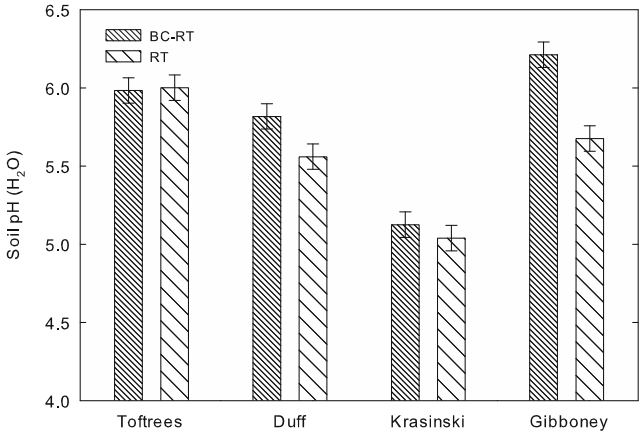

Fig. 6 Mean (± SEM) soil pH (determined in H_2O) in BC-RT and RT plots at the four sites, averaged across years 2012, 2013, and 2014 (n = 12)

best evidence of this is the fact that yield was significantly increased in the rototilled plots but not in the chisel-plowed plots. When biochar is thoroughly mixed with soil, it has the opportunity to affect soil physical properties. When it is not mixed, such as when it remains concentrated in the inter-row position, the properties of the remaining soil cannot be altered as readily. Moreover, because root growth is inhibited in 100% biochar, any trait of the biochar itself, such as improved water availability, cannot be realized by the crop.

In coarse-textured soils such as at the Toftrees and Duff sites, biochar may have increased water holding capacity, plant available water content, or soil hydraulic conductivity as has been shown elsewhere [18, 24–26, 37, 38, 63]. Indeed, Jeffery et al. [61] and Liu et al. [62] found that the greatest yield increases occurred in coarse- or medium-textured soils, and Omondi et al. [36] found, on average, that biochar increased available water holding capacity by 15.1%, with the effect being larger on coarse-textured soils than on fine-textured soils. In fine-textured soils such as at Gibboney and Krasinski, biochar may have improved yield by increasing soil aggregation, reducing bulk density and increasing soil porosity or aeration [9, 22–25, 36].

In some cases, biochar additions appear to be more beneficial at low soil pH and less beneficial at neutral pH [62]. This suggests that the biochar effect may be at least partly due to its alkalinity. However, in our study increased pH does not appear to be the major mechanism by which biochar increased switchgrass yield across all sites. There was no significant relationship between soil pH and yield during 2012–2014 for the BC-RT and RT plots across all sites (results not shown). While it is common for biochar additions to increase soil pH [40] as it did in this study, many have found that biochar improves crop yield even in neutral soils [61, 62] for which an increased pH is not likely to improve yield. Therefore, an increase in pH due to biochar may not have been of overriding importance with respect to yield increase in this study, and other mechanisms, such as improvement in soil physical properties, may have been more important.

Biochar applications may enhance the availability of certain nutrients, especially P and K [40, 64]. We found that biochar amendment significantly increased soil extractable K concentration, as well as the soil extractable concentrations of Mg, Ca, and Zn. It is unclear how the biochar increased the availability of these three, particularly Zn. In other studies, biochar additions and pH increases were associated with significant *decreases* in Zn availability [65–67]. In any case, the increased availability of these elements may have contributed to the yield increase, although biochar did not increase leaf concentrations of any of these nutrients (data not shown). In the rototilled plots, biochar significantly increased in leaf P concentration, but leaf P concentrations in the plots without biochar averaged approximately 0.25% of dry matter, which does not appear to be limiting [68], so increased P availability does not appear to be the cause of increased growth. And, although biochar has been known to improve plant N uptake [12], in our study, biochar did not significantly influence leaf N concentration. Enhanced nutrient availability, therefore, does not appear to be the major mechanism by which biochar increased yield in this study.

In some instances, biochar additions have increased mycorrhizal colonization [69, 70], and this may contribute to increased yield. However, in our study biochar addition did not significantly influence mycorrhizal colonization, so this mechanism could not have been responsible for the biochar effect on yield.

By rototilling biochar into soil, its color was darkened significantly. Darker soils may heat more rapidly in the spring and may thus increase seed germination in cool springs if water is not limiting [50]. Although we transplanted switchgrass seedlings into the field, switchgrass production normally requires sowing seeds that must germinate in the field [47]). Because switchgrass seed germination and seedling emergence are temperature dependent [71], the darkening of the soil by biochar application may be significant to switchgrass production in systems where seeds are directly sown into the field. However, darker and thus warmer soils cannot easily account for the increased yield due to biochar addition in this study.

Soil C

Without biochar addition, switchgrass production had a largely negative impact on soil C concentration compared with the control vegetation. Such has been previously predicted [3]. However, *with* biochar addition the C concentration in soil supporting switchgrass was generally better than in the ConVeg treatment, suggesting that conversion from idle, naturally vegetated, marginal lands to switchgrass production

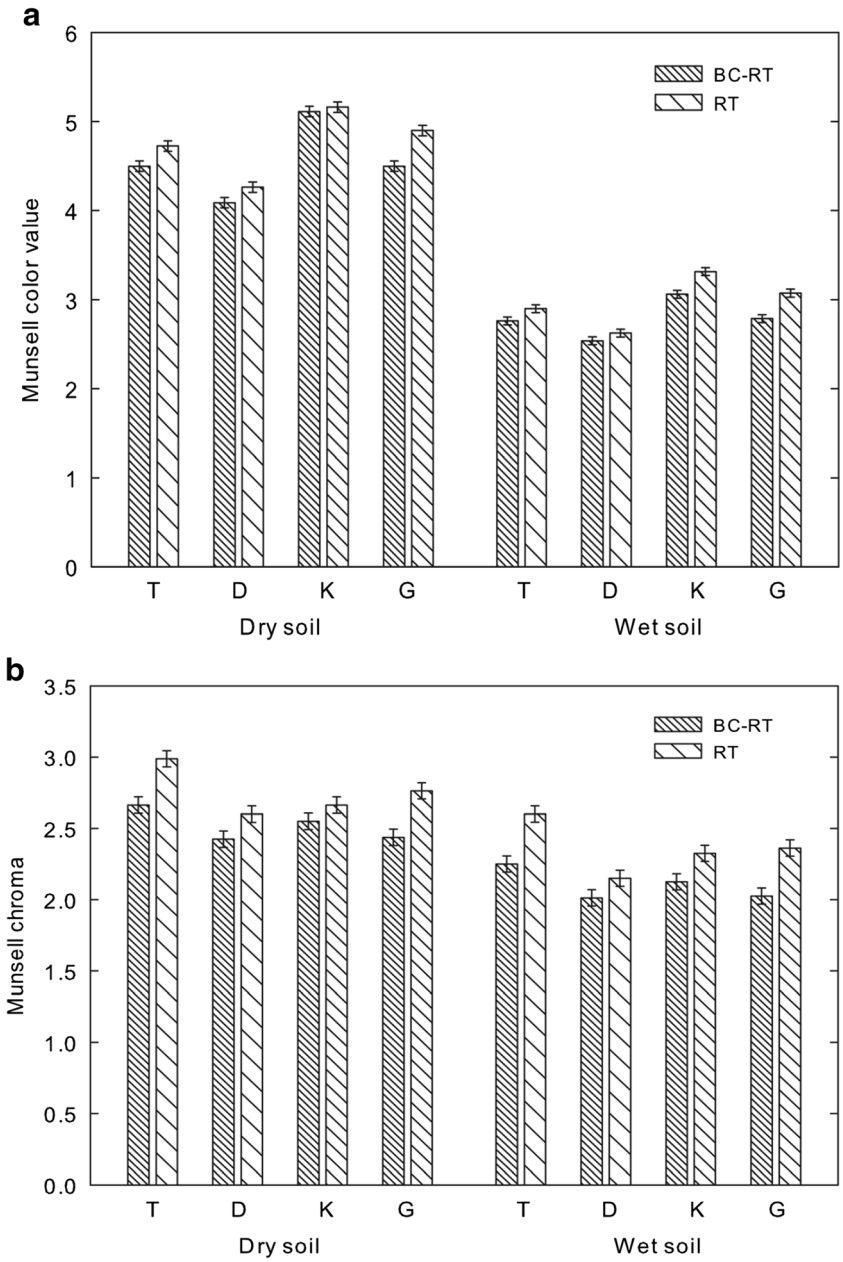

Fig. 7 **a** Mean (± SEM) Munsell color value for soils from the four locations (T, Toftrees; D, Duff; K, Krasinski; G, Gibboney), both in the dry and wet conditions and either in the BC-RT (+ biochar) or RT (control) treatments ($n = 4$). **b** Mean (± SEM) Munsell chroma for soils from the four locations (T, Toftrees; D, Duff; K, Krasinski; G, Gibboney), both in the dry and wet conditions, and either in the BC-RT (+ biochar) or RT (control) treatments ($n = 4$)

need not result in a net loss of soil C and may even result in a net gain in soil C if biochar is added.

More C was sequestered with the biochar addition in the chisel-plowed plots than in the rototilled plots despite the fact that the total amount of added biochar was the same. There are several mechanisms that may contribute to this. First, compared with biochar in the rototilled plots, the biochar in the chisel-plow furrows was better protected from losses due to surface water or wind action. Second, the high concentration of biochar in the inter-rows of the chisel-plowed plots may have reduced decomposition of native soil organic matter, i.e., it may have resulted in negative priming as shown elsewhere [72]. Third, the high concentration of biochar in the inter-rows of the chisel-plowed plots may have increased microbial biomass as has been shown previously [73]. Fourth, the reduced soil disturbance in the chisel-plowed plots compared with the rototilled plots may have resulted in less decomposition of indigenous soil organic matter [33]. Fifth, the difference in biochar C sequestration may relate to differences in average biochar age and thus the amount of biochar the decomposed [28]. In 2015, the average age of the biochar in the rototilled plots (applied in 2011) was 4 years. The average age of the biochar in the chisel-plowed plots, which had been added over the course of 4 years (2011–2014), was 2.5 years.

In the chisel-plowed plots at the Toftrees, Duff, and Gibboney sites, the addition of biochar resulted in the expected amount of C sequestration based on the total C content of the biochar, while at Krasinski, there was significantly greater than

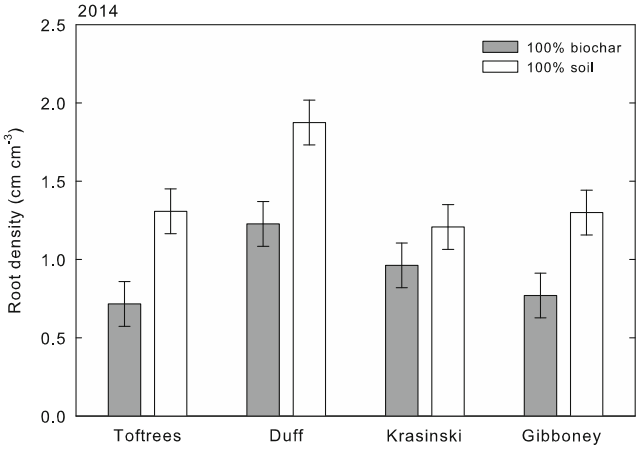

Fig. 8 Mean (± SEM) switchgrass root density within either 100% switchgrass biochar or 100% soil at the respective sites. Root growth occurred from May to July 2014 ($n = 4$)

expected C sequestration in the BC-CP plots. Again, biochar added in a concentrated fashion between crop rows in the chisel-plowed plots may have stimulated microbial biomass as shown previously [73], but why that may have occurred at only one site is not known. The result was apparently not due to greater switchgrass productivity at Krasinski, as it did not have greater switchgrass yields than at the other sites. Possibly, the sampling (30 cores per plot) was inadequate to accurately estimate the true value of soil C in the chisel-plowed plots in which the biochar was heterogeneously distributed.

In litter or soil, most C is assumed to be available to soil microbes, so C/N ratios help to indicate whether N might be mobilized or immobilized by the microbial community. Biochar is different as borne out in our study. Biochar generally sequestered the expected amount of C based on the C content of the biochar, but it did not sequester the amount of N based on the N content of the biochar. The most logical explanation is simply that the biochar N was labile while the C was not. Thus, C/N ratios of biochar are not meaningful in the same way that litter C/N or soil C/N ratios are.

Biochar Application Method

As indicated above, the net soil C sequestration with biochar was generally better with the incremental, chisel-plow application compared with the all-at-once, rototilled application. However, the chisel-plow method required very careful manipulation of the implement in order to minimize damage to the crop, especially in later years when root systems were large. Moreover, that method required machinery in the field each year and, in some years, that proved difficult for us. Because the crop was harvested in the late fall, biochar incorporation had to be accomplished either after harvest but before the ground froze, or in the spring before regrowth. If the spring were particularly rainy, finding a time when the soil was not excessively wet could be difficult. The resulting high concentration of biochar between rows may have been inhibitory to root growth and may therefore have constrained crop yield. Therefore, when sufficient biochar is available before planting, it may be practical to till in the entire desired amount prior to crop establishment, but the intensive soil disturbance, the resultant loss of indigenous soil organic matter via enhanced decomposition, and the erosion risk associated with tillage would need to be carefully considered.

Earthworms, Mycorrhizal Fungi, Soil Fungal and Bacterial Communities, and Soil Enzymes

At 10 t ha^{-1}, switchgrass biochar did not have significant impacts on mycorrhizal fungi or earthworms, and generally did not impact soil fungal and bacterial communities or soil enzyme activities in any consistent way, despite having a positive effect on yield and C sequestration. Thus, in this study, there did not appear to be any obvious tradeoffs between yield increases/enhanced C sequestration and other ecosystem functions influenced by

Table 3 Significance (p values) of biochar treatment on the structure of soil fungal and bacterial communities for each site × year combination, established by the PerMANOVAs ($n = 24$)

	Toftrees Biochar (BC-RT vs. RT)	Duff Biochar (BC-RT vs. RT)	Krasinski Biochar (BC-RT vs. RT)	Gibboney Biochar (BC-RT vs. RT)
Fungi				
May 2013	NS	NS	NS	NS
July 2014	0.007	NS	NS	0.001
June 2015	NS	NS	NS	0.004
Bacteria				
May 2013	NS	NS	NS	NS
July 2014	NS	NS	NS	0.001
June 2015	–	–	–	–

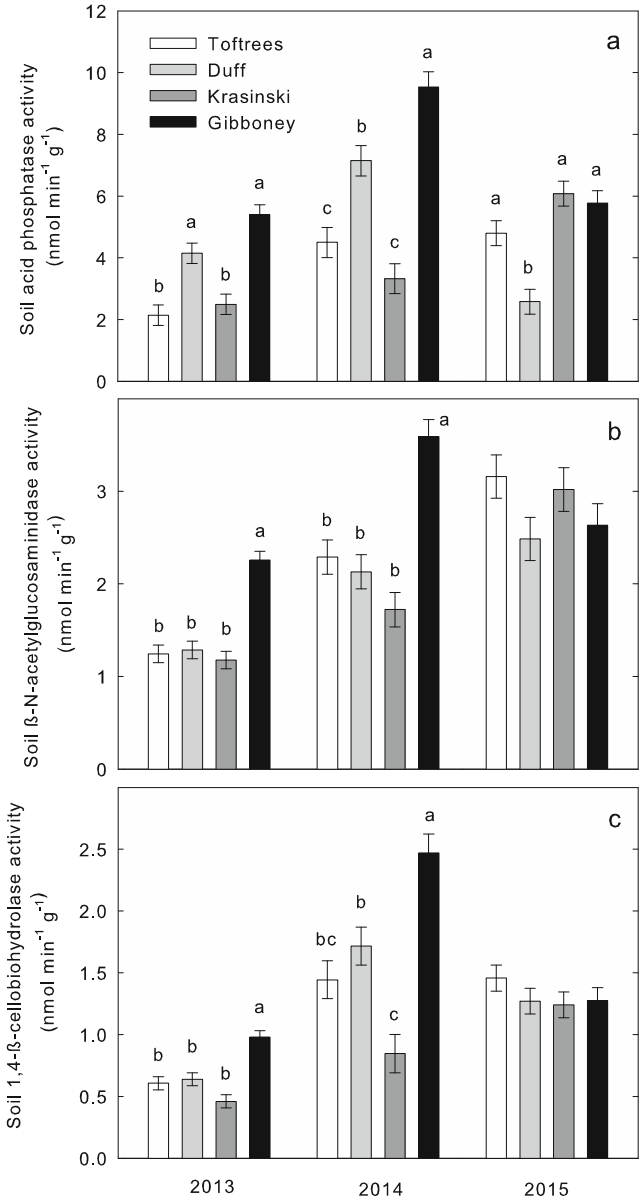

Fig. 9 Activities of three soil enzymes in years 2013, 2014, and 2015. Within a given year and enzyme, different letters indicate significant differences among means according to Tukey 95% method. **a** Acid phosphatase; **b** *N*-acetylglucoaminidase; **c** cellobiohydrolase. Mean separations for a given year are based on Tukey method. For years where no mean separations are given, sites were not significantly different ($n = 4$)

earthworms, mycorrhizal fungi, soil bacteria and fungi, and soil enzymes such as soil hydrology, nutrient uptake by the crop, organic matter decomposition, or nutrient cycling. At concentrations significantly higher than 10 t ha^{-1}, biochar may more strongly alter soil pH or other soil properties that have result in larger impacts on mycorrhizal fungi or earthworm activity or microbial community structure, and thus possibly have a significant impact on various ecosystem functions.

Conclusion

We performed this research at four sites in Pennsylvania, USA, that are economically marginal for food crop production. Two have soils that are coarse-textured and excessively drained. Two have soils that are fine-textured and poorly drained. All four appear to be suitable for growth of switchgrass as a biofuel crop.

The all-at-once incorporation of 10 t switchgrass biochar ha^{-1} (by rototilling in prior to crop establishment) significantly improved crop yield while ameliorating the negative impact of cultivation on soil C stocks. However, rototilling may have increased the probability of soil erosion and promoted indigenous soil organic matter decomposition. Incrementally incorporating biochar between crop rows via chisel plow lowered the risk of soil erosion and increased C sequestration compared with rototilling and would certainly be necessary if biochar availability did not permit adding the full amount in year 1. Nevertheless, the incremental application between crop rows did not have a positive effect on switchgrass yield, and the chisel plow posed some risk to the root systems unless great care was taken. We suggest, therefore, that the method of application be determined by the relative importance of crop yield, C sequestration, soil erodibility, biochar availability and ease of application.

When biochar is not thoroughly mixed with soil but remains concentrated, such as it was in the inter-row positions of the BC-CP plots, it is not as likely to improve the physical characteristics of either heavy- or light-textured soils as biochar that is thoroughly mixed with soil. When concentrated, therefore, biochar may not be able to increase crop yield when it is limited by unfavorable soil physical characteristics. Concentrated biochar may even have significant, negative impacts such as we observed for root growth. However, we found that when biochar was mixed evenly into the soil by rototilling, it increased crop yield without having significant negative impacts on the earthworm population, the colonization of roots by mycorrhizal fungi, soil fungal or bacterial community structure, or soil enzyme activities.

We conclude, therefore, that incorporation of 10 t biochar ha^{-1} may be part of an effective strategy for switchgrass production on certain economically marginal lands in PA, USA. However, the general considerations concerning biochar application methods and their impacts on soil physical properties, crop yield, soil C stocks, and soil organisms are applicable elsewhere.

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