DOI: https://doi.org/10.24297/jaa.v10i0.8035

Impact of Biochar and Different Nitrogen Sources on Forage Radish Production in Middle Tennessee

Todd Pirtle[a], Lee Rumble[b], Michael Klug[a], Forbes Walker[c], Song Cui[a], Nate Phillips[a*],

[a]School of Agriculture, Middle Tennessee State University, Murfreesboro, TN 37132, USA,

[b]Department of Biology, Middle Tennessee State University, Murfreesboro, TN 37132, USA

[c]Department of Biosystems Engineering and Soil Science, University of Tennessee, Knoxville, TN 37996, USA,

nate.phillips@mtsu.edu

Abstract

Short-season forage radish (*Raphanus sativus* L. var. longipinnatus) has recently gained great popularity in Middle Tennessee and many parts of the world used as a high-quality vegetable crop for human consumption or a forage crop for winter grazing and cover cropping. In this study, we (i) estimated soil pH buffering capacity and microbial activity, (ii) quantified crop productivity influenced by different biochar amendment rates and N fertilizer management practices based on a factorial treatment design. Particularly, biochar was amended at rates of 0, 5, 20, and 40 Mg/ha; N fertilizer was applied at zero (N0), 122 kg/ha of urea (56 kg/ha of N; N1) and 4.8 Mg/ha of aged dairy cattle manure (56-60 kg/ha of N), providing a total of 12 treatments (four biochar rates × three fertilization practices). The combination of biochar and inorganic N fertilizer such as urea appeared to have positive impacts on the short-term biomass production, soil pH buffering capacity, and enhanced soil microbial activity for short-season forage radish production ($P < 0.05$). Future research is warranted to evaluate the use of biochar in field-based forage/vegetable studies in Tennessee.

Keywords: Biochar; crop productivity; manure; soil respiration; fertilization; forage radish

Introduction

Despite the small total area of farmlands, Tennessee is one of the leading states for vegetable, forage, and beef cattle production in the nation (USDA-NASS, 2016). Located in the climatic transition zone, middle Tennessee has excellent weather for vegetable and forage production, including those in the *Brassicaceae* family which have become very popular in the region over the past decade. Particularly, short-season forage radish (*Raphanus sativus* L. var. *longipinnatus*) has recently gained great popularity in the state and many parts of the world used as a high-quality vegetable crop for human consumption or a forage crop for winter grazing and cover cropping (White and Weil, 2011). As a brassica species, forage radish features deep fleshy taproots, slow nutritive value decline, high tissue P concentration, excellent cold tolerance, rapid biomass production in the fall, and high nitrate/ammonium scavenging ability to reduce N losses such as volatilization and leaching (Chen et al., 2007; Dean and Weil, 2009; Weil et al., 2009; Wang et al., 2010; White and Weil, 2011; Gieske et al., 2016). Additionally, it has been reported that forage radish possesses great weeds suppression capacity through fall-season cover cropping induced interspecific competition (Lawley et al., 2011, 2012). Forage radish is typically sensitive to soil physical property, moisture content, and N deficiency (Dean and Weil, 2009; Weil et al., 2009). However, the soil type in the state of Tennessee is largely diverse due to the complexity of topography and hydrological conditions due to the abundance of rivers and lakes. Additionally, the state soil is often acidic because of ample precipitation, which can greatly affect N availability for radish production. Therefore, better soil remediation and/or improvement practices are warranted, such as incorporation of biochar and organic N sources.

Biochar is the product of the thermal degradation process of organic materials in the absence of air called pyrolysis, and is distinguished from charcoal by its primary use as a soil amendment (Lehmann et al., 2011).

Application of biochar has been proposed as a novel approach to increase soil C sequestration and reduce greenhouse gas emission (Lehmann, 2007). In addition to reduced carbon footprint, many soil physical and biochemical processes could also be affected by biochar amendment (Petersen et al., 2001; Liang et al., 2006; Atkinson et al., 2010; Laird et al., 2010a; Chen et al., 2011; Schmidt et al., 2014). There is also strong evidence that biochar amendment can significantly alter soil nutrient dynamics (Anderson et al., 2011; Dempster et al., 2012; Hossain et al., 2011; Laird et al., 2010b; Lehmann, 2003; Chimayo et al., 2010), soil microbial community/population and activities (Anderson et al., 2011; Castaldi et al., 2011; Jindo et al., 2012; Steinbeiss et al., 2009; Zhang et al., 2010). In general, the content and stability of biochar-induced soil organic C are known as very important factors for maintaining agronomic productivity and soil functionality (Lal, 2004; Liu et al., 2013; Pan et al., 2009) and have been investigated in details by many scientists. However, information relating to using biochar as soil amendment for improving brassica species production, such as short-season forage radish, in the transitional climate condition remains limited. We expect this kind of information could be of great importance because there is a growing interest in adopting brassica-incorporated cover cropping practices in this region.

The overall purpose of this research was to assess the efficacy and effectiveness of using biochar as a soil amendment for radish (*Raphanus sativus* L.) production by evaluating soil chemical and biological properties as well as plant production affected by different rates of biochar amendment and fertilization regimes in the middle Tennessee region. Specifically, based on a factorial treatment arrangement; we (i) estimated soil pH buffering capacity and microbial activity, (ii) quantified crop productivity influenced by the combinations of four different biochar amendment rates and three fertilization practices, including both organic and inorganic N sources.

Materials and methods

Experiments were conducted on an outdoor testing bench of the Middle Tennessee State University Greenhouse Unit, Murfreesboro, Tennessee from early October to mid-November in 2015 (48-day fall growing season) and repeated during similar growing season in 2016 (56-day production). All plants were manually irrigated using a watering can every two days throughout the growing season. Outdoor weather conditions were continuously monitored using a scientific weather station system consisting of an HMP60 probe (Campbell Scientific, Logan, UT 84321) for measuring relative humidity and air temperature, a 014A anemometer (Met One Instrument, Grants Pass, OR 97526) for wind speed, an LI190SB quantum sensor (Li-Cor Biosciences, Lincoln, NE 68504) for photosynthetic active radiation (PAR), an LI200S pyranometer (Li-Cor Biosciences, Lincoln, NE 68504) for global irradiance, an NR-LITE2 net radiometer (Kipp & Zonen, Bohemia, NY 11716) for net radiation, and a TE525 tipping bucket rain gauge (Texas Electronics, Dallas, TX 75237) for daily precipitation.

An early-season brassica radish cultivar 'Daikon' was used for this study. Five to six seeds were planted 1-cm deep in 15-cm diameter plastic pots (volume = 1800 cm^3) kept on an outdoor testing bench. Four plants per pot were maintained throughout the growing season and additional plants in each pot were removed after germination and before the beginning of data collection. The growth medium consisted of an Armour silt loam soil (2 to 5 percent slope) collected from the Middle Tennessee State University Farm Research Laboratory, Lascassas, TN during each year. Soil samples were analyzed by the University of Tennessee Soil, Plant and Pest Center verifying that no nutrients other than N were at "low" status. Particularly, a TL-2800 Ammonia Analyzer (Timberline Instrument Inc. Boulder, CO 80301) was used to measure ammonia and nitrate in soil and a "low" status indicates N concentration is below 20 ppm in the extraction solution. This 2-yr experiment was a Completely Randomized Design (CRD) with factorial arrangement and three replications. The treatments consisted of the combinations of four biochar amendment rates and three N fertilizer management practices. Biochar was amended at rates of 0, 5, 20, and 40 Mg/ha. Fertilizers were applied at zero (N0), 122 kg/ha of urea (56 kg/ha of N; N1) and 4.8 Mg/ha of aged dairy cattle manure (56-60 kg/ha of N). This level of N was recommended by local Extension scientists and producers. We used Wakefield Biochar Soil Conditioner (Wakefield Biochar, Columbia, MO 65203), which is primarily made of pine tree ashes. Dairy cattle manure was collected from the Middle Tennessee State University Dairy Center, Lascassas, TN. Manure samples were analyzed by the University of Arkansas Agricultural Diagnostic Laboratory for standard nutrient concentration, indicating an average concentration of 2.7, 0.8, 2.8 % of N, P, and K, respectively. Both biochar amendments and N sources were applied at planting.

Plant height was measured for each plant on a weekly basis using a ruler. Foliar chlorophyll concentration was estimated weekly from four most representative leaves from each plant using a SPAD meter (Spectrum Technologies, Inc., Aurora, IL 60504). Photosynthetic intensity and stomatal conductance were measured once each year during the middle of the growing season (20 days after planting) from four most representative leaves using the Li-Cor LI6400XT portable photosynthesis system (Licor, Inc., Lincoln, NE 68504). Particularly, we performed standard checking of flow rate, CO_2 and H_2O scrubbing efficiency, as well as mixer and lamp calibration before making the photosynthetic and soil respiration measurements using the LI6400XT. Additionally, a one-time matching was also performed to ensure the photosynthetic activity was at zero when there were no differences between the reference and sample cell CO_2 concentration. Light intensity, flow rate, and reference CO_2 mixer rate were set at 1000 µmol /m^{2}s, 500 µmol/s and 380 µmol/mol, respectively, to simulate natural growing conditions of middle Tennessee. Plant biomass (both above and below-ground biomass) was measured at the end of the growing season by gently digging out the entire radish plant with minimum disturbance of soil surface and clipping each plant at the crown region to separate the above and below-ground portions. All fresh biomass samples were immediately placed in paper bags and dried at 60°C using a drying oven till constant weight. Total biomass was calculated by adding both above and below-ground biomasses. Soil microbial respiration was measured for each pot immediately after the removal of all plants using the Licor 6400-09 Soil CO_2 Flux Chamber (Li-Cor Biosciences, Lincoln, NE 68504), which can be directly attached to the LI-6400XT sensor head. A target value of 360 ppm, a delta value of 20 ppm, and a cycle number of 3 was selected for making soil respiration measurement. Meanwhile, soil temperatures were also recorded by a Licor 6000-09TC soil temperature probe (Li-Cor Biosciences, Lincoln, NE 68504) to ensure no significant temperature differences were found across different pots which could significantly affect soil microbial activity. A one-time soil pH measurement was performed at the end of each experiment using a Fieldscout SoilStik pH Meter (Spectrum Technologies, Inc., Aurora, IL 60504).

Statistical analysis was performed using the MIXED Procedure in SAS release 9.2 (SAS Institute, 2003). The entire dataset was analyzed as a CRD with factorial treatment arrangements and repeated measures. Particularly, main treatment effects included fertilization source and biochar amendment rate. Thus, if no two-way interactions were detected ($P \geq 0.05$), only main treatment effects were evaluated. However, if both main factors interacted with each other ($P < 0.05$), results will be evaluated according to both main factors as well as different fertilizer source and biochar rate combinations (three fertilization sources × four biochar amendment rates = 12). Year effect was included in the model as fixed effect to account for the potential treatment × year interactions caused by differences in weather conditions and growing season. Data recorded multiple times within the same year (e.g. plant height and SPAD) was analyzed as repeated measures because multiple measurements of response variables were collected from the same experimental unit over time. The "REPEATED" statement was used in SAS to control for autocorrelation of observations over time. The first-order autoregressive AR (1) covariance matrix was selected as the covariance structure. Means of different variables were separated using the PDMIX800 macro, which is capable of grouping means based on pair-wise comparisons (Saxton, 1998).

Results

The air temperature at the study site ranged from 4.8 to 20.6 °C and averaged at 14.2 °C during the first experimental period in 2015. The average daily net radiation intensity was 47.6 W m^2. The average reference evapotranspiration was 1.6 mm/d. Cumulative precipitation was 63.7 mm. The average air temperature, net radiation, and reference evapotranspiration during the second experimental period in 2016 were 20.2 °C, 83.1 W m^2, and 3.8 mm/d, respectively. Total rainfall received was 29 mm. The dramatic weather differences in these two years resulted in significant amount of interactions with years (year × biochar treatment, fertilizer sources, sampling date, and etc), thus, data were reported by years. Statistical significance was evaluated at P = 0.1, 0.05, and 0.001 level. Detailed results from statistical analysis were summarized in Table 1. Interestingly, no statistical significances were detected from photosynthetic intensity data or stomatal conductance data (Table 1), therefore, mean separation was not conducted (the average photosynthetic intensity was 13.13 with SE = 3.91, and 18.47 with SE = 2.43 μmol CO_2 $m^{-2}s^{-1}$ for yr 1 and 2, respectively; stomatal conductance was 0.57 with SE = 0.03 and 0.46 with SE = 0.03 mol H_2O $m^{-2}s^{-2}$ for yr 1 and 2, respectively). Additionally, no soil temperature differences were found when soil respiration intensity was measured in each year (biochar effect P = 0.9995, fertilization P = 1.0, two-way interaction P = 1.000), ensuring no interference was induced by soil temperature differences on soil respiration. Furthermore, neither below-ground biomass nor total biomass production indicated significant responses to our treatments (Table 1), thus, no means separation was conducted (the average below-ground biomass was 1.11 with SE = 0.57 and 1.38 with SE = 0.89 kg ha^{-1} for yr 1 and 2, respectively; total biomass was 1.79 with SE = 0.59 and 2.17 with SE = 1.47 kg ha^{-1} for yr 1 and 2, respectively.

Table 1. Statistical significance results (F and P values) of plant height, SPAD reading, soil pH, soil respiration intensity, above-ground (AG) biomass, below-ground (BG) biomass, total biomass, photosynthetic intensity (Pho intensity), and stomatal conductance (Stomatal Cond) affected by different biochar amendment rates (B), fertilization sources (F), sampling date (D), and their interactions in a 2yr study (2015 and 2016) in Murfreesboro, TN.

Year 1	B		F		B × F		D		D × B		D × F		D × B × F	
Effect	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Plant height	5.11	0.006**	281.2	<0.0001***	32.78	<0.0001***	181.95	<0.0001***	3.03	0.0048**	21.35	<0.0001***	2.24	0.0135**
SPAD	2.07	0.2376	16.99	0.0087**	1.66	0.3146	0.29	0.6010	0.74	0.5557	0.44	0.6567	0.77	0.6145
Soil pH	12.24	0.0657*	2.91	0.2428	0.54	0.7628								
Soil respiration	23.42	0.0392**	1.89	0.3436	2.12	0.3508								
AG biomass	15.88	0.0283**	198.58	0.0009***	9.7	0.0514*								
BG biomass	0.31	0.8561	0.29	0.7812	0.21	0.9531								
Total biomass	0.32	0.8267	2.52	0.4067	0.24	0.9103								
Pho intensity	2.88	0.4031	2.08	0.4404	0.13	0.9674								
Stomatal Cond	2.21	0.4510	0.49	0.7118	0.46	0.8076								
Year 2	B		F		B × F		D		D × B		D × F		D × B × F	
Effect	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Plant height	3.51	0.032**	137.4	<0.0001***	4.64	0.0035**	260.74	<0.0001***	1.21	0.3167	5.5	<0.0001***	0.63	0.8941

SPAD	41.74	<0.0001***	31.68	<0.0001***	4.62	0.0015**	14.24	<0.0001***	1.37	0.2034	1.65	0.1231	0.91	0.5999
Soil pH	21.95	0.0558*	3.24	0.2156	0.89	0.9861								
Soil respiration	3.92	0.0797*	5.8	0.0996*	2.83	0.3786								
AG biomass	0.07	0.9677	0.07	0.9389	0.06	0.9947								
BG biomass	0.25	0.5984	0.34	0.4698	0.18	0.7991								
Total biomass	0.28	0.8464	0.24	0.8228	0.18	0.9455								
Pho intensity	0.18	0.8995	1.94	0.4523	0.44	0.8155								
Stomatal Cond	2.41	0.5671	0.52	0.7480	0.67	0.7125								

*significant at the 0.1 probability level.

**significant at the 0.05 probability level.

***significant at the 0.001 probability level.

Above-ground biomass was significantly affected by different fertilization practices and biochar amendment levels in yr 1 (Table 1; Fig. 1). However, no two-way interaction was detected between two main factors (Table 1), therefore, only main effects were evaluated. During yr 1, medium-level biochar amendment increased above-ground biomass production compared to either no-biochar control or the high-rate treatments. Additionally, fertilization of urea increased more above-ground growth compared to control and manure treatments. No effects were detected in yr 2. Plant height indicated good responses to biochar and fertilization across both years (Fig. 2). In yr 1, low and medium biochar amendment levels increased plant height compared to high-level. Fertilization of urea indicated the greatest plant height responses compared to manure or control, and manure treatment increased plant height compared to control. In yr 2, low-level biochar treatment increased plant height more than the high-level treatment. Urea increased plant height more than control, and manure increased plant height compared with either control or urea fertilizer. Due to the existence of the two-way interaction, we further analyzed the combinational effects of both factors (Table 2). In yr 1, zero, low, and medium rates of biochar plus urea resulted in the greatest plant height. Interestingly, zero, low, and medium rates of biochar combined with manure increased the greatest height growth than other treatments in yr 2. Across both years, plants without N inputs indicated least height growth generally across all biochar treatments. The treatment effects on soil respiration were only significant in yr 1, indicated by the increased respirational intensity of medium-level biochar treatment compared with no-biochar control (Fig. 3). The foliar SPAD values agreed well with different treatments (Fig. 4). In yr 1 and 2, urea fertilizer increased SPAD readings compared to manure or no-N control. In yr 2, both low-level biochar treatment and no-biochar control had greater SPAD values than medium and high biochar treatments. Again, due to the existence of two-way interaction between two main factors in yr 2, means were separated according to different biochar amendment and fertilization combinations (Table 3).

Particularly, zero and low biochar rates with urea fertilization generally provided the greatest foliar SPAD values. Additionally, medium and high biochar rates with manure or without any N inputs tended to provide lower SPAD values. In our study, soils treated with high-rate biochar amendment had higher soil pH values than no-biochar control across two different years (Fig. 5), which agrees well with the assumption. Fertilization appeared to have no effect on soil pH values. Altogether, medium-level biochar amendments with low N fertilization rate indicated the greatest above-ground biomass yield (1206 kg/ha). Biochar treatments can help retain higher soil pH levels and soil microbial activities particularly at those low N fertilization rates. Urea fertilizer provided the greatest effect on leaf chlorophyll concentration measured as SPAD values.

Table 2. Mean separation results of plant height (cm) affected by different biochar amendment rates (Control, 0 Mg/ha; Low, 5 Mg/ha; Medium, 20 Mg/ha; High, 40 Mg/ha) and fertilization sources (Control, 0 kg N/ha; Urea, 56 kg N/ha; Manure, 56-60 kg N/ha) combinations. Letters separate means based on P < 0.05 level by pair-wise comparison. The greatest and the least means are bolded.

Year 1	Biochar Rates				
		Control	Low	Medium	High
Fertilization	Control	$12.62^{e,f}$	11.30^{f}	$14.39^{c,d}$	$12.76^{e,f}$
Sources	Urea	18.77^{a}	19.04^{a}	18.03^{a}	$15.02^{b,c,d}$
	Manure	$13.95^{d,e}$	$15.56^{b,c}$	$13.93^{d,e}$	15.89^{b}
Year 2					
Fertilization	Control	8.39^{g}	$9.81^{e,f,g}$	$9.11^{f,g}$	$9.14^{f,g}$
Sources	Urea	$12.02^{c,d,e}$	$13.54^{b,c,d}$	$11.20^{d,e,f}$	12.20^{d}
	Manure	16.09^{a}	$14.51^{a,b,c}$	$14.87^{a,b}$	$13.09^{b,c,d}$

Table 3. Mean separation results of foliar SPAD values affected by different biochar amendment rates (Control, 0 Mg/ha; Low, 5 Mg/ha; Medium, 20 Mg/ha; High, 40 Mg/ha) and fertilization sources (Control, 0 kg N/ha; Urea, 56 kg N/ha; Manure, 56-60 kg N/ha) combinations in 2016. Letters separate means based on P < 0.05 level by pair-wise comparison. The greatest and the least means are bolded.

Year 1	Biochar Rates				
		Control	Low	Medium	High
Fertilization	Control	$33.04^{b,c}$	$30.80^{c,d}$	$29.12^{d,e}$	$29.60^{d,e}$
Sources	Urea	Urea	37.12^{a}	$35.54^{a,b}$	$30.53^{c,d}$
	Manure	$31.52^{c,d}$	$32.12^{c,d}$	26.86^{e}	$29.63^{d,e}$

Fig. 1.

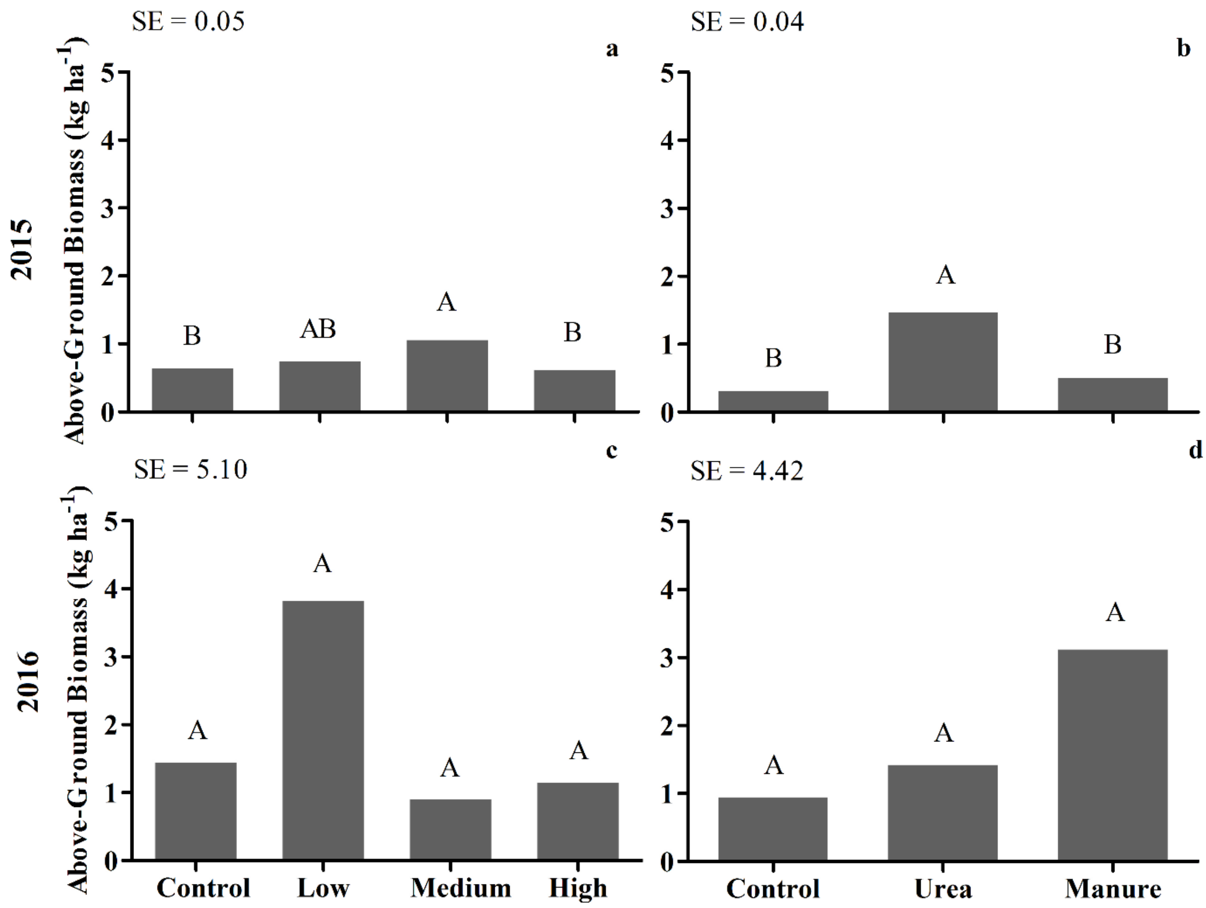

Fig. 1. Above-ground biomass production of an early-season radish variety affected by various levels of biochar amendments (a & c; Control, 0 Mg/ha; Low, 5 Mg/ha; Medium, 20 Mg/ha; High, 40 Mg/ha) and different N sources (b & d; Control, 0 kg N/ha; Urea, 56 kg N/ha; Manure, 56-60 kg N/ha) in middle Tennessee during 2015 and 2016. Letters separate means based on P < 0.05 level by pair-wise comparison.

Fig. 2.

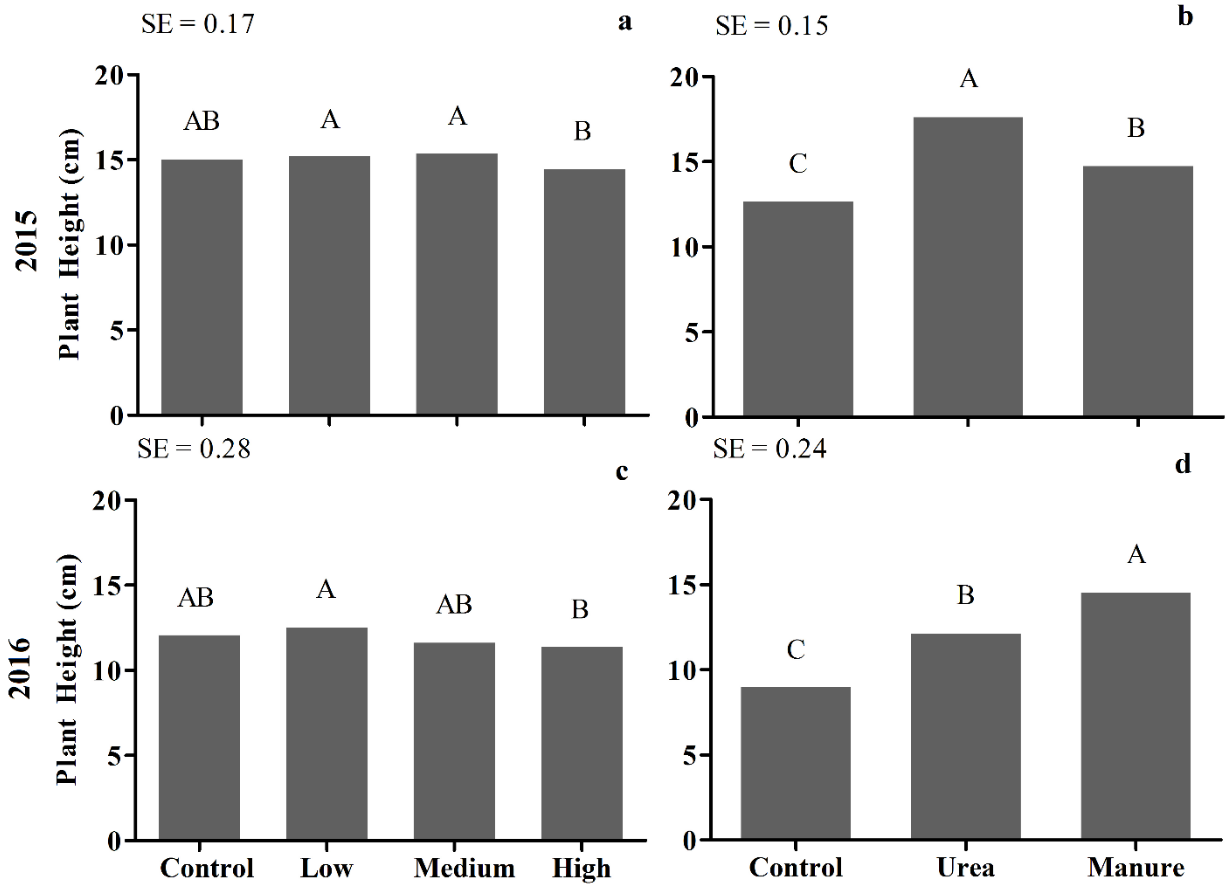

Fig. 2. Plant height of an early-season radish variety affected by various levels of biochar amendments (a & c; Control, 0 Mg/ha; Low, 5 Mg/ha; Medium, 20 Mg/ha; High, 40 Mg/ha) and different N sources (b & d; Control, 0 kg N/ha; Urea, 56 kg N/ha; Manure, 56-60kg N/ha) in middle Tennessee during 2015 and 2016. Letters separate means based on $P < 0.05$ level by pair-wise comparison.

Fig. 3.

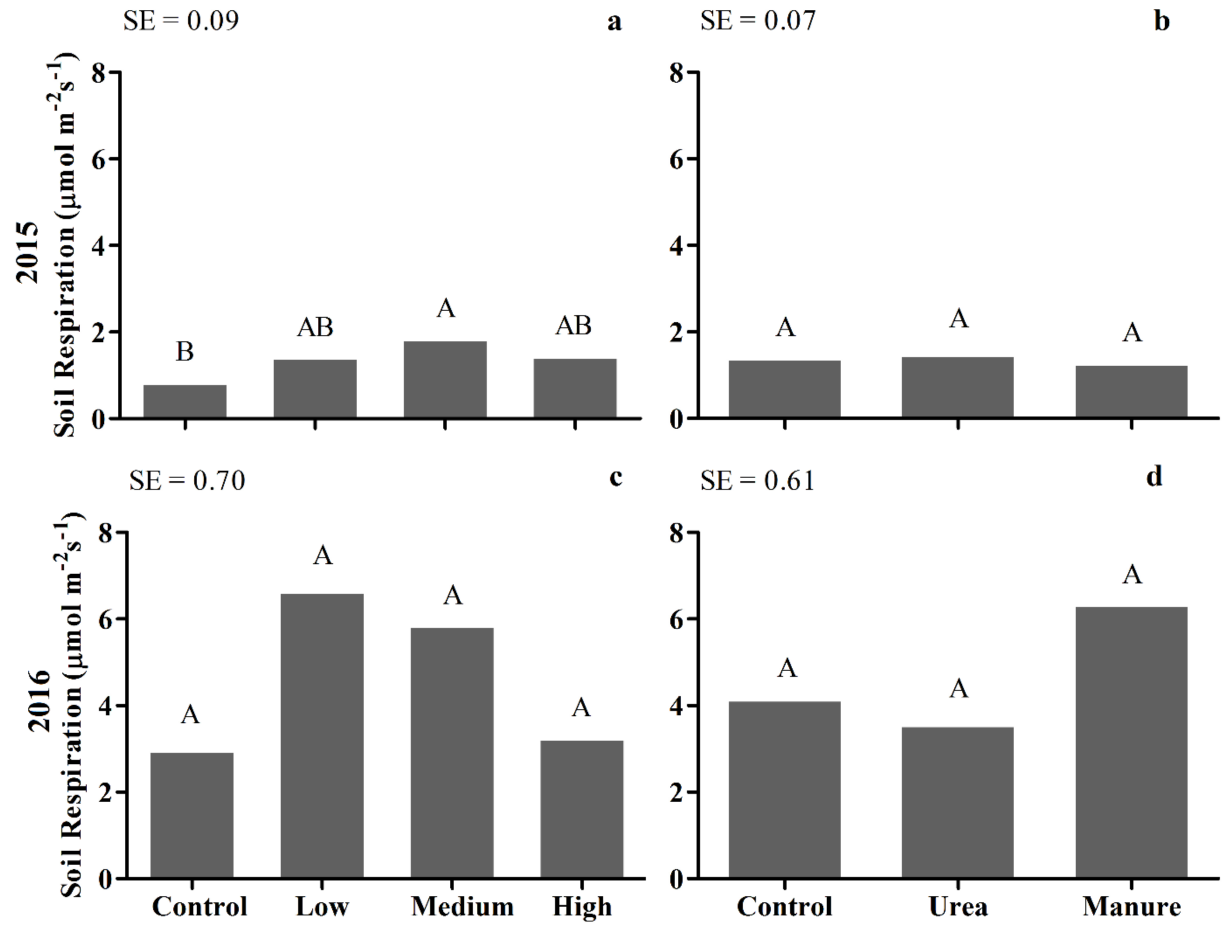

Fig. 3. Microbial respiration intensity of soils under an early-season radish variety affected by various levels of biochar amendments (a & c; Control, 0 Mg/ha; Low, 5 Mg/ha; Medium, 20 Mg/ha; High, 40 Mg/ha) and different N sources (b & d; Control, 0 kg N/ha; Urea, 56 kg N/ha; Manure, 56-60kg N/ha) in middle Tennessee during 2015 and 2016. Letters separate means based on $P < 0.05$ level by pair-wise comparison.

Fig. 4.

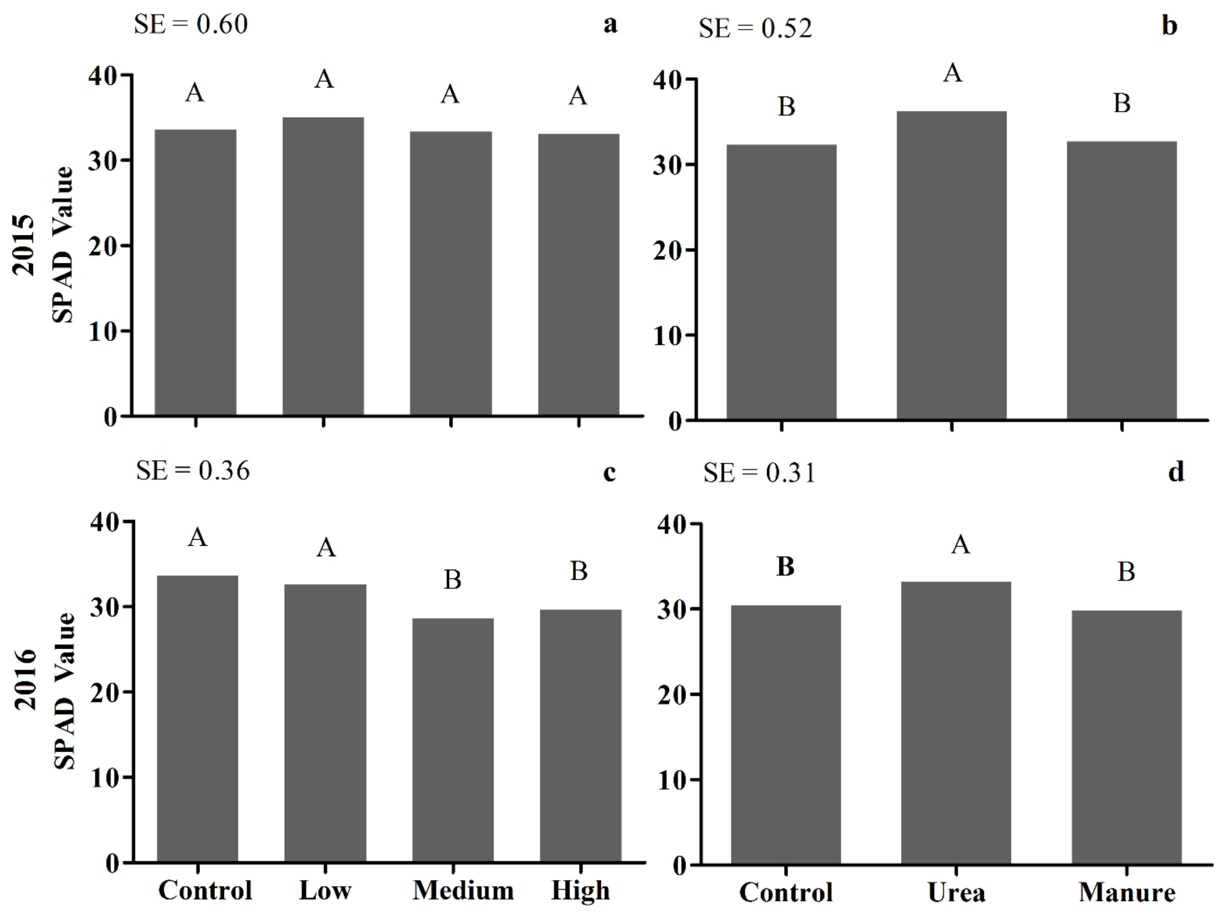

Fig. 4. Foliar SPAD values of an early-season radish variety affected by various levels of biochar amendments (a & c; Control, 0 Mg/ha; Low, 5 Mg/ha; Medium, 20 Mg/ha; High, 40 Mg/ha) and different N sources (b & d; Control, 0 kg N/ha; Urea, 56 kg N/ha; Manure, 56-60kg N/ha) in middle Tennessee during 2015 and 2016. Letters separate means based on $P < 0.05$ level by pair-wise comparison.

Fig. 5.

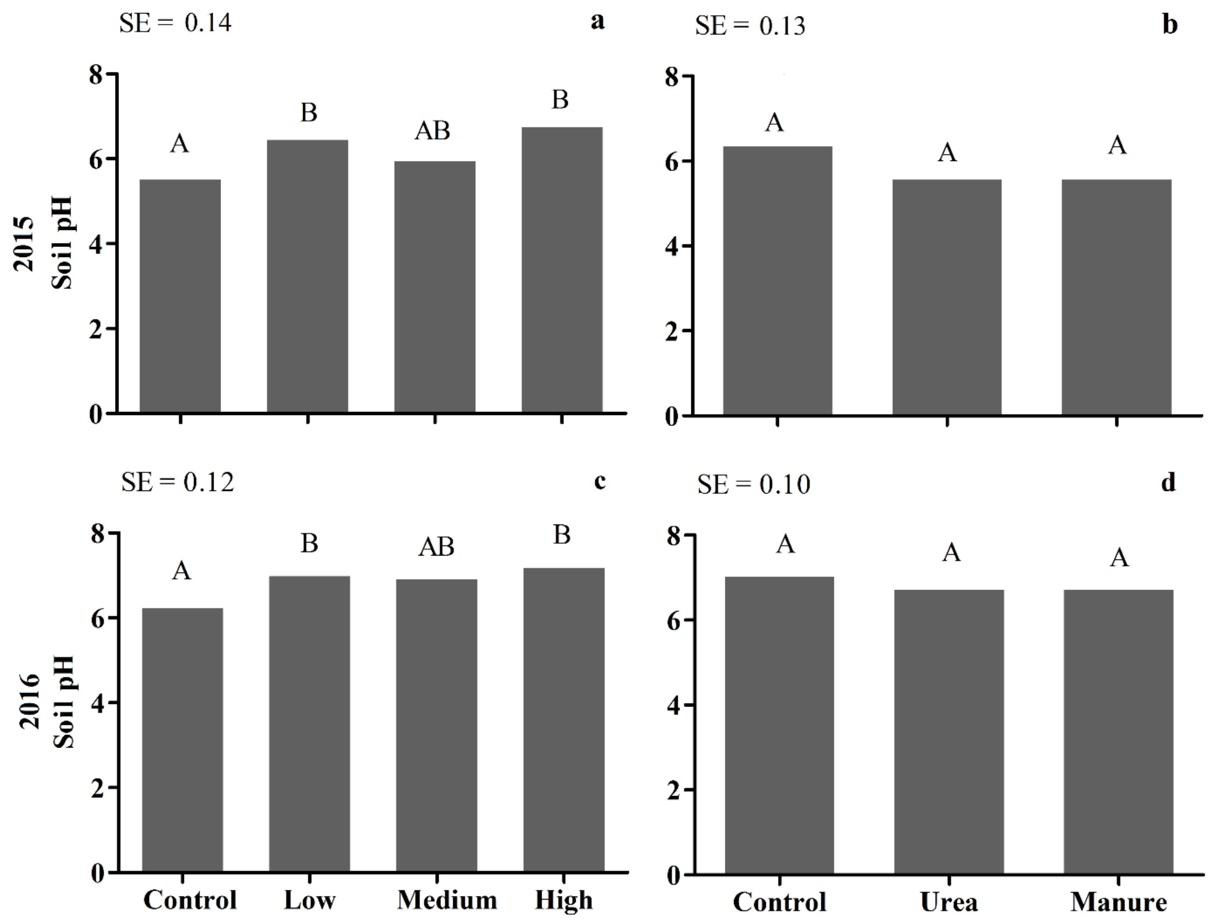

Fig. 5. Soil pH values of soils under an early-season radish variety affected by various levels of biochar amendments (a & c; Control, 0 Mg/ha; Low, 5 Mg/ha; Medium, 20 Mg/ha; High, 40 Mg/ha) and different N sources (b & d; Control, 0 kg N/ha; Urea, 56 kg N/ha; Manure, 56-60kg N/ha) in middle Tennessee during 2015 and 2016. Letters separate means based on $P < 0.1$ level by pair-wise comparison.

Discussion

Many studies have found that adding biochar to soils could increase crop productivity (Major et al., 2010; Jeffery et al., 2011; Vaccari et al., 2011). Additionally, the majority of these studies focused on the crops that are primarily harvested for their above-ground portion (e.g. stem/leaf biomass or grain yield), including rice (*Oryza sativa* L., Asai et al., 2009), sugarcane (*Saccharum officinarum* L., Chen et al., 2010), tomato (*Lycopersicon esculentum* L., Hossain et al., 2010), beans (*Phaseolus vulgaris* L., Rondon et al., 2007), etc. For example, Major et al. (2010) reported increased maize (*Zea mays* L.) grain yield from high-level (20 Mg/ha) biochar amendment starting from the second year in a four-year cropping system study. In another study, Vaccari et al. (2011) found that biochar application increased up to 30% on biomass production and grain yield of durum wheat (*Triticum aestivum* L.). Likewise, a meta-analysis study conducted in 2011 indicated that biochar could lead to an average increase of 10% on crop above-ground productivity nationwide (Jeffery et al., 2011). These findings agreed with our finding, which indicated increased above-ground biomass from medium-level biochar amendment treatments. Furthermore, only a few studies have investigated below-ground biomass production such as root biomass and root/shoot ratio. Lentz et al. (2014) showed that biochar had no/negative effects on maize root biomass when added alone, which was similar to our research findings (Table 1). Interestingly, the combination of additions of biochar and manure significantly increased root biomass production. Likewise, an early review indicated that crop yield could be enhanced if charcoal amendments are applied with fertilizers (Glaser et al., 2002). Steiner et al. (2007) found that the combined application of charcoal and inorganic fertilizer to soil significantly improved plant growth and doubled grain production compared with soil receiving fertilizer only. In the long run, crop biomass production should be enhanced through increased fertility and nutrient status of soil as reported by other studies (Igalavithana et al., 2016). We attributed the lack of similar responses (enhanced effects of adding both biochar and N inputs) in our research primarily to the short duration of this study. Additionally, the enhanced N immobilization activities within the first few months following the application of high-carbon-content biochar is a typical effect of biochar on altering soil N dynamics (Gundale and DeLuca, 2007; Deenik et al., 2008; Sarkhot et al., 2012). These findings emphasize the importance of conducting long-term field studies in the future.

Plant height provides key indications on plant growth and nutrient status particularly during the vegetative growth stages of various agronomic crops (Katsvairo et al., 2003; Yin et al., 2011). The ability to measure plant height in various easy and nondestructive manners makes it one of the most popular methods for early-stage yield prediction and nutrient status evaluation. Therefore, we used height measurements to monitor the growth of radishes affected by additions of biochar and different N sources. Obviously, plant height responded greatly to various N sources but less obvious to different biochar levels. Particularly, the slightly decreased height from medium and/or high biochar levels did not contradict with the above-ground biomass results as increased number of leaves and total leaf area were observed from these treatments in our study (data not shown). The interactive effects of both biochar and fertilization in yr 1 indicated that low-level biochar application and urea fertilization could provide superior plant height increase particularly during a wet year, likely due to limited volatilization. However, prolonged drought during the growing season should greatly favor organic N inputs (e.g. manure) with low-rate biochar application as indicated in yr 2.

In general, the effects of biochar addition on soil biological property in the temperate region is not well understood (Lentz et al., 2014). Soil heterotrophic respiration can provide a general indication of soil biological activity affected by different management practices. However, soil respiration responses measured as carbon fluxes (CO_2 flux) could be complicated by the complexity of soil organic carbon stocks. Additionally, the effect of biochar application on soil respiration could be affected by many factors, including soil types, soil pH, and property of biochar related to pyrolysis conditions and production procedures (Paz-Ferreiro et al., 2016), leading to a wide range of responses from different studies. For example, some studies indicated that biochar addition could suppress CO_2 flux on a cropping system basis (Spokas and Reicosky, 2009; Sarkhot et al., 2012; Lentz et al., 2014). Some studies found similar (Zavalloni et al., 2011) or even increased soil respiration rate (Smith et al., 2010) compared with no-biochar control. In our research, soils amended with biochar provided slightly greater CO_2 emission compared with no-biochar control. We expected this response was primarily caused by the fact that the Wakefield Biochar Soil Conditioner, primarily made of pine tree ashes under low-temperature pyrolysis, contains high concentration of labile carbon that could be easily used by soil microorganisms as substrates for generating energy and releasing CO_2.

The SPAD (Soil Plant Analysis Development) meter, which was originally designed in the 1990s for *in vivo* Chl measurements, and had indicated strong correlation between N inputs and leaf chlorophyll concentration; was therefore widely used in many agronomic studies for estimating crop N status (Hussain et al., 2000; Singh et al., 2002). Again, due to the increased N immobilization caused by biochar addition (the majority of biochar typically has high C: N ratio), both medium and high rates of biochar treatments decreased immediate-plant available N, leading to lower SPAD readings. Similarly, Asai et al. (2009) also reported reduced leaf SPAD values of rice caused by biochar application. This biochar-induced N immobilization process can be very active during the third or fourth month following application (Ippolito et al., 2014). Biochar has been well recognized for its buffering capacity against soil pH change, thus, plays important roles in improving soil nutrient status. For example, Chan et al., (2007) observed various improvements in soil chemical properties under biochar amendments, including increased pH buffering capacity and cation exchange capacity. Particularly, the liming effect of biochar on acidic soils such as the soils in Tennessee could significantly increase the availability of alkaline cations and phosphorus for crop production. This effect was observed in our study indicated by increased soil pH of biochar treatments.

Taken altogether, biochar application has been recognized as a good strategy for enhancing soil carbon sequestration, improving soil quality, and increasing agronomic productivity (Zheng et al., 2016). Integrating biochar application with addition of organic and/or inorganic N sources (such as chemical fertilizers or compost) has indicated positive effect on crop productivity and nutrient status (Otterpohl, 2012; Schmidt et al., 2014; Wilson, 2014). This study has indicated various benefits induced by biochar application and suggested that the observed crop production effects could be mainly caused by ameliorated soil acidity status and increased soil microbial activities.

Conclusion

The combination of biochar and inorganic N fertilizer such as urea appeared to have positive impacts on the short-term biomass production, soil pH buffering capacity, and enhanced soil microbial activity for short-season forage radish production in Middle Tennessee, particularly in a year with ample precipitation. However, organic N inputs such as manure should be considered if prolonged drought conditions were anticipated. The long-term effects of using biochar on the productivity and quality of brassica and other vegetable species production still need further investigation (Glaser et al., 2002; Sohi et al., 2010). Specifically, we expect the short-term biochar-induced N immobilization effect will be weakened and the cumulative net N mineralization will dominate in the long run (> three years), leading to enhanced biomass production and foliar chlorophyll/protein content. With the continual population increase, more frequent incidences of extreme weather, exacerbating soil degradation and erosion, and rising carbon footprint from agronomic production; adopting climate-smart and sustainable agricultural management practices becomes more and more important. Investigating the effects of using biochar as a soil amendment for short-season radish production (a very versatile crop) could provide important insights for forage, row crop, and vegetable growers in the southeastern region. Future research is warranted to evaluate the incorporation of biochar into cover cropping and vegetable cropping systems in Tennessee.

Acknowledgements

This project is supported by USDA National Institute of Food and Agriculture Grant Number: 2015-70001-23418, 2015-70001-24636 and 2015-68007-23212. We also would like to acknowledge James C. Kathilankal (Application Scientist, Li-Cor Inc.) for providing technical support for the data acquisition using the LI6400XT instrument.

References

1. Anderson C., Condron L., Clough T., Fiers M., Stewart A., Hill R., Sherlock R. (2011): Biochar induced soil microbial community change: Implications for biogeochemical cycling of carbon, nitrogen and phosphorus. Pedobiologia 54: 309–320.

2. Asai H., Samson B., Stephan H., Songyikhangsuthor K., Homma K., Kiyono Y., Inoue Y., Shiraiwa T., Horie T. (2009): Biochar amendment techniques for upland rice production in Northern Laos: 1. Soil physical properties, leaf SPAD and grain yield. Field Crops Res. 111: 81–84.

3. Atkinson C., Fitzgerald J., Hipps N. (2010): Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review. Plant Soil 337: 1–18.

4. Castaldi S., Riondino M., Baronti S., Esposito F., Marzaioli R., Rutigliano F., Vaccari F., Miglietta F. (2011): Impact of biochar application to a Mediterranean wheat crop on soil microbial activity and greenhouse gas fluxes. Chemosphere 85: 1464–1471.

5. Chan K., Downie A., Joseph S., Meszaros I., Van Zwieten L. (2007): Agronomic values of greenwaste biochar as a soil amendment. Australian J. of Soil Res. 45 (8): 629–634.

6. Chen G., Clark A., Kremen A., Lawley Y., Price A., Stocking L., Weil R. (2007): Brassicas and mustards. In: A. Clark, editor, Managing cover crops profitably. 3rd ed. Sustainable Agric. Res. and Education, College Park, MD. p. 81–90.

7. Chen Y., Shinogi Y., Taira M. (2010): Influence of biochar use on sugarcane growth, soil parameters, and groundwater quality. Aust. J. Soil Res. 48: 526–530.

8. Chen H., Du Z., Guo W., Zhang Q.Z. (2011): Effects of biochar amendment on farmland soil bulk density, cation exchange capacity, and particulate organic matter content in North China Plain. (in Chinese.) Chinese J. Appl. Ecol. 22: 2930–2934.

9. Dean J., Weil R. (2009): Brassica cover crops for nitrogen retention in the mid-Atlantic coastal plain. J. Environ. Qual. 38: 520–528.

10. Deenik J., Uehara G., Sumiyoshi Y., Sidibe A., McClellan A., Antal M. (2008): Charcoal volatile matter content and its effects on plant growth and biological properties of an infertile tropical soil. Abstract #665-665. Proc. Joint Annual Meeting of GSA, ASA-CSSA-SSSA, and GCAGS 2008.

11. Dempster D., Jones D., Murphy D. (2012): Organic nitrogen mineralisation in two contrasting agro-ecosystems is unchanged by biochar addition. Soil Biol. Biochem. 48: 47–50.

12. Gieske M., Ackroyd V., Baas D., Mutch D., Wyse D., Durgan B. (2016): Brassica cover crops effects on nitrogen availability and oat and corn yield. Agron. J. 108: 151–161.

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

14. Gundale M., DeLuca T. (2007): Charcoal effects on soil solution chemistry and growth of Koeleria macrantha in the ponderosa pine/Douglas-fir ecosystem. Biol. Fertil. Soils 43: 303–311.

15. Hossain M., Strezov V., Chan K., Nelson P. (2010): Agronomic properties of wastewater sludge biochar and bioavailability of metals in production of cherry tomato (Lycopersicon esculentum). Chemosphere 78: 1167–1171.

16. Hossain M., Strezov V, Chan K., Ziolkowski A., Nelson P. (2011): Influence of pyrolysis temperature on production and nutrient properties of wastewater sludge biochar. J. Environ. Manage. 92: 223–228.

17. Hussain F., Bronson K., Yadvinder-Singh, Bijay-Singh, Peng S. (2000): Use of chlorophyll meter sufficiency indices for nitrogen management of irrigated rice in Asia. Agron. J. 92: 875–879.

18. Igalavithana A., Ok Y., Usman A., Al-Wabel M., Oleszczuk P, Lee S. (2016): The Effects of biochar amendment on soil fertility. In: Guo M., He Z., Uchimiya S., editors, Agricultural and environmental applications of biochar: advances and barriers, SSSA Spec. Publ. 63. SSSA, Madison, WI. p. 123–144.

19. Ippolito J., Stromberger M, Lentz R., Dungan R. (2014): Hardwood biochar influences calcareous soil physiochemical and microbiological status. J. Environ. Qual. 43: 681–689.

20. Jeffery S., Verheijen F., van der Velde M., Bastos A. (2011): A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agric. Ecosyst. Environ. 144: 175–187.

21. Jindo K., Sanchez-Monedero M., Hernandez T., Garcia C., Furukawa T., Matsumoto K., Sonoki T., Bastida F. (2012): Biochar influences the microbial community structure during manure composting with agricultural wastes. Sci. Total Environ. 416: 476–481.

22. Katsvairo T., Cox W., Van Es H. (2003): Spatial growth and nitrogen uptake variability of corn at two nitrogen levels. Agron. J. 95: 1000–1011.

23. Laird D., Fleming P., Davis D., Horton R., Wang B., Karlen D. (2010a): Impact of biochar amendments on the quality of a typical midwestern agricultural soil. Geoderma 158: 443–449.

24. Laird D., Fleming P., Wang B., Horton R., Karlen D. (2010b): Biochar impact on nutrient leaching from a midwestern agricultural soil. Geoderma 158: 436–442.

25. Lal R. (2004): Soil carbon Sequestration impacts on global climate change and food security. Science 304: 1623–1627.

26. Law Y., Weil R., Teasdale J. (2011): Forage radish cover crop suppresses winter annual weeds in fall and before corn planting. Agron. J. 103: 137–144.

27. Law Y., Teasdale J., Weil R. (2012): The mechanism for weed suppression by forage radish cover crop. Agron. J. 104: 205–214.

28. Lawley Y., Weil R., Teasdale J. (2011): Forage radish cover crop suppresses winter annual weeds in fall and before corn planting. Agron. J. 103: 137–144.

29. Lawley Y., Teasdale J., Weil R. (2012): The mechanism for weed suppression by a forage radish cover crop. Agron. J. 104: 205–214.

30. Lehmann J. (2003): Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the central Amazon basin: Fertilizer, manure and charcoal amendments. Plant Soil 249: 343–357.

31. Lehmann J. (2007) A handful of carbon. Nature 447: 143–144.

32. Lehmann J. (2007): Bio-energy in the black. Front. Ecol. Environ 5: 381–387.

33. Lehmann J., Rillig M., Thies J., Masiello C., Hockaday W., Crowley D. (2011) Biochar effects on soil biota—A review. Soil Biol. Biochem. 43: 1812–1836.

34. Lentz R., Ippolito J., Spokas K. (2014): Biochar and manure effects on net nitrogen mineralization and greenhouse gas emissions from calcareous soil under corn. Soil Sci. Soc. Am. J. 78: 1641–1655.

35. Liang B., Lehmann J., Solomon D., Kinyangi J., Grossman J., O'Neill B., Skjemstad J., Thies J., Luizão F., Petersen J., Neves E. (2006): Black carbon increases cation exchange capacity in soils. Soil Sci. Soc. Am. J. 70: 1719–1730.

36. Liu X., Zhang A., Ji C., Joseph S., Bian R., Li L., Pan G., Paz-Ferreiro J. (2013): Biochar's effect on crop productivity and the dependence on experimental conditions-a meta-analysis of literature data. Plant Soil 373: 583–594.

37. Major J., Rondon M., Molina D., Riha S., Lehmann J. (2010): Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. Plant Soil 333: 117–128.

38. Otterpohl R. (2012): Boosting compost with biochar and bacteria. Nature 486: 187–188.

39. Pan G., Smith P., Pan W. (2009): The role of soil organic matter in maintaining the productivity and yield stability of cereals in China. Agric. Ecosyst. Environ. 129: 344–348.

40. Paz-Ferreiro J., Méndez A., Gascó G. (2016): Application of biochar for soil biological improvement. In: Guo M., He Z., Uchimiya S., editors, Agricultural and Environmental Applications of Biochar: Advances and Barriers, SSSA Spec. Publ. 63. SSSA, Madison, WI. p. 145–174.

41. Petersen J., Neves E., Heckenberger M. (2001): Gift from the past: Terra preta and prehistoric Amerindian occupation in Amazonia. In: McEwan C., Barreto C., Neves E., editors, Unknown Amazon. The British Museum Press, London, UK. p. 86–105.

42. Rondon M., Lehmann J., Ramirez J., Hurtado M. (2007): Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. Biol. Fertil. Soils 43: 699–708.

43. Sarkhot D., Berhe A., Ghezzehei T. (2012): Impact of biochar enriched with dairy manure effluent on carbon and nitrogen dynamics. J. Environ. Qual. 41: 1107–1114.

44. Saxton A. (1998): A macro for converting mean separation output to letter groupings in Proc Mixed. In: Proceedings 23rd SAS Users Group International Conference, Nashville, TN. 22-25 Mat. 1998. SAS Inst., Cary, NC. p. 1243–1246.

45. Schmidt H., Kammann C., Niggli C., Evangelou M., Mackie K., Abiven S. (2014): Biochar and biochar-compost as soil amendments to a vineyard soil: Influences on plant growth, nutrient uptake, plant health and grape quality. Agric. Ecosyst. Environ. 191: 117–123.

46. Schmidt M., Rapp Py-Daniel A., de Paula Moraes C., Valle R., Caromano C., Texeira W., Barbosa C., Fonseca J., Magalhães M., Silva do Camo Santos D., da Silva e Silva R., Guapindaia V., Moraes B., Lima H., Neves E., Heckenberger M. (2014): Dark earths and the human built landscape in Amazonia: A widespread pattern of anthrosol formation. J. Archaeol. Sci. 42: 152–165.

47. Singh B., Singh Y., Ladha J., Bronson K., Balasubramanian V., Singh J., Khind C. (2002): Chlorophyll meter- and leaf color chart-based nitrogen management for rice and wheat in Northwestern India. Agron. J. 94: 821–829.

48. Smith J., Collins H., Bailey V. (2010): The effect of young biochar on soil respiration. Soil Biol. Biochem. 42: 2345–2347.

49. Sohi S., Krull E., Lopez-Capel E., Bol R. (2010): A review of biochar and its use and function in soil. Adv. Agron. 105: 47–82.

50. Spokas K., Reicosky D. (2009): Impacts of sixteen different biochars on soil greenhouse gas production. Ann. Environ. Sci. 3: 179–199.

51. Steinbeiss S., Gleixner G., Antonietti M. (2009): Effect of biochar amendment on soil carbon balance and soil microbial activity. Soil Biol. Biochem. 41: 1301–1310.

52. Steiner C., Teixeira W., Lehmann J., Nehls T. de Macêdo J., Blum W., Zech W. (2007): Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. Plant Soil 291: 275–290.

53. Uchimiya M., Lima I., Klasson K, Wartelle L. (2010): Contaminant immobilization and nutrient release by biochar soil amendment: Roles of natural organic matter. Chemosphere 80: 935–940.

54. USDA-NASS. (2016): Organic Survey. Volume 3 Special Studies Par 4. U.S. Dep. Agric.-National Agric. Statistics Serv., Washington, DC. Available at https://www.agcensus.usda.gov/Publications/2012/Online_Resources/Organics/ (accessed 23 Jan. 2017). USDA-NASS, Washington, DC.

55. Vaccari F., Baronti S., Lugato E., Genesio L., Castaldi S., Fornasier F., Miglietta F. (2011): Biochar as a strategy to sequester carbon and increase yield in durum wheat. Eur. J. Agron. 34: 231–238.

56. Wang G., Ngouajio M., Charles K. (2010): Brassica biofumigants improve onion (Allium cepa L.) and celery (Apium graveolens) production systems. J. Sustain. Agric. 34(1): 2–14.

57. Weil R., White C., Lawley Y. (2009): Forage radish: New multi-purpose cover crop for the Mid-Atlantic. Fact sheet 824. Available at https://enst.umd.edu/sites/enst.umd.edu/files/_docs/FactSheet824%20Weil%20et%20al%202009.pdf (verified 12 Apr. 2017). Maryland Coop. Ext., College Park.

58. White C., Weil R. (2011): Forage radish cover crops increase soil test phosphorous surrounding radish taproot holes. Soil Sci. Soc. Am. J. 75: 121–130.

59. Wilson K. (2014): How biochar works in soil. The Biochar Journal, Arbaz, Switzerland. Available at www.biochar-journal.org/en/ct/32 (accessed 23 Jan. 2017).

60. Yin X., McClure M., Jaja N., Tyler D., Hayes M. (2011): In-season prediction of corn yield using plant height under major production systems. Agron. J. 103: 923–929.

61. Zavalloni C., Alberti G., Biasiol S., Delle Vedove G., Fornasier F., Liu J., Peressotti A. (2011): Microbial mineralization of biochar and wheat straw mixture in soil: A short-term study. Appl. Soil Ecol. 50: 45–51.

62. Zhang A., Cui L., Pan G., Li L., Hussain Q., Zhang X., Zheng J., Crowley D. (2010): Effect of biochar amendment on yield and methane and nitrous oxide emissions from a rice paddy from Tai Lake plain, China. Agric. Ecosyst. Environ. 139: 469–475.

63. Zheng W., Holm N., Spokas K. (2016): Research and Application of Biochar in North America. In: Guo M., He Z., Uchimiya S., editors, Agricultural and Environmental Applications of Biochar: Advances and Barriers, SSSA Spec. Publ. 63. SSSA, Madison, WI. p. 475–494.