International Journal of Research Studies in Science, Engineering and Technology
Volume 5, Issue 5, 2018, PP 9-18
ISSN : 2349-476X

Effects of Biochar Application on Beneficial Soil Organism Review

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ABSTRACT

Understanding the key roles of biochar properties on microbial activity in different soil types are essential to know the conditions of biochar to reach the desired benefits, and how can the trade-offs between various environmental and the biological effects of biochar. Focused on certain key functions of biochar, this review synthesizes the biochar-microbe interaction mechanisms pertinent to those questions, covering. The microbial community modification by biochar, via alteration of nutrient availability and soil characters, and the electron transfer between microbial cells and contaminants facilitated by biochar. In generally biochar and soil beneficial microorganism interaction cannot be determined in a single conclusion as biochar can affect soil beneficial microorganism positively and as well as negatively. This study provided evidence that beneficial soil microorganism improvements were directly or circuitously affected by biochar incorporation into soil results from the combination of a direct effect that is dependent on the type of char and a microbiome shift in root-associated beneficial bacteria. Therefore, in order to use in biochar for soil amendment, biochar feedstock, soil type, microorganism species, pyrolysis temperature must be considered.

Keywords: *Biochar, Microorganism, pH, pyrolysis, Microbe.*

INTRODUCTION

Biochar is the carbon-rich product obtained when biomass is heated in a closed container with little or no available air with the purpose to amend soil and means to sequester carbon (C) and maintain or improve soil functions (Lehmann and Joseph, 2009). Currently, in response to the need of more sustainable agricultural production and in order to tackle global warming, there are attempts to recreate Terra Preta (ancient soils amended with black carbon) by incorporating biochar to soils as means of increasing soil fertility and carbon sequestration (Lehmann et al., 2006). Biochar addition to soil has a great impact on plant development and root colonization by microorganisms (e.g. mycorrhizal fungi) and nematodes (Sławomir G et al., 2017). Interactions between biochar, soil, microbes, and plant roots were known to occur within a short period after application to the soil (Lehmann and Joseph 2009). According to Lehmann and Joseph (2009), Dissolution, hydrolysis, carbonation, and decarbonation, hydration, and redox reactions are the major process affecting biochar weathering in the soil, as well as interactions with soil biota. The rates at which these reactions occur depending on the nature of the reactions, type of biochar, and climatic conditions. Biochar can influence physical and chemical properties as well as beneficial soil microorganisms like bacteria, fungi, and invertebrates, both in field and laboratory conditions (Sławomir G et al., 2017).

Biochar has also been shown to enhance nutrient availability over longer time scales by enhancing nitrogen (N) mineralization or nitrification (DeLuca et al., 2006; Ameloot et al., 2015) as a result of enhanced microbial growth and activity (Lehmann et al., 2011) and by reducing soil nutrient losses due to its high ion exchange capacity (Atkinson et al., 2010). Numerous recent studies have shown that the positive effects of biochar on soil fertility can result in enhanced plant growth (Lehmann, 2007a; Jeffery et al., 2011; Biederman & Harpole, 2013), thereby having an indirect positive effect on net ecosystem C uptake.

Biochar, as a soil amendment, can increase microbial biomass (Kolb et al., 2009), stimulate soil microbial activity (Lehmann et al., 2011), change microbial community in soil (Pietikainen et al., 2000). Biochars application in the soil can affect soil microbial community structure due to their high sorption capacity (Lehmann et al., 2011), changing the soil pH (Rousk et al., 2010) as well as modification of microbial environment. (Lehmann et al., 2011) also reported that biochars contain compounds such as polycyclic aromatic hydrocarbons and other toxic carbonyl compounds that can have bactericidal or fungicidal activity. The experiment conducted by hypothesizing that, the application of biochar (pyrolyzed at 600°C with high pH value) improves soil organic carbon and soil pH and would have an effects on soil enzymes, microbial biomass, and community that support many key ecosystem functions essential for soil quality. It was found that soil pH, total organic carbon (TOC) and urease increased significantly with increasing biochar rate while the activity of acid phosphatase decreased, the reason can be the inverse correlation of this enzyme with soil pH. TOC had a positive correlation with urease. The β-glucosidase correlated positively with dissolved organic carbon (DOC) and negatively with C/N, suggesting that mineralization of organic matter provides substrates for this enzyme. The highest microbial biomass C as well as total Phospholipid fatty acid analysis (PLFA) were observed at the lowest rates, particularly the treatment of W0.5 had a higher relative abundance of soil bacteria, fungi and gram-positive bacteria (Walelign Demisie and Mingkui Zhang 2015).

Generally, Biochar addition to soils increases soil microbial biomass and changes microbial community structure and competition for available nutrients (Pietikäinen et al., 2000) by changing physicochemical properties of soils (Steiner et al., 2007). Bacteria and fungi were the primary decomposers of available soil organic matter and comprise 90 % of the soil microbial biomass (Pietikäinen et al., 2000). Therefore, the aim of this topic was to review the potential effects of biochar application on soil microorganisms.

Biochar – Microbe Interaction

Biochar affects the soil microbial activity and biomass, changes the soil bacteria to fungi ratio and soil enzyme activity, and reshapes the microbial community structure (Ahmad et al., 2016). Note that biochar application may significantly alter the microbial community structure even when it does not change the overall microbial activity and biomass. To clearly interpret the microbial responses to biochar application in soils, gene copy numbers serve as a more sensitive parameter than microbial biomass (Chen et al., 2013). Various techniques used to test microbial activity and community structure, including fluorescence in situ hybridization (FISH), phospholipids fatty acid quantitation (PLFA), and molecular fingerprinting of 16S rRNA gene fragments. Changes in the relative abundances of Acidobacteria, Actinobacteria, Gemmatimonadetes, and Verrucomicrobia were frequently detected using high-through sequencing, under treatment with biochar (Nielsen et al., 2014; Mackie et al., 2015).

Since the mechanisms, underlying biochar's effects on microbes and related soil functions and processes are still not quite clear; this review focuses on the synthesis of several possible mechanisms based on the published research and reviewed literature. The influences of biochar on microbial activity are diverse and seven possible mechanisms were demonstrated in the central circle of Fig. 1 (from which points 1 to 3 can be classified into direct influences and points 4 to 7 indirect influences). (1) Biochar provides shelter for soil microbes with pore structures and surfaces (Quilliam et al., 2013a). (2) Biochar supplies nutrients to soil microbes for their growth with those nutrients and ions adsorbed on biochar particles (Joseph et al., 2013). (3) Biochar triggers potential toxicity with environmentally persistent free radicals (Fang et al., 2014a). (4) Biochar modifies microbial habitats by improving soil properties that are essential for microbial growth (including aeration conditions, water content, and pH) (Quilliam et al., 2013a). (5) Biochar induces changes in enzyme activities that affect soil elemental cycles related to microbes (Lehmann et al., 2011; Yang et al., 2016b). (6) Biochar interrupts microbial intra and inter-specific communication between microbial cells via a combination of sorption and the hydrolysis of signaling molecules (Gao et al., 2016; Massillon et al., 2013). It should be noted that biochar might contain some molecules that can work as signals for microbial communication. (7) Biochar enhances the sorption and degradation of soil contaminants and reduces their bioavailability and toxicity to microbes (Stefaniuk and Oleszczuk, 2016).

Earthworms

Earthworms have very well known contribution in soil fertility by altering soil physical, chemical and biological properties, through grazing, burrowing and casting and dispersal. Earthworms alter soil structure and fertility, through their direct and indirect contribution to the creation of organic structures (biopores, biogenic aggregates) which alter microbial mineralization of organic matter and nutrient cycling (Lal, R., 2001) and can change soil enzymatic activity (Lal, R., 2001). This could lead to drastic shifts in the microbial communities within the bulk of soil and there has been some attention devoted to the interaction between earthworms and biochar. Earthworms cannot only ingest soil but also biochar (Downie A, et al. 2010), which could alter its distribution in the soil profile or biochar intrinsic properties. Earthworm-biochar interactions have been addressed in the context of soil protein turnover (Downie A, et al. 2010), plant resource allocation (Lavelle P, et al. 2010) enzyme activity or greenhouse gas emissions, but not with respect to microbial community structure. It has also been suggested that earthworms and, in particular, grinding and mixing activity of the circum tropical species Pontoscolex corethrurus, could have favored the genesis of Amazonian Dark Earths.

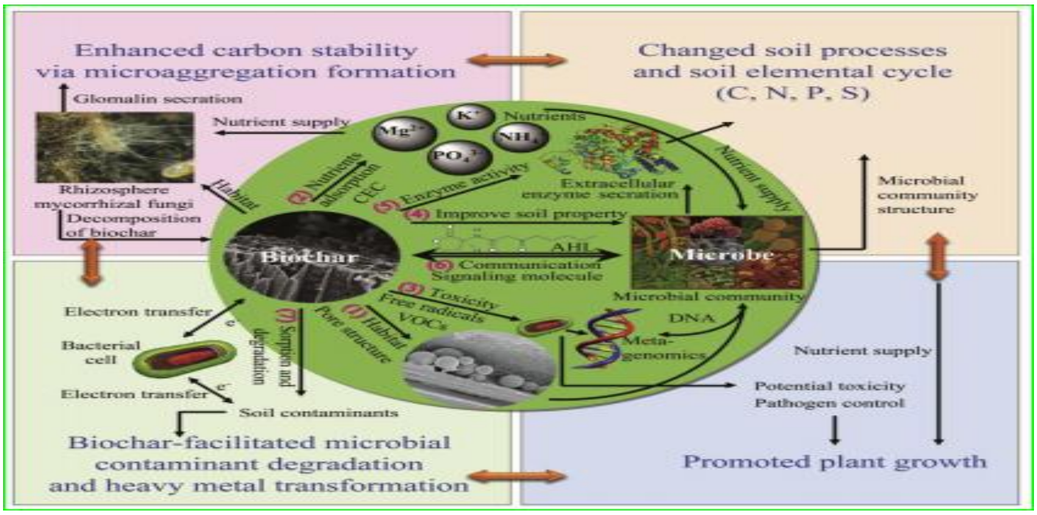

Figure1. *Proposed mechanisms of biochar-microbe interactions and the environmental effects of biochar. prsented by (Xiaomin Z., et al 2017)*

Bacterial

Jones, D.L., et al (2011), conductedthe experiment by the Enumeration of Microbes and Isolation of Root-Associated Bacteria revealed the results of the pot experiments as, Biochar at a concentration of 2% stimulated the growth of soybeans and thus was used for the characterization of root-associated plant growth promoting bacteria. The bacteria were enumerated after 48 h in the plate count agar medium. The total numbers of cultivable bacteria isolated from the rhizosphere of plants grown in soil without biochar were 1.5 × 107 CFU (colony-forming units, per gram fresh weight) and 5.3 × 107 CFU (per gram fresh weight) in soil with 2% biochar-amended soil. In total, 200 bacterial strains were isolated from the rhizosphere of soybeans. Among these, 90 isolates were selected from plants grown in control soil, and 110 isolates, from plants grown in char, amended the soil. All strains were tested for their abilities to stimulate root and shoot growth of soybeans under greenhouse conditions in the loamy sand soil. Root and shoot growth stimulating abilities (>20%) were observed in 27–32% of isolates from plants grown in soil without biochar and in 45–57% of isolates from soil amended with 2% char, respectively. A total of 32 isolates from the control plants and 43 isolates from the char amended soil induced stimulatory effects on plant growth compared with the non-treated control plants. Soil samples taken from the replicate field plots in summer 2010 and 2011 were used to assess bacterial growth and fungal growth and biomass. The bacterial growth was estimated using leucine incorporation in bacteria extracted from soil using the homogenization/centrifugation technique with modifications (Rousk and Bååth, 2011). The amount of Leu incorporated into extracted bacteria per h and g soil was used as a measure of bacterial growth. In the case of bacteria in 2010, their growth was stimulated by about 80%, from 47. ± 6.9 pmol Leu h1 g1 in the control soil to 85.5 ± 10.8 pmol Leu h1 g1in the presence of biochar ($P<0.05$).

Another study presented by De Tender, C.A et al., (2016) demonstrates the effect of oak wood pyrolysis biochar on strawberry grown in white peat and lettuce grown in field soil. In the strawberry bioassay, the addition of 3% w/w biochar to peat resulted in Changes in the rhizosphere microbiology such as an increase of bacterial diversity and a shift in the composition of the rhizosphere microbiota.

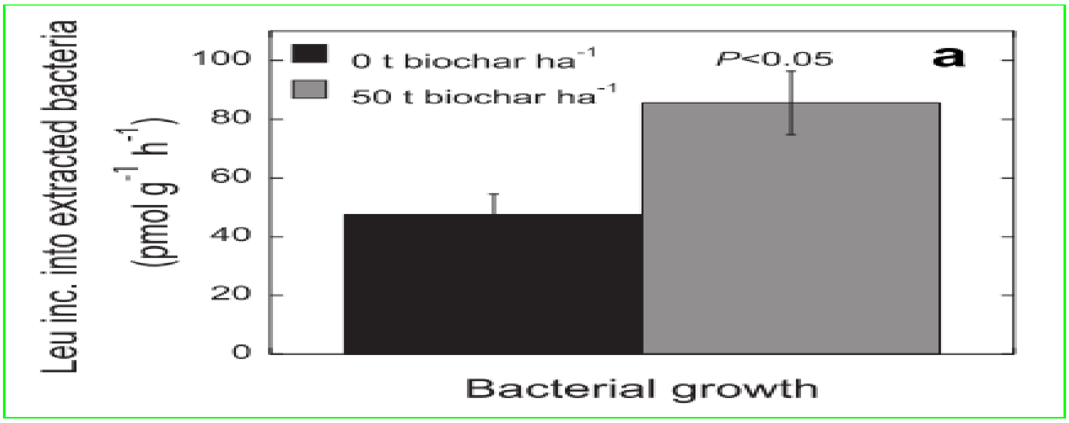

Figure3. *Jones, D.L., Edwards-Jones, G., Murphy, D.V., 2011. (Biochar mediated alterations in herbicide breakdown and leaching in soil. Soil Biol. Biochem. 43, 804e813)*

Biochar Influence on Rhizosphere Microorganisms

The kind of influence of biochar on the number and biomass of microorganisms and their effectiveness in colonizing plant roots were most likely associated with the type of the soil into which it has been introduced. Biochar can increase the biomass of microorganisms and their activity in soils. Kolb et al. (2009) observed that increased doses of charcoal increase the populations of soil microbes as measured by their respiration activity. Opposite effect of different kinds of biochar added into the soil on microbial activity was observed by Chintala et al. (2014). Corn stover biochar (CS), switchgrass biochar (SG), and Ponderosa pine wood residue biochar (WC) decrease of microorganism's activity measures as the activity of dehydrogenase and esterase. Miscanthus biochar addition increase abundances of genera of phosphorus and sulfur mobilizing bacteria like Acidothermus, Bacillus, Isosphaera, Planctomyces, Bradyrhizobium, Rhodobium, Pseudorabies and Rhodanobacter (Fox et al., 2016). Rice stem biochar (3% in soil) increased the abundance of living cells of Neorhizobium T1-17 strain in the soil in a pot experiment conducted by (Wang et al., 2016).

Table1. *Different biochar influence on soil microorganisms in different soils type*

Type of biochar/raw material	Effect on soil microorganisms	Soil type	Reference
Willow wood and swine manure digestate feedstock:	Increased microbial biomass in both cases and:	Sandy loam	Ameloot *et al.*, 2013
slowly pyrolyzed at 350°C biochar	-Increased dehydrogenase activity		
slowly pyrolyzed at 700°C biochar	-Decreased dehydrogenase activity		
Biochars: poultry litter (PL) and pine chips (P) at 400 or 500°C	Increased SOM and microbial biomass, higher N mineralization in (PL)	Silt loam	Ameloot *et al.*, 2015
Mixed leafy tree chipped trunks and branches biochar	increased soil respiration, fungal and bacterial growth rate	Eutric cambisol	Jones *et al.*, 2012
Fast-pyrolysis wood-derived biochar	increased microbial abundance with	Sandy loam/ clay/clay loam Gram-negative bacteria-domination	Gomez *et al.*, 2014
Wheat straw pyrolysis between 350°C and 550°C	-increased in bacterial 16S rRNA gene copy -decreased fungal 18S rRNA gene copy	Hydragric anthrosol	Chen *et al.*, 2013
Compost inoculated or not with AMF as a control and:		Sterilized soil-sand-clay mixture inoculated or not	Akhter *et al.*, 2015
Wood biochar + compost	-Increased root colonization by AMF in Fol+ treatment in comparison to Fol-	with *F. oxysporum* f.sp. *lycopersici* (Fol+ or Fol-)	
Green waste biochar + compost	-Decreased root colonization by AMF in Fol+ treatment in comparison to Fol-		
Empetrum nigrum L. twigs charcoal (EmpCh) forest humus charcoa (HuCh), both prepared at 450°C for 30 min	Imcreased microbial biomass carbon and number of cell in both biochar treatments in comparison to control	Scots pine and Norway spruce forest humus	Pietikäinen *et al.*, 2000

Source *Slawomir G.et al., 2016*

Microorganisms can also change the properties of biochar, especially when causing it to oxidize the surface of particles, which increases the oxygen content (from 7% to 24%). Carbon content decreases (from 91% to 71%) in biochar particles (Cheng et al., 2008a). These results in the formation of oxygen-containing groups, which form negatively, charged surfaces, leading to a greater cation exchange capacity (CEC) of biochar (Glaser et al., 2002) and nutrient retention in soil (Liang et al., 2006), in comparison to new, nonoxidized biochar. Microbial oxidation of biochar is more effective when it is conducted in the presence of organic matter, whereas in the absence of organic matter it does not produce oxidation effects as measured by CEC (Cheng et al., 2006).

Abundance of Microorganisms in Biochar-Amended Soil

Domene et al. (2014) indicated that microbial abundance could increase from 366.1 (control) to 730.5μgCg−1 after an addition of 30 t ha−1 biochar. Similarly, microbial abundance increased by 5–56 % with the increase of corn Stover biochar rates (from 0 to 14 %) for the different pre-incubation times of 2–61 days (Domene et al.2015). The different author lists some possible reasons that may be responsible for the increase of microbial abundance as follows. The higher availability of nutrients or labile organic matter on biochar surface (Pietikäinen et al.2000; Bruun et al. 2012), less competition (Lehmann et al.2011), are the major case of microbial abundance increments. Microbial abundance has been determined in biochar-amended soil by various methods. This method including total genomic DNA extracted (Grossman et al., 2010), phospholipids fatty acid (PLFA) extraction (Birk et al., 2009). The study conducted by (Thomas F, et al., 2015) focusing on two common soil series in the southeastern coastal plain utilizing feedstocks endemic to the area in four ratios (100% pine chip; 80:20 mixture of pine chip to poultry litter; 50:50 mixture of pine chip to poultry litter;100% poultry litter) using phospholipids fatty acid analysis (PLFA).

Table2. *Relative abundances (% of total) of PFA groups in biochar treatments.*

Soil	PLFA Group	Control	PC:PL 100:0	PC:PL 80:20	PC:PL 50:50	PC:PL 0:100
Coxville	Gram-positive	37.39 ± 0.18 †	36.82 ± 0.20	37.03 ± 0.88	35.86 ± 0.32 *	34.99 ± 0.20 *
	Gram-negative	36.04 ± 0.03	36.35 ± 0.32	36.17 ± 0.22	37.15 ± 0.34 *	38.44 ± 0.48 *
	Actinomycetes	19.90 ± 0.27	20.10 ± 0.14	19.94 ± 0.35	19.61 ± 0.18	19.49 ± 0.31
	Fungi	1.52 ± 0.18	1.59 ± 0.08	1.62 ± 0.43	1.81 ± 0.04	1.74 ± 0.16
	Eukarya	0.58 ± 0.30	0.42 ± 0.08	0.55 ± 0.32	1.00 ± 0.02	0.96 ± 0.11
Norfolk	Gram-positive	34.80 ± 0.44	33.91 ± 0.93	33.55 ± 1.17	29.93 ± 0.68 *	28.63 ± 0.38 *
	Gram-negative	35.24 ± 0.74	36.58 ± 0.48	36.24 ± 0.89	38.68 ± 0.81 *	41.00 ± 1.09 *
	Actinomycetes	18.25 ± 0.37	17.69 ± 0.93	17.64 ± 0.44	17.22 ± 0.23 *	18.36 ± 1.32
	Fungi	4.61 ± 1.55	4.19 ± 2.00	4.59 ± 1.56	5.61 ± 1.18	4.61 ± 1.18
	Eukarya	0.74 ± 0.02	0.97 ± 0.21	0.82 ± 0.10	1.35 ± 0.86	1.12 ± 0.04 *

† Mean and standard deviations; * indicates statistically significant differences ($p < 0.05$) compared to control sample.

Source: (Thomas F, et al., 2015)

Then the results demonstrated significant shifts in microbial community composition in response to biochar amendment (Thomas F, et al., 2015).

The microbial reproduction rate has also been shown to increase in some biochar-amended soils (Pietikäinen et al., 2000; Steiner et al., 2009), and in wastewater. Similarly, in biodigesters used to generate methane (CH4) as an energy source, additions of biochar (commercial wood charcoal) led to an increase in anaerobic and cellulose-hydrolyzing bacterial abundance. The reasons for changes in microbial abundance may differ for different groups of microorganisms. The two most commonly occurring types of mycorrhizal fungi (arbuscular (AM) and ectomycorrhizal (EM), are often positively affected by biochar presence, as reviewed in (Warnock et al., 2007). The mycorrhizal response in the host plant is most commonly assessed by measuring root colonization; that is, the abundance of fungal tissue in the host. Both formation rate and tip number of EM infection of larch seedling roots were increased by 157% with biochar additions.

Summary of possible mechanisms by which microbial abundance is affected by biochar additions to soil was reviewed on Biochar-Rhizosphere Interactions – a Review by (Slawomir G.et al., 2016).

Negative Effects Of Biochar On Soil Biota

Negative, null, or positive effects of biochar on soil microbial community may depend on the biochar and soil type. Organic pyrolytic products, such as phenolics and polyphenolics, may be present in biochar and were harmful to soil microorganisms. (Ding, Liu Y. et al., 2016) indicated that mycorrhizae and total microbial biomass decreased after biochar application. (Gell et al., 2011) and (Ennis et al., 2012) reported that the decrease in microbial abundance and activities might expected with an enhanced retention of toxic substances, such as heavy metals and pesticides, and the release of pollutants from biochar, such as bio-oil and polycyclic aromatic hydrocarbons. Moreover, some biochars might pose a direct risk to soil biota and their functions (Liesch et al. 2010) and that need to be taken seriously in consideration and be evaluated for their suitability as a soil amendment. Therefore, it is not valid to conclude that a special biochar, which has positive effects on one soil biota, would also have similar effects on others. Several factors are likely to be responsible for the negative effects of biochar on soil biota, including the volatile matters, properties of biochar as well as salts, such as Cl or Na amount in the soil.

Table3. *Summary of mechanisms by which microbial abundance is affected by biochar*

Mode of action	Rhizobia or other N assimilators	Other bacteria	Mycorrhizal fungi	Other fungi	References
Better soil hydration	rnk	+	rnk	+	Pietikäinen *et al.*, 2000; Thies and Rilling, 2009
Increased N availability	+ or –	rnk	rnk	rnk	Laird *et al.*, 2010; Wang *et al.*, 2013; Güereña *et al.*, 2015; Wang *et al.*, 2015,;
Improved other macronutrient availability	rnk	+	+	rnk	Laird *et al.*, 2010; Yao *et al.*, 2012; Postma *et al.*, 2010; Hammer *et al.*, 2014
Increased pH	+	+	rnk	rnk	Beesley *et al.*, 2010
Habitat formation and/or protection from grazers	rnk	+	+ or -	rnk	Ishii and Kadoya, 1994; Pietikäinen *et al.*, 2000; Gryndler *et al.*, 2006, Birk *et al.*, 2009, Rillig *et al.*, 2010; Warnock *et al.*, 2010, Jaafar, 2014
Sorption/transformation of inhibitory compounds	rnk	+	rnk	+	Kim *et al.*, 2013; Mitchell *et al.*, 2015
Sorption of signalling compounds	rnk or -	rnk	rnk	rnk	Ni *et al.*, 2011, Masiello *et al.*, 2013
Biofilm formation	+	+	rnk	rnk	Piscitelli *et al.*, 2015
Sorption of dissolved OM as an energy source for microorganisms	rnk	+	nc	rnk	Pietikäinen *et al.*, 2000; Steiner *et al.*, 2008

Note: (+) indicates that relative abundance may increase (not necessarily better growth conditions); (–) indicates that relative abundance decreases; (nc) – no change; (rnk) – reaction not known.

Source: (Slawomir G.et al., 2016)

Interactions Of Biochar And Microorganisms In Soil.

Biochar affects the soil microbial activity and biomass, changes the soil bacteria to fungi ratio and soil enzyme activity, and reshapes the microbial community structure (George et al., 2012; Mackie et al., 2015; Ahmad et al., 2016). Note that biochar application may significantly alter the microbial community structure even when it does not change the overall microbial activity and biomass. To clearly interpret the microbial responses to biochar application in soils, gene copy numbers can serve as a more sensitive parameter than microbial biomass (Chen et al., 2013). Biochar soil amendment increased bacterial but decreased fungal gene abundance with shifts in community structure in a slightly acid rice paddy in Southwest China (Chen et al. 2013). In greenhouse studies, biochar added at 1.5 & 3.0% (wt/wt) to field soil caused proportional increases in root weights and linear reductions in the percentage of root lesions caused by Fusarium species.

Soybean plants grown in soil amended with HC char (2%) showed the best performance and were collected for isolation and further characterization of root-associated bacteria for multiple plant growth promoting traits. Only HTC char amendment resulted in a statistically significant increase in the root and shoot dry weight with higher diversity than the rhizosphere isolates from the control soil of soybeans (Wirth S, et al., 2016). In addition, a higher proportion of isolates from HTC char amended soil compared with control soil was found to express plant growth promoting properties and showed antagonistic activity against one or more phytopathogenic fungi. That study provided evidence that improved plant growth by biochar incorporation into soil results from the combination of a direct effect that is dependent on the type of char and a microbiome shift in root-associated beneficial bacteria (Egamberdieva D, et al., 2016)

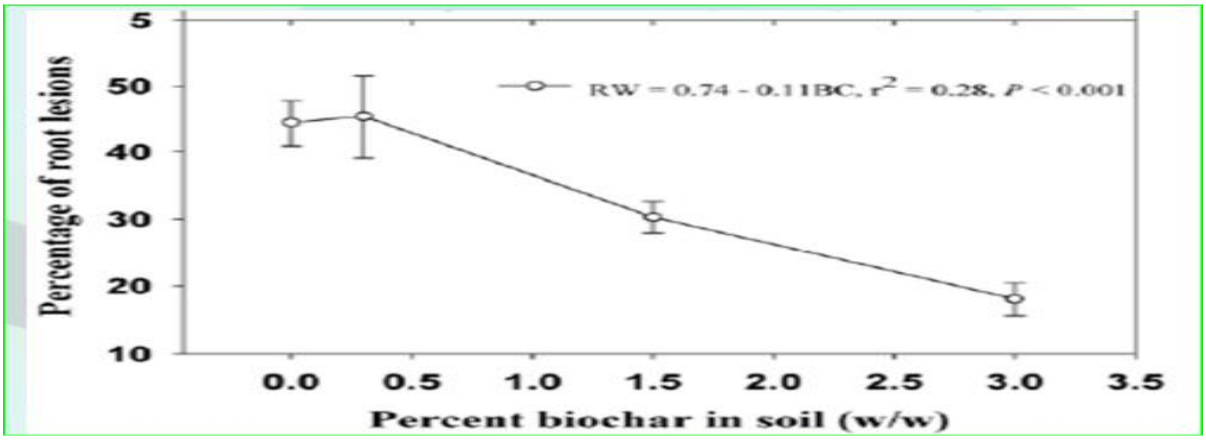

Figure3. *Addition of aromatic acids that are known allelopathic agents reduced AM colonization but the deleterious effects were not observed following the application of biochar at the higher rate. Effect of Biochar Amendments on Mycorrhizal Associations and Fusarium Crown and Root Rot of Asparagus in Replant Soils (Elmer, et al. 2011).*

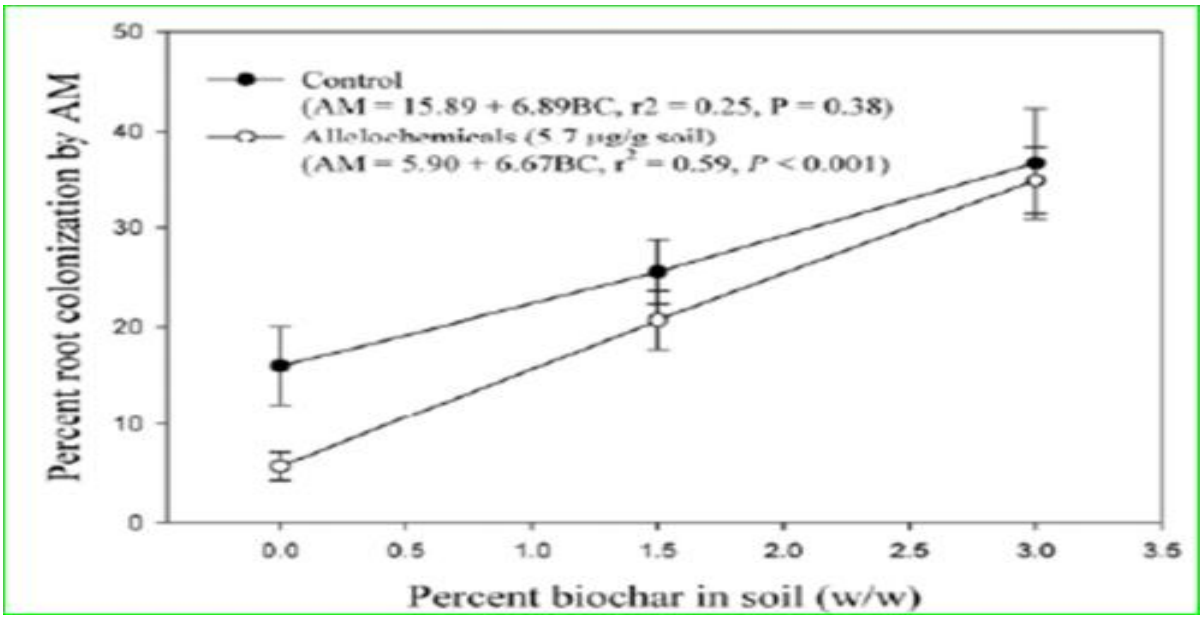

Figure4. *Elmer, W. H., and Pignatello, J. J. 2011.*

(Wirth S, Behrendt U, Abd_Allah EF and Berg G 2016), Reported that the application of biochar to soil is considered to have the potential for long-term soil carbon sequestration, as well as for improving plant growth and suppressing soil pathogens. The evaluated the effect of biochar on the community composition of root-associated bacteria with plant growth promoting traits by using three types of biochar, namely, maize biochar (MBC), wood biochar (WBC), and hydrochar (HC) were used for pot experiments to monitor plant growth.

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Citation: *Leta, A. (2018).“Effects of Biochar Application on Beneficial Soil Organism Review” International Journal of Research Studies in Science, Engineering and Technology, 5(5), pp.9-18.*