Journal of Plant Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/lpla20

Plant-soil nitrogen, carbon and phosphorus content after the addition of biochar, bacterial inoculums and nitrogen fertilizer

Irina Mikajlo, Bertrand Pourrut, Brice Louvel, Jaroslav Hynšt & Jaroslav Záhora

To cite this article: Irina Mikajlo, Bertrand Pourrut, Brice Louvel, Jaroslav Hynšt & Jaroslav Záhora (2023) Plant-soil nitrogen, carbon and phosphorus content after the addition of biochar, bacterial inoculums and nitrogen fertilizer, Journal of Plant Nutrition, 46:4, 541-555, DOI: 10.1080/01904167.2022.2043369

To link to this article: https://doi.org/10.1080/01904167.2022.2043369

© 2022 The Author(s). Published with license by Taylor and Francis Group, LLC

Published online: 06 Mar 2022.

Submit your article to this journal

Article views: 1246

View related articles

View Crossmark data

Citing articles: 2 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=lpla20

JOURNAL OF PLANT NUTRITION
2023, VOL. 46, NO. 4, 541–555
https://doi.org/10.1080/01904167.2022.2043369

Taylor & Francis
Taylor & Francis Group

OPEN ACCESS Check for updates

Plant-soil nitrogen, carbon and phosphorus content after the addition of biochar, bacterial inoculums and nitrogen fertilizer

Irina Mikajlo[a,b], Bertrand Pourrut[b,c], Brice Louvel[b], Jaroslav Hynšt[a], and Jaroslav Záhora[a]

[a]Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Brno, Czech Republic; [b]Laboratoire de Génie Civil et géo-Environnement, Univ. Lille, IMT Lille Douai, Univ. Artois, JUNIA Lille, UMR 4515 - LGCgE, Lille, France; [c]Laboratoire d'Ecologie Fonctionnelle, Université de Toulouse, CNRS, INPT, UPS, Auzeville-Tolosane, France

ABSTRACT

The use of biochar in combination with mineral or biological amendments to improve its influence on soil-plant properties has received growing attention. The changes of nitrogen, carbon and phosphorus content in *Lactuca sativa var. capitata* aboveground plant biomass and soil after the addition of beech wood biochar combined with the addition of bacterial inoculums (Bacofil and Novarefm) and nitrogen fertilizer have been studied using spectrophotometry methods. Pots were filled with the arable soil from the plots in the protection zone of water sources (Březová nad Svitavou, South Moravia, Czech Republic). Biochar with inoculums decreased plant growth in the first yield of Novaferm treatment and both yields of Bactofil treatment. Increased plant biomass growth was observed with Novaferm addition in the second yield. Total nitrogen increase has been obtained in the plant aboveground biomass and soil of the treatments amended with inoculums and nitrogen fertilizer. The decrease of phosphorus content has been observed in plant aboveground biomass in the biochar amended samples.

ARTICLE HISTORY
Received 9 September 2019
Accepted 27 January 2022

KEYWORDS
Biochar; carbon; inoculum; nitrogen; phosphorus; plant biomass; soil

Introduction

Agricultural lands have been exposed to anthropogenically soil degradation resulting in its productivity loss, frequently caused by overfertilization and poor water management involving soil erosion (Tilman et al. 2002). Worldwide, approximately 45% of arable soils are degraded (Lal 2007) and annually 0.3–0.8% of global arable land is considered improper for agricultural production (Osman 2014). In the Czech Republic, soil erosion caused by water, soil compaction, and reduction in soil organic matter (SOM) are the most common types of soil degradation that were induced by past intensive farm practices (Prazan and Dumbrovsky 2011).

Thus, soil restoration strategies have to be implemented aiming to mitigate soil degradation trends (Lal 2004). In the last decade, biochar (BCH) has attracted an increasing interest to improve the arable soil due to its potential agronomic benefits and carbon sequestration ability. BCH can have an effect on soil-forming processes that in turn determine the transformation, translocation and accumulation of soil constituents, changing soil pedogenic activity, morphology, and productivity from the long term perspective (Richter 2007). BCH could also play a role as a soil conditioner for improving soil fertility and crop productivity (Lehmann, Gaunt, and Rondon

CONTACT Irina Mikajlo irin.mikaylo@gmail.com Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic.

© 2022 The Author(s). Published with license by Taylor and Francis Group, LLC
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Table 1. Basic properties of soil used in experiment (adapted from Plošek 2016).

CEC (mekv kg^{-1})	pH (H_2O)	Conductivity ($\mu S\ cm^{-1}$)	N_{tot}	C_{tot} (mg g^{-1})	C_{org}	C:N	Humus content %	Ca	K (mg kg^{-1})	Mg	P
103.33	6.3	106.4	1.6	17.7	11.3	19.8	1.95	1449	167.8	52.5	180.6

2006; Major et al. 2010). Overall, BCH influence on soil fertility depends on the amounts of carbon and macro or micronutrients derived from the BCH feedstock and pyrolysis temperature (Spokas et al. 2012; Wiedner et al. 2015). Though, improvements in global soil nutrient availability may take a certain period of time to be observed (Spokas et al. 2012).

Soil amendment with biochar has a direct influence on soil microbial communities and their activity (Rondon et al. 2007; Warnock et al. 2007). According to Martínez-Viveros et al. (2010) plant growth-promoting bacteria (PGPB) create significant component of the soil microflora improving plant health along with productivity, while BCH with its porous structure of specific appropriate dimensions may serve as an effective inoculum carrier providing a protected habitat for bacteria, inaccessible for predators (Warnock et al. 2010). In addition, BCH can retain organic and inorganic nutrients from the feedstock and adsorb nutrients from root exudates thus successfully supporting inoculum development after its introduction into the soil (Zimmerman, Gao, and Ahn 2011).

On the other hand, BCH could reduce plant growth (Deenik et al. 2010). This phenomenon could be explained by the presence of toxic compounds due to the charring process, like polycyclic aromatic hydrocarbons (PAHs), cresols, xylenols, formaldehyde, acrolein and volatile compounds such as pyroligneous acids (PA) (Gundale and DeLuca 2006) or the immobilization of nutrients by BCH (Liang et al. 2006; Yao et al. 2010), and especially nitrogen (Rondon et al. 2007; Asai et al. 2009; Verheijen et al. 2009).

Some researchers have shown interest in applying BCH in combination with mineral or organic amendments to mitigate potential BCH negative effects. Plant growth responses are significant when charcoal and fertilizers are combined, assuming a synergistic relationship (Chan et al. 2007; Steiner et al. 2007). According to Steiner et al. (2007), BCH combined with nitrogen (N) fertilizer is efficient for crop yield enhancement while reducing the N application. Considering the influence of BCH on soil microorganisms, the co-application of BCH with biological amendments could be also of interest. Several studies demonstrate the effect of inoculated BCH by bacterial inoculums mixed with fertilizers effect (Major et al. 2010; Chia et al. 2014; Conversa et al. 2015; Fazal and Bano 2016), however there are still missing data about their combined acting after the plant influence and persistence in soil. Moreover, there are also insufficient studies regarding the comparison of freshly applied BCH with the persisted in soil BCH after the second yield as the vast majority of research is based on one growth cycle investigation. In addition, the BCH influence is broadly dependent on the BCH feedstock and production temperature.

In this study, we investigated the influence of BCH combined with other soil amendments (bacterial inoculums and nitrogen fertilizer) on lettuce (*Lactuca sativa var. capitata*) growth and soil-plant nutrient content (nitrogen, carbon and phosphorus) during two growth cycles.

Materials and methods

Soil sampling and preparation

Soil has been collected from permanent experimental Banín plots situated in the protection zone of the underground drinking water source 'Brezova nad Svitavou' (Czech Republic; 49°40.409'N, 16°27.545'E.). The soil was sampled with a spade according to Czech Technical Standard ISO 10381-6 (2009) from the topsoil horizon (till 0–30 cm) in summer 2015. The soil is characterized

Table 2. Physicochemical characteristics of studied biochar.

Ash (%)	Conductivity ($mS.cm^{-1}$)	Dry matter (%)	pH (H_2O)	N_{tot} (%)	C_{tot} (%)	Ca	K ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	P ($mg\ g^{-1}$)
32.72	4.22	95.47	10.12	0.37	56.05	51.23	16.36	6.13	2.61

as a sandy loam type of luvisol (Table 1). Fresh soil samples were air-dried, homogenized, and sieved through a 10-mm sieve.

Before an establishment of the experiment, basic soil parameters were determined. Content of available nutrients was measured using the Mehlich III method. Soil pH value was determined in H_2O, soil total C and total N were measured on the LECO CNS 2000 analyzer (Plošek 2016). According to Pokorný, Šarapatka, and Hejátková (2007) this sandy loam soil is characterized by lightly acidic pH, lower organic carbon (Corg) content, low total nitrogen—N_{tot} (0.1–0.3%), weak humus content, high phosphorus (P) content, low magnesium (Mg) content which is typical for Czech soils. The conductivity index suggested increased salt content (60–120 $\mu S\ cm^{-1}$) in soil.

Biochar material

Beech wood biochar (*Fagus silvatica L.*) was manufactured by the company BIOUHEL.CZ s.r.o. It was produced by slow pyrolysis with the use of low temperature 470 °C (Table 2).

The C_{tot} percentage with the high ash content of 32.72% is commonly revealed in wood-derived BCH that was produced in high temperatures (Domingues et al. 2017). An alkaline pH 10.12 is directly correlated with the higher pyrolysis temperature of BCH production (Ippolito et al. 2012) and the high content of basic elements calcium (Ca) and potassium (K) in wood. N_{tot} and C_{tot} content were close to the BCH produced from the wood matter (Van Zwieten et al. 2010).

Experimental design and plant cultivation

Lettuce plants (*Lactuca sativa var. capitata*) were planted in plastic square containers (10x10x11 cm) filled with 800 g of topsoil. Five different treatments types including control were prepared with four replications of each treatment: control (C) without any amendment, addition of biochar and Bactofil (B), addition of biochar and Bactofil with urea ammonium nitrate (BD), addition of biochar and Novaferm (N) and addition of biochar and Novaferm with urea ammonium nitrate (ND) (Table 3).

In biochar amended treatments, BCH was mixed into the whole volume of soil in the quantity of 6.5% per weight with the first plant growth cycle. The BCH quantity used for the experiment was chosen as a high concentration to obtain distinguished results (Chan et al. 2007). Pots were inoculated with the commercial bacterial inoculums 'Bactofil' (B) from BioFil Ltd (Budapest, Hungary) while the others were inoculated with 'NovaFerm' (N) from Nova Scienta Kft (Soltvadkert, Hungary) at the beginning of the experiment. Inoculums were applied in 10 ml suspension to the soil surface of each pot with the lettuce at growth stage 13 of BBCH-scale (Jenni and Bourgeois 2008). Later at BBCH-scale 15–18, the urea ammonium nitrate fertilizer (UAN 390) was added to BD and ND pots with the dose recommended by the supplier (140 kg N ha^{-1}). UAN 390 is a liquid fertilizer of ammonium nitrate with urea and with ammonium (NH_4-N) nitrogen, nitrate (NO_3-N) nitrogen and amide (N-NH_2) nitrogen. It contains 30% nitrogen; the ratio of ammonium, nitrate, and amide nitrogen is 1:1:2.

The whole experiment was performed in the CLF PlantClimatics® growth chamber with ambient air and light conditions and day temperature variations that remained in the range of

Table 3. Characteristics of all the applied treatments.

Amendment					
Description	Biochar	Bacterial inoculum	Mineral fertilizer	Dose per pot	Treatment
Without amendment	–	–	–	–	C
'Bactofil' inoculum (B)	+ 60 g	*Azospirillum brasilense, Azotobacter vinelandii, Bacillus megaterium, Bacillus polymyxa, Pseudomonas fluorescens, Streptomyces albus*	–	0.1 ml	B
			+UAN 390 fertilizer (D)	0.359 ml	BD
'Novaferm' inoculum (N)	+ 60 g	*Azospirillum* spp., *Azotobacter* spp., *B. megaterium, Bacillus subtilis*	–	1 ml	N
			+UAN 390 fertilizer (D)	0.359 ml	ND

16–22 °C. Pots were irrigated with deionized water during two months of one growth cycle, to maintain soil humidity for approximately 70% of WHC.

After two months of one growth cycle, leaf biomass was harvested, weighed, and prepared for nutrient analysis. Soil (e.g. with the same BCH) was used again without additional BCH amendment. Root biomass from the first growth cycle of plants was removed, and experimental soils were homogenized again and put back into the containers. Lettuce was seeded into the pots for the second experiment. Inoculums and UAN 390 were applied again using the same experimental design as in the first growth cycle. The inoculation by 'Bactofil' (B) and 'NovaFerm'(N) additives and the application of UAN 390 fertilizer (D) were done at the same BBCH-scale as in the first plant growth cycle.

At the end of the second experiment in the next two months, soil and plant samples were collected and analyzed for Corg, total N and P contents.

Nutrient determination in soil and Lactuca sativa aboveground biomass

Plant and soil samples preparation

Lettuce aboveground biomass was cut and then dried at 40 °C to an invariable weight. After the determination of total dry plant biomass, plants were ground into a fine powder utilizing a knife mill (GM200, Retsch). Soil samples were cleared from plant residues, sieved through a 2-mm sieve and transferred into plastic trays to dry at 40 °C in the oven.

Nutrient content analysis

500 mg of soil sample and 50 mg of dry plant sample were used for nutrient content determination. Organic carbon (Corg) levels were determined with a spectrocolorimetric dosing according to the sulphochromic oxidation method (NFX 31 109-1 1993). 5 mL of potassium dichromate ($K_2Cr_2O_7$, 80 g L^{-1}) and 7.5 mL of sulfuric acid (H_2SO_4, 95%) were added to a glass tube containing 500 mg of soil sample sieved to 250 µm, or 50 mg of dry plant sample ground with a mill. Then, the glass tube was stirred thoroughly and placed on a heating block (DK Heating Digester Velp Scientifica®) at 140 °C for 30 min. The following solution was cooled and poured in a 100 mL graduated flask, where the level was adjusted with osmotic water. After stirring, an aliquot of 20 mL was taken and centrifuged for 10 min (4500 rpm; 2000 g). 200 µl of all samples were placed on a microplate and the absorbance was measured at the wavelength λ= 585 nm using a spectrophotometer Multiskan® GO.

Nitrogen (N) and phosphorus (P) were analyzed in the same way for soil and plant extracts prepared in accordance with the Kjeldahl digestion procedure, modified by Saha, Sonon, and Kissel (2012). For N and P estimation in soil, 0.5 g of dry soil sample was weighed in a glass

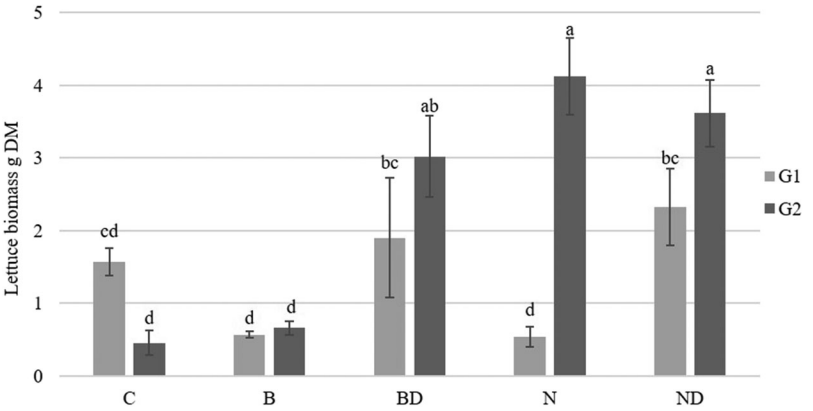

Figure 1. Aboveground biomass production (g DM) within two plant growing cycles—the first (G1) and the second (G2) in pots with treatments (C, B, BD, N and ND). Values are presented as means ± SD. Different letters refer to significant differences between plants (Tukey HSD test, n = 4, $p \leq 0.05$).

digestion tube with the addition of 3.5 g of a catalyst mixture (K_2SO_4, $CuSO_4$ x 5 H_2O, and TiO_2; the proportion of 33:1:1). Then, samples were vortexed.

For N and P estimation in plant aboveground biomass, 50 mg of dry plant biomass sample was weighed to a glass digestion tubes with adding 3.5 g of the same catalysts. A volume of 10 mL of concentrated (96.8%) H_2SO_4 and 10 mL of 30% H_2O_2 was successively added slowly to each tube of soil and aboveground biomass samples and then mixed by vortexing. Thereafter, the digestion tubes were placed on a digestion block (DK Heating Digester, Velp Scientifica), heated at 200 °C for 20 min and 390 °C for 45 min. The samples were digested until the solution was clear green. The final N extraction followed the ascorbic/molybdate method described in the French standard NFX 31-161, where digests were cooled for 15 min at room temperature and diluted by adding distilled water to constitute a solution of 100 mL volume and measured by UV/VIS spectrophotometer (Multiskan® GO) at a wavelength $\lambda = 660$ nm.

The final P extraction was done in accordance with Joret and Hébert (1955), where solutions after were measured spectrophotometrically (UV − 1800 Shimadzu) at wavelength $\lambda = 825$ nm.

Data analysis

Nutrient concentrations in soil and aboveground biomass of lettuce are expressed and presented as the means and standard deviations of replicates for each treatment within two plant growth cycles. Analysis of variance (ANOVA) was accomplished to estimate between two plant growth cycle differences concerning elements concentration in soil and including the aboveground biomass weight across the treatments. The normal distribution of data (Shapiro-Wilk test) and equality of variances (Bartlett test) were checked. When both tests proved conformity, Fisher statistics was considered for significance ($p \leq 0.05$) and the Tukey (HSD) test was used for pair-wise comparisons of statistical groups. The Kruskal-Wallis test was performed for data that were not distributed normally. All statistic tests were conducted in XLSTAT software (AddinsoftTM software 2016).

Results

Lettuce growth/plant biomass measurements

In the first growth cycle (G1) biomass yield ranged from 0.5 to 2.3 g DM (Figure 1). No significant differences were found between the treatments and control (1.6 g DM). However, treatments amended with UAN 390 fertilizer had significantly higher values by 3.2 times in BD treatment

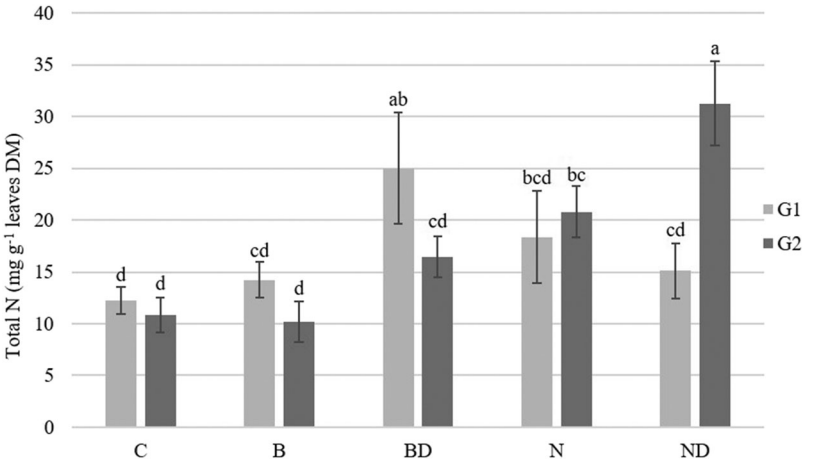

Figure 2. Nitrogen content (mg g^{-1} leaves DM) in aboveground lettuce biomass of the first (G1) and the second (G2) growing cycles in five different soil treatments (C, B, BD, N and ND). Values are presented as means ± SD. Different letters refer to significant differences between plants (Tukey HSD test, n = 4, $p \leq 0.05$).

and by 4.6 times in ND treatment compared to the same treatments amended just with inoculums, B and D respectively).

In the second growth cycle (G2) values ranged from 0.5 till 4.1 g DM. No significant differences were found between the C and B treatment. Significantly increased values were found in BD treatment (by 6 times), ND treatment (by 7.2 times) and N treatment (by 8.3 times) compared to the control plant.

Taking into comparison two growth cycles G1 and G2, no significant differences were determined within the treatments, except the Novaferm inoculated treatments, where N treatment showed a significant increase by 8.2 times in G2 compared to G1 and ND treatment by 1.6 times respectively.

Total N in biomass/soil

In G1, N content values in lettuce biomass fluctuated from 12.3 mg g^{-1} leaves DM to 25 mg g^{-1} leaves DM (Figure 2). No significant differences have been observed between the C, B, N and ND treatments in G1. Although BD value was significantly higher by 1.6–2 times compared to the C, B and ND treatments. In G2, no significant differences have been detected within B, BD compared to the control (10.8 mg g^{-1} leaves DM), where N treatment differed from C, B and ND treatment, but not the BD treatment. ND value was significantly higher than all the other treatments and by 2.9 times compared to the C.

Comparing G1 and G2 reveals that no significant differences were observed that characterize the C, B and N treatments, although UAN 390 amended treatments showed a decrease up to 34% in the case of its combination with Bactofil inoculum (BD) and an increase in 51.8% with the Novaferm inoculum combination (ND).

Figure 3 shows that BCH amended treatments exhibited significantly higher N values in BD, N and ND treatments compared to control soil (0.6 mg g^{-1} soil DM), though B treatment did not show any significant differences compared to the rest of the treatments.

Organic carbon content in biomass/soil

No statistically significant differences have been detected in G1 and G2 regarding organic carbon (Corg) content in the lettuce biomass (Figure 4). Corg content fluctuated in G1 from 337 to 364 mg g^{-1} leaves DM and in G2 from 317 to 347 mg g^{-1} leaves DM.

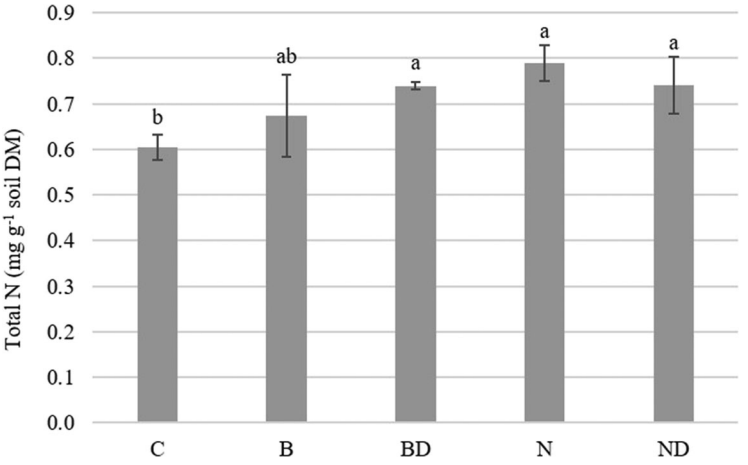

Figure 3. Total soil N content (mg g^{-1} soil DM) after G2 plants harvesting in five different soil treatments (C, B, BD, N and ND). Values are presented as means ± SD. Different letters refer to significant differences between plants (Tukey HSD test, n = 4, $p \leq 0.05$).

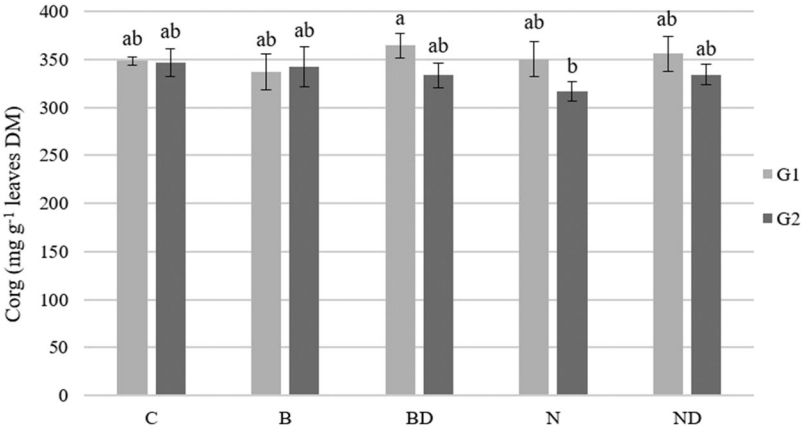

Figure 4. Corg content (mg g^{-1} leaves DM) in aboveground lettuce biomass of the first (G1) and the second (G2) growing cycles in five different soil treatments (C, B, BD, N and ND). Values are presented as means ± SD. Different letters refer to significant differences between plants (Tukey HSD test, n = 4, $p \leq 0.05$).

Soil Corg content has been detected in significantly higher amounts by 3.6–5 times in the BCH amended treatments compared to the control soil (8.7 mg g^{-1} soil DM), Figure 5.

No differences have been found between B, N and ND treatments itself, while BD treatment was significantly higher by 1.3–1.4 times compared to B and ND treatments, but meantime having no significant difference with the N treatment.

Phosphorus in biomass/soil

In G1 the highest P content was observed in the C plant: 13.8 mg g^{-1} leaves DM (Figure 6). The other lettuce plants aboveground biomass had significantly lower P content compared to the control plant: by 1.6 times in B treatment and by 3.7–5.3 times in the rest N, BD and ND treatments.

In G1, no statistically significant differences in plant P content have been found between UAN 390 amended treatments (BD and ND) and the N inoculated plants. In G2, no statistically significant differences have been found within the treated plants including the C plant. P contents

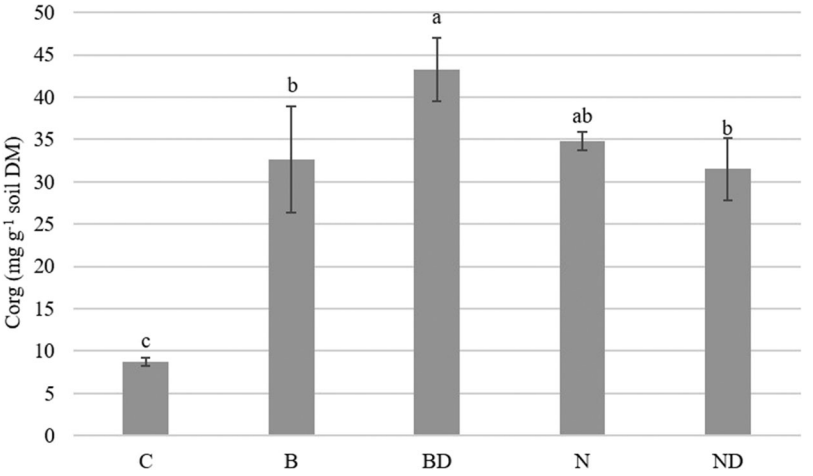

Figure 5. Soil Corg content (mg g^{-1} soil DM) after G2 plants harvesting in five different soil treatments (C, B, BD, N and ND). Values are presented as means ± SD. Different letters refer to significant differences between plants (Tukey HSD test, n = 4, $p \leq 0.05$).

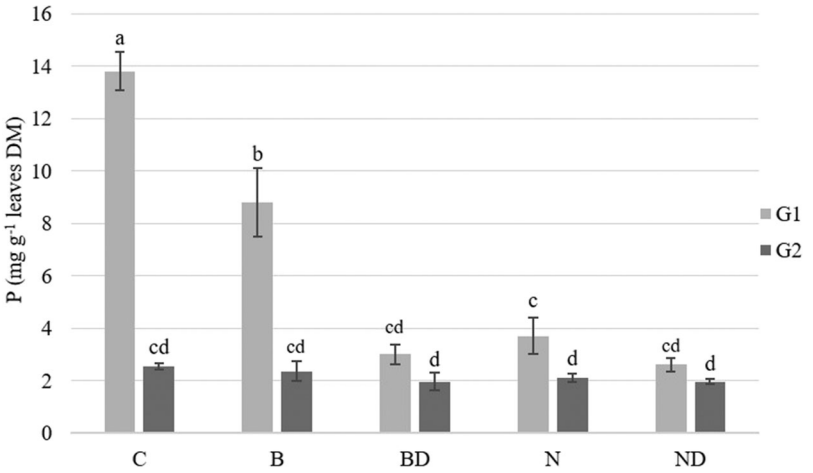

Figure 6. P content (mg g^{-1} leaves DM) in aboveground lettuce biomass of the first (G1) and the second (G2) growing cycles in five different soil treatments (C, B, BD, N and ND). Values are presented as means ± SD. Different letters refer to significant differences between plants (Tukey HSD test, n = 4, $p \leq 0.05$).

varied from 2–2.5 mg g^{-1} leaves DM. Comparing G1 and G2, P concentration decreased in all the treatments of G2, except for the UAN 390 amended ones (BD and ND) having no statistical differences between the growth cycles.

In G2, biomass P content in the control plant dropped by 5.5 times compared to G1. In B treatment biomass P level has dropped by 3.6 times compared to G1 and by 1.8 times in N treatment respectively.

No statistically significant differences of soil extractable P have been found between the control soil along with the treated by BCH soils (Figure 7). Values fluctuated from 0.5–0.6 mg g^{-1} soil DM.

Discussion

The differences between two plant growth cycles grown in the same BCH amended soil and influenced by the two bacterial inoculums with mineral fertilizer have been investigated throughout

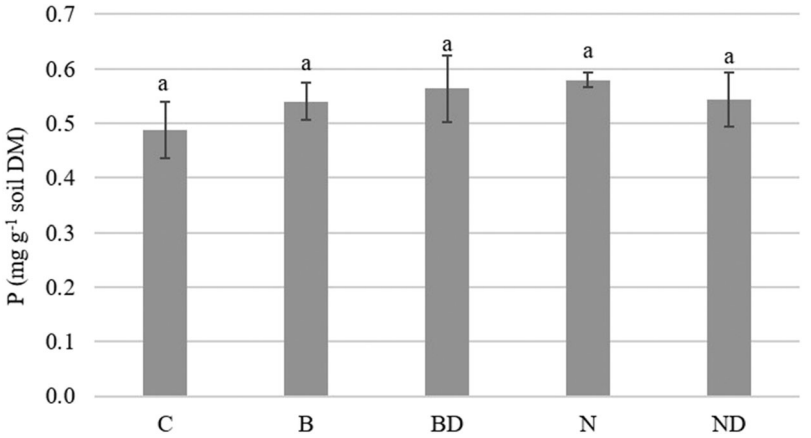

Figure 7. Soil P content (mg g^{-1} soil DM) of second plant growing cycle (G2) in five different soil treatments (C, B, BD, N and ND). Values are presented as means ± SD. Different letters refer to significant differences between plants (Tukey HSD test, n = 4, $p \leq 0.05$).

the experiment. As hypothesized, the plants from two plant growth cycles differed while growing in the same soil, although with the altered soil physicochemical and biological properties throughout the experiment, as a result of microorganisms activity-soil-plant interactions.

Influence of the BCH mixed with bacterial inoculums

Plant biomass of control plants in G1 and G2 had lower values compared to the amended treatments (Figure 1). This might have been caused by the consequent nutrient depletion due to plant growth in G1 and the lack of those nutrients in G2 for the lettuce plants. B plants in G1 and G2 and N plants in G1 had decreased values equal to the control in G2. According to Joseph et al. (2015) BCH in high concentrations added into the rhizosphere established contrasting from the typical one environment that would naturally develop there from the typical soil clays, silt, sand and organic matter components, resulting in changed redox potential around the BCH particle. Low biomass N content in control and Bactofil treatment of both growth cycles has been related to the limitation by the N lack in the soil as well (Figures 2 and 3). Initial soil N amount (Table 1) compared to G2 C soil showed a decrease by 62.5% which can be explained by the lettuce nutrient consumption. The results are in the line with the former studies on lettuce growth and sandy soil with compost (Brito 2001) and with hydroponic solution (Domingues et al. 2012).

The highest plant P content in G1 control plant and the following decreased plant P values in the amended treatments may state on P allocation where in G1 microorganisms in inoculated soils had concurrence for available P. Whereas in the B treated soil no concurrence occurred that consequently resulted into a higher P content in aboveground biomass (Figure 6). Studies of Rodríguez and Fraga (1999) on phosphate solubilizing bacteria and P uptake support this hypothesis. On the other side, lower P content in the amended lettuce plants compared to the higher P ratio of the C plant and B treated plant could have been related to the nutrient dilution within the plant growth resulting in decreased P concentration. Initial soil P content (Table 1) was higher by 64% compared to the soil P in G2 C soil (Figure 7) might be also explained by the reduced biomass development of C plant in G2 with the decreased P uptake which differs from G1 lettuce plant (Figures 1 and 6). The results are in accordance with the studies on lettuce P uptake in silty clay loam soil (Chabot, Antoun, and Cescas 1996) and quartz sand with P solutions (Xu et al. 2004).

Biomass of BCH amended plants with inoculums reduced in G1 (Figure 1) and this might be related to BCH ability to bend nutrients, as demonstrated with burcucumber BCH on lettuce that

led to the suppressed plant growth (Rajapaksha et al. 2014). B treated soils showed a decrease in lettuce growth of both G1 and G2 compared to the other treatments. In the studies of Jaiswal et al. (2015) on beans and four BCH types (feedstock: eucalyptus wood chips and greenhouse pepper plant wastes) studied 'Shifted Rmax-Effect' revealed the effective BCH dose for disease reduction that was lower than that for plant growth promotion, whereas at higher BCH doses no UAN ping-off suppression had been detected, even was promoted in certain cases. From the other hand, Novaferm inoculated soil stimulated lettuce growth in G2 by 87.8% compared to G1 and the C plant in G2. This might be explained by the B inoculum type which could need a longer period to fully realize its potential, as its application into the BCH amended soil did not lead to the lettuce development. In addition, biomass values in the B treatment of both growth cycles remained unchanged (Figure 1). Our results are in accordance with the studies of Asai et al. (2009) where BCH without N fertilizer led to the reduction of rice N uptake and caused decreased grain yield in G1. Decreased N values in the BCH amended treatments with the B inoculum could have been related to N immobilization by BCH and this hypothesis might explain the reduced plant growth in B amended treatments of both growth cycles. Results are in accordance with the experiments on corn stover and hardwood BCH with silt loam soil and corn (Fidel, Laird, and Parkin 2017), maize stover BCH and ryegrass with loamy/sandy soil (Liu et al. 2017). Otherwise, N content reduction in B treated plants with the reduced biomass might be explained by the B inoculum lower N_2-fixators activity with its BCH combination compared to the Novaferm one, with perhaps inefficient microbial population establishment of the added inoculum as suggested in studies of Dempster et al. (2012) where jarrah wood BCH suppressed microbial development. However, the amount of total soil N (Figure 3) has increased by the application of BCH up to 24% (N treatment), some of which can be explained by the direct input from BCH(Zhang, Voroney, and Price 2015; Nguyen et al. 2017). Some portion of N and P is released from the charcoal residues, but these compounds are rather immediately involved in a soil-plant nutrient cycle (Gul and Whalen 2016). Soil P allocation without any significant differences among the treatments might be explained by the better phosphate solubilizing bacteria development in the BCH amended soils with inoculums that had the other strategy than releasing P available to plants (Khan et al. 2009). Basically, greater P allocation took place within BCH amended soils, as the lettuce development, in that case, was higher (Figures 1 and 7). Another explanation could be related to BCH as an available P adsorbent from the soil as in the studies of Yao et al. (2011) on sugar beet biochar removing P from phosphate solution. Consequently, it could have led to the low biomass P values obtained in BCH amended soils of both growth cycles, as the soil mixed with BCH makes P partly unavailable for the plants.

The effect of the BCH mixed with the bacterial inoculums and UAN fertilizer

BCH combination with inoculums and N fertilizers resulted in a biomass increase in both growth cycles (Figure 1). Overall, practically all the BCH amended treatments showed lettuce biomass increase in G2 (except the upper mentioned B and control). This trend confirms our hypothesis of BCH persistence in soil and consequent pores inoculation in course of time that drives to plant better growth and therefore biomass increase as suggested previously (Jones et al. 2012; Biederman and Stanley Harpole 2013). UAN fertilizer combined with inoculums also initiated a total N content increase in biomass of BD treatment compared to the C (Figure 2). This can be explained by the application of N additives into the soil and thus N availability that correlates with increased biomass growth (Liu et al. 2018). The plant N content of ND treatment was not significantly different in G1 compared to C, that might be due to microbial N immobilization as in the studied N microsites suggested by Schimel and Bennett (2004). In the case of ND treatment in G1 the tendency of a decreased plant N amounts up to 48% compared to G2 could have stated on its use primarily by soil bacteria in G1, as suggested by Kaye and Hart (1997)

considering N competition within plants and soil microorganisms. Soil N content remained unchanged between BCH amended treatments (Figure 3), but was significantly higher than the C soil (treatments BD, N and ND). BCH may also limit soil N availability in N deficient soils due to the high C/N ratio and temporarily reduce crop productivity (Lehmann et al. 2003a).

Hence, significantly raised Corg values have been found in all the BCH treated soils with the highest value in BD treated soil by 80% (Figure 5). Obtained results where the Corg doubled in the BCH amended soils are confirmed as well by the studies of Lehmann et al. (2003b) involving Ferrasol and using cowpea as a test plant. Other studies confirm, that for example maize BCH addition in augmented amounts led to great soil Corg rise and total N as well (Wang et al. 2015). Moreover, BCH addition associated with the increased soil Corg contents might enhance the nutrient retention capacity of the soil due to the higher CEC with organo-mineral complexes forming (Glaser, Lehmann, and Zech 2002). On the other side, BCH application in high rates like 16 t ha^{-1} caused N limitation, even with N fertilizer addition, and thus low grain yields with the reduction of rice plant N uptake (Asai et al. 2009). In the other studies, maize BCH and urea fertilizer lead to the short-term reductions in soil mineral N availability as a result of probable BCH negative effect on soil quality and fertility characteristics (Wang et al. 2015). It might be also caused by the significant absorption of N up to 22.1% with the ash/charcoal woody residues according to the studies of Dünisch et al. (2007), where actually the same has been observed in the case of available P compounds (up to 11.7%). Biomass and soil P content remained without any significant differences between the G2 treatments (Figures 6 and 7). The explanation can be found in the blocked soil P due to the increased pH that is caused by the BCH amendment (Takaya et al. 2016). BCH absorbs P and also increases P fertilizer retention in soils, though its acting intensity broadly depends on BCH feedstock type (Zhang et al. 2016) or can also adsorb phosphate efficiently from solutions being a potential P source (Liang et al. 2018). The other investigation, that studied the influence of BCH and N fertilizers on different soil types from several locations in northern Laos, found yield rise in soils with low P availability and also enhanced plant reaction to additional fertilizers with BCH additions (Asai et al. 2009).

Conclusion

This research proved the importance of BCH enrichment during physicochemical and biological alterations in soil stimulated by crop growth and its consequent positive influence on plant development. The resulting nutrient depletion by plants of G1 can be compensated by the BCH bacterial inoculation and the addition of mineral fertilizer. Comparing the two bacterial additives mixed with BCH and their acting in the soil it has been concluded, that growth-promoting effect of inoculums was supported by N fertilization. Regarding the influence on the total N content in plants, Novaferm bacteria composition promotes better N availability and assimilation possibility, and consequently the plant growth. Thus, the Novaferm inoculum tends to increase N bonding in G2 via bacterial N_2-fixation to a greater extent than Bactofil. BCH most probably decreases N availability in soil. Higher C and P content in all biochar amended soils should be attributed to C and nutrient input in added BCH. Nevertheless, in both inoculums, Bactofil and Novaferm are phosphate solubilizing bacteria not efficient enough. Observed effects need to be investigated further aiming to analyze BCH aging in soil for a longer period and its functioning in soil-plant nutrient cycling.

Acknowledgements

This paper was supported by the project ATCZ42 INTEKO: 'Innovation technologies in composting, its application and soil protection'; by the Internal Grant Agency IGA FA MENDELU (Faculty of Agriscience, Mendel University

in Brno) No. TP 3/2015 with the support of the Specific University Research Grant (Ministry of Education, Youth and Sports, Czech Republic); by the Erasmus + mobility program realizing the internship at JUNIA, Lille (France).

Disclosure statement

No potential conflict of interest was reported by the authors.

Authors' contribution statement

Irina Mikajlo: All steps of the study from conception to manuscript drafting and approval of the final version. **Bertrand Pourrut**: Conception and design of the study, analyzing and interpretation of data, critical revision of the manuscript and approval of the final version. **Brice Louvel**: Conception and design of additional experiments, Acquisition of data, critical revision of the manuscript and approval of the final version. **Jaroslav Hynšt**: Acquisition of data, critical revision of the manuscript and approval of the final version. **Jaroslav Záhora**: Conception and design of the study, analyzing and interpretation of data, critical revision of the manuscript.

References

Asai, H., B. K. Samson, H. M. Stephan, K. Songyikhangsuthor, K. Homma, Y. Kiyono, Y. Inoue, T. Shiraiwa, and T. Horie. 2009. Biochar amendment techniques for upland rice production in Northern Laos. 1. Soil physical properties, leaf SPAD and grain yield. *Field Crops Research* 111 (1–2):81–4. doi: 10.1016/j.fcr.2008.10.008.

Biederman, L. A., and W. Stanley Harpole. 2013. Biochar and its effects on plant productivity and nutrient cycling: A meta-analysis. *GCB Bioenergy* 5 (2):202–14. doi: 10.1111/gcbb.12037.

Brito, L. M. 2001. Lettuce (Lactuca sativa L.) and Cabbage (Brassica oleracea L. var. capitata L.) growth in soil mixed with municipal solid waste compost and paper mill sludge composted with bark. *Acta Horticulturae* 563 (563):131–7. doi: 10.17660/ActaHortic.2001.563.16.

Chabot, R., H. Antoun, and M. P. Cescas. 1996. Growth promotion of maize and lettuce by phosphate-solubilizing Rhizobium leguminosarum biovar. phaseoli. *Plant and Soil* 184 (2):311–21. doi: 10.1007/BF00010460.

Chan, K. Y., L. Van Zwieten, I. Meszaros, A. Downie, and S. Joseph. 2007. Agronomic values of green waste biochar as a soil amendment. *Soil Research* 45 (8):629–34. doi: 10.1071/SR07109.

Chia, C. H., B. P. Singh, S. Joseph, E. R. Graber, and P. Munroe. 2014. Characterization of an enriched biochar. *Journal of Analytical and Applied Pyrolysis* 108:26–34. doi: 10.1016/j.jaap.2014.05.021.

Conversa, G., A. Bonasia, C. Lazzizera, and A. Elia. 2015. Influence of biochar, mycorrhizal inoculation, and fertilizer rate on growth and flowering of Pelargonium (Pelargonium zonale L.) plants. *Frontiers in Plant Science* 6 (429):429.

CSN ISO 10381-6. 2009. Czech Technical Standard. Soil quality - Sampling - Part 6: Guidelines for the collection, manipulation and storage of soil samples intended for the study of aerobic microbial processes in the laboratory.

Deenik, J. L., T. McClellan, G. Uehara, M. J. Antal, and S. Campbell. 2010. Charcoal volatile matter content influences plant growth and soil nitrogen transformations. *Soil Science Society of America Journal* 74 (4):1259–70. doi: 10.2136/sssaj2009.0115.

Dempster, D. N., D. B. Gleeson, Z. M. Solaiman, D. L. Jones, and D. V. Murphy. 2012. Decreased soil microbial biomass and nitrogen mineralisation with Eucalyptus biochar addition to a coarse textured soil. *Plant and Soil* 354 (1–2):311–24. doi: 10.1007/s11104-011-1067-5.

Domingues, D. S., H. W. Takahashi, C. A. P. Camara, and S. L. Nixdorf. 2012. Automated system developed to control pH and concentration of nutrient solution evaluated in hydroponic lettuce production. *Computers and Electronics in Agriculture* 84:53–61. doi: 10.1016/j.compag.2012.02.006.

Domingues, R. R., P. F. Trugilho, C. A. Silva, I. C. N. A. De Melo, L. C. A. Melo, Z. M. Magriotis, and M. A. Sánchez-Monedero. 2017. Properties of biochar derived from wood and high-nutrient biomasses with the aim of agronomic and environmental benefits. *PLoS One* 12 (5):e0176884. doi: 10.1371/journal.pone.0176884.

Dünisch, O., V. C. Lima, G. Seehann, J. Donath, V. R. Montóia, and T. Schwarz. 2007. Retention properties of wood residues and their potential for soil amelioration. *Wood Science and Technology* 41 (2):169–89. doi: 10.1007/s00226-006-0098-1.

Fazal, A., and A. Bano. 2016. Role of plant growth-promoting rhizobacteria (PGPR), biochar, and chemical fertilizer under salinity stress. *Communications in Soil Science and Plant Analysis* 47 (17):1985–93. doi: 10.1080/00103624.2016.1216562.

Fidel, R. B., D. A. Laird, and T. B. Parkin. 2017. Impact of six lignocellulosic biochars on C and N dynamics of two contrasting soils. *GCB Bioenergy* 9 (7):1279–91. doi: 10.1111/gcbb.12414.

Glaser, B., J. Lehmann, and W. Zech. 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal - A review. *Biology and Fertility of Soils* 35 (4):219–30. doi: 10.1007/s00374-002-0466-4.

Gul, S., and J. K. Whalen. 2016. Biochemical cycling of nitrogen and phosphorus in biochar-amended soils. *Soil Biology and Biochemistry* 103:1–15. doi: 10.1016/j.soilbio.2016.08.001.

Gundale, M. J., and T. H. DeLuca. 2006. Charcoal effects on soil solution chemistry and growth of Koeleria macrantha in the ponderosa pine/Douglas-fir ecosystem. *Biology and Fertility of Soils* 43 (3):303–11. doi: 10.1007/s00374-006-0106-5.

Ippolito, J. A., J. M. Novak, W. J. Busscher, M. Ahmedna, D. Rehrah, and D. W. Watts. 2012. Switchgrass biochar affects two aridisols. *Journal of Environmental Quality* 41 (4):1123–30. doi: 10.2134/jeq2011.0100.

Jaiswal, A. K., O. Frenkel, Y. Elad, B. Lew, and E. R. Graber. 2015. Non-monotonic influence of biochar dose on bean seedling growth and susceptibility to Rhizoctonia solani: The "Shifted Rmax-Effect". *Plant and Soil* 395 (1–2):125–40. doi: 10.1007/s11104-014-2331-2.

Jenni, S., and G. Bourgeois. 2008. Quantifying phenology and maturity in crisphead lettuce. *HortTechnology* 18 (4): 553–8. doi: 10.21273/HORTTECH.18.4.553.

Jones, D. L., J. Rousk, G. Edwards-Jones, T. H. DeLuca, and D. V. Murphy. 2012. Biochar-mediated changes in soil quality and plant growth in a three year field trial. *Soil Biology and Biochemistry* 45:113–24. doi: 10.1016/j.soilbio.2011.10.012.

Joret, F., and J. Hébert. 1955. Contribution à la détermination du besoin des sols en acide phosphorique. *Annales Agronomiques* 2: 233–99.

Joseph, S., O. Husson, E. R. Graber, L. Van Zwieten, S. Taherymoosavi, T. Thomas, S. Nielsen, J. Ye, G. Pan, C. Chia, et al. 2015. The electrochemical properties of biochars and how they affect soil redox properties and processes. *Agronomy* 5 (3):322–40. doi: 10.3390/agronomy5030322.

Kaye, J. P., and S. C. Hart. 1997. Competition for nitrogen between plants and soil microorganisms. *Trends in Ecology & Evolution* 12 (4):139–43. doi: 10.1016/S0169-5347(97)01001-X.

Khan, A. A., G. Jilani, S. M. Saqlan Naqvi, M. H. Rasheed, and M. Saleem Akhtar. 2009. Phosphorus solubilizing bacteria: Occurrence, mechanisms and their role in crop production. *Journal of Agriculture and Biological Sciences* 1 (1):48–58.

Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. *Science (New York, N.Y.)* 304 (5677):1623–7. doi: 10.1126/science.1097396.

Lal, R. 2007. Anthropogenic influences on world soils and implications to global food security. *Advances in Agronomy* 93 (Suppl):69–93.

Lehmann, J., J. Gaunt, and M. Rondon. 2006. Bio-char sequestration in terrestrial ecosystems - A review. *Mitigation and Adaptation Strategies for Global Change* 11 (2):403–27. doi: 10.1007/s11027-005-9006-5.

Lehmann, J., D. Kern, L. German, J. Mccann, G. C. Martins, and A. Moreira. 2003a. Soil fertility and production potential. In *Amazonian Dark Earths*, 105–24. Dordrecht: Kluwer Academic Publishers.

Lehmann, J., J. Pereira da Silva, C. Steiner, T. Nehls, W. Zech, and B. Glaser. 2003b. Nutrient availability and leaching in an archaeological Anthrosol and Ferralsol of the Central Amazon basin: Fertilizer, manure and charcoal amendments. *Plant and Soil* 249 (2):343–57. doi: 10.1023/A:1022833116184.

Liang, B., J. Lehmann, D. Solomon, J. Kinyangi, J. Grossman, B. O'Neill, J. O. Skjemstad, J. Thies, F. J. Luizão, J. Petersen, et al. 2006. Black carbon increases cation exchange capacity in soils. *Soil Science Society of America Journal* 70 (5):1719–30. doi: 10.2136/sssaj2005.0383.

Liang, X., Y. Jin, M. He, C. Niyungeko, J. Zhang, C. Liu, G. Tian, and Y. Arai. 2018. Phosphorus speciation and release kinetics of swine manure biochar under various pyrolysis temperatures. *Environmental Science and Pollution Research International* 25 (26):25780–9. doi: 10.1007/s11356-017-0640-8.

Liu, X., J. Xu, L. Yu, P. C. Brookes, C. Tang, M. Yu, and X. Lu. 2018. Combined application of biochar and nitrogen fertilizer benefits nitrogen retention in the rhizosphere of soybean by increasing microbial biomass but not altering microbial community structure. *Science of the Total Environment* 640–641:1221–30. doi: 10.1016/j.scitotenv.2018.06.018.

Liu, Z., T. He, T. Cao, T. Yang, J. Meng, and W. Chen. 2017. Effects of biochar application on nitrogen leaching, ammonia volatilization and nitrogen use efficiency in two distinct soils. *Journal of Soil Science and Plant Nutrition* 17 (2):515–528. doi: 10.4067/S0718-95162017005000037.

Major, J., M. Rondon, D. Molina, S. J. Riha, and J. Lehmann. 2010. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. *Plant and Soil* 333 (1–2):117–128. doi: 10.1007/s11104-010-0327-0.

Martínez-Viveros, O., M. Jorquera, D. Crowley, G. Gajardo, and M. Mora. 2010. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *Journal of Soil Science and Plant Nutrition* 10 (3): 293–319. doi: 10.4067/S0718-95162010000100006.

NFX 31 109-1. 1993. *Qualite des sols - Methodes chimiques - Determination du carbone organique par oxydation sulfochromique.*

Nguyen, T. T. N., C. Y. Xu, I. Tahmasbian, R. Che, Z. Xu, X. Zhou, H. M. Wallace, and S. H. Bai. 2017. Effects of biochar on soil available inorganic nitrogen: A review and meta-analysis. *Geoderma* 288:79–96. doi: 10.1016/j.geoderma.2016.11.004.

Osman, K. T. 2014. *Soil degradation, conservation and remediation*.

Plošek, L. 2016. *Aplikace zpracovaného biologicky rozložitelného odpadu v kombinaci s průmyslovými hnojivy do půdy. Application of processed biodegradable waste in combination with industrial fertilizers to the soil*, 144p. Czech Republic: Mendel University in Brno.

Pokorný, E., B. Šarapatka, and K. Hejátková. 2007. *Hodnocení kvality půdy v ekologicky hospodařícím podniku*. Náměšt and Oslavou: ZERA.

Prazan, J., and M. Dumbrovsky. 2011. Soil conservation policies: Conditions for their effectiveness in the Czech Republic. *Land Degradation & Development* 22 (1):124–133. doi: 10.1002/ldr.1066.

Rajapaksha, A. U., M. Vithanage, J. E. Lim, M. B. M. Ahmed, M. Zhang, S. S. Lee, and Y. S. Ok. 2014. Invasive plant-derived biochar inhibits sulfamethazine uptake by lettuce in soil. *Chemosphere* 111:500–504. doi: 10.1016/j.chemosphere.2014.04.040.

Richter, D. D. B. 2007. Humanity's transformation of earth's soil: Pedology's new frontier. *Soil Science* 172 (12): 957–967. doi: 10.1097/ss.0b013e3181586bb7.

Rodríguez, H., and R. Fraga. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances* 17 (4–5):319–339. doi: 10.1016/S0734-9750(99)00014-2.

Rondon, M. A., J. Lehmann, J. Ramírez, and M. Hurtado. 2007. Biological nitrogen fixation by common beans (Phaseolus vulgaris L.) increases with bio-char additions. *Biology and Fertility of Soils* 43 (6):699–708. doi: 10.1007/s00374-006-0152-z.

Saha, U. K., L. Sonon, and D. E. Kissel. 2012. Comparison of conductimetric and colorimetric methods with distillation–titration method of analyzing ammonium nitrogen in total Kjeldahl digests. *Communications in Soil Science and Plant Analysis* 43 (18):2323–2341. doi: 10.1080/00103624.2012.708081.

Schimel, J. P., and J. Bennett. 2004. Nitrogen mineralization: Challenges of a changing paradigm. *Ecology* 85 (3): 591–602. doi: 10.1890/03-8002.

Spokas, K. A., K. B. Cantrell, J. M. Novak, D. W. Archer, J. A. Ippolito, H. P. Collins, A. A. Boateng, I. M. Lima, M. C. Lamb, A. J. McAloon, et al. 2012. Biochar: A synthesis of its agronomic impact beyond carbon sequestration. *Journal of Environmental Quality* 41 (4):973–989. doi: 10.2134/jeq2011.0069.

Steiner, C., W. G. Teixeira, J. Lehmann, T. Nehls, J. L. V. De MacÊdo, W. E. H. Blum, and W. Zech. 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant and Soil* 291 (1–2):275–290. doi: 10.1007/s11104-007-9193-9.

Takaya, C. A., L. A. Fletcher, S. Singh, K. U. Anyikude, and A. B. Ross. 2016. Phosphate and ammonium sorption capacity of biochar and hydrochar from different wastes. *Chemosphere* 145:518–527. doi: 10.1016/j.chemosphere.2015.11.052.

Tilman, D., K. G. Cassman, P. A. Matson, R. Naylor, and S. Polasky. 2002. Agricultural sustainability and intensive production practices. *Nature* 418 (6898):671–677. doi: 10.1038/nature01014.

Van Zwieten, L., S. Kimber, S. Morris, K. Y. Chan, A. Downie, J. Rust, S. Joseph, and A. Cowie. 2010. Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant and Soil* 327 (1–2):235–246. doi: 10.1007/s11104-009-0050-x.

Verheijen, F., S. Jeffery, A. C. Bastos, M. Van Der Velde, and I. Diafas. 2009. *Biochar application to soils: A critical review of effects on soil properties, processes and functions*, 149p. Luxembourg: EUR 24099 EN, Office for the Official Publications of the European Communities.

Wang, X., D. Song, G. Liang, Q. Zhang, C. Ai, and W. Zhou. 2015. Maize biochar addition rate influences soil enzyme activity and microbial community composition in a fluvo-aquic soil. *Applied Soil Ecology* 96:265–272. doi: 10.1016/j.apsoil.2015.08.018.

Warnock, D. D., J. Lehmann, T. W. Kuyper, and M. C. Rillig. 2007. Mycorrhizal responses to biochar in soil - Concepts and mechanisms. *Plant and Soil* 300 (1–2):9–20. doi: 10.1007/s11104-007-9391-5.

Warnock, D. D., D. L. Mummey, B. McBride, J. Major, J. Lehmann, and M. C. Rillig. 2010. Influences of non-herbaceous biochar on arbuscular mycorrhizal fungal abundances in roots and soils: Results from growth-chamber and field experiments. *Applied Soil Ecology* 46 (3):450–456. doi: 10.1016/j.apsoil.2010.09.002.

Wiedner, K., J. Schneeweiß, M. A. Dippold, and B. Glaser. 2015. Anthropogenic Dark Earth in Northern Germany - The Nordic analogue to terra preta de Índio in Amazonia. *Catena* 132 (September):114–125. doi: 10.1016/j.catena.2014.10.024.

Xu, G., I. Levkovitch, S. Soriano, R. Wallach, and A. Silber. 2004. Integrated effect of irrigation frequency and phosphorus level on lettuce: P uptake, root growth and yield. *Plant and Soil* 263 (1):297–309. doi: 10.1023/B:PLSO.0000047743.19391.42.

Yao, F. X., M. C. Arbestain, S. Virgel, F. Blanco, J. Arostegui, J. A. Maciá-Agulló, and F. Macías. 2010. Simulated geochemical weathering of a mineral ash-rich biochar in a modified Soxhlet reactor. *Chemosphere* 80 (7): 724–732. doi: 10.1016/j.chemosphere.2010.05.026.

Yao, Y., B. Gao, M. Inyang, A. R. Zimmerman, X. Cao, P. Pullammanappallil, and L. Yang. 2011. Biochar derived from anaerobically digested sugar beet tailings: Characterization and phosphate removal potential. *Bioresource Technology* 102 (10):6273–6278. doi: 10.1016/j.biortech.2011.03.006.

Zhang, H., C. Chen, E. M. Gray, S. E. Boyd, H. Yang, and D. Zhang. 2016. Roles of biochar in improving phosphorus availability in soils: A phosphate adsorbent and a source of available phosphorus. *Geoderma* 276:1–6. doi: 10.1016/j.geoderma.2016.04.020.

Zhang, H., R. P. Voroney, and G. W. Price. 2015. Effects of temperature and processing conditions on biochar chemical properties and their influence on soil C and N transformations. *Soil Biology and Biochemistry* 83: 19–28. doi: 10.1016/j.soilbio.2015.01.006.

Zimmerman, A. R., B. Gao, and M. Y. Ahn. 2011. Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biology and Biochemistry* 43 (6):1169–1179. doi: 10.1016/j.soilbio.2011.02.005.