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Review

Physico-chemical properties and microbial responses in biochar-amended soils: Mechanisms and future directions

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A B S T R A C T

Soil microbial communities are responsive to biochar amendments. As the residence time of biochar in soil is expected to be hundreds to thousands of years, the changes in microbial community structure and functions could persist for a long period of time. Given that biochar is being applied as a soil amendment in many parts of the world, the long-term consequences for soil microbial communities need to be considered. The objective of this review is to document how biochar creates new habitats and changes the soil environment for microorganisms, which may lead to changes in microbial abundance, community structure and activities. Our meta-analysis revealed that slow pyrolyzed biochars produced from various feedstocks at temperatures from 300 °C to 600 °C consistently increased some physico-chemical properties (i.e., pH, cation exchange capacity and aggregation) and microbial parameters (i.e., abundance and community structure of microorganisms) in a vast number of soils during short ($\leq$90 days) laboratory incubations and longer (1–3 years) field studies. The biochar-mediated changes in soil physico-chemical and biological properties appeared to be a function of soil texture and biochar type based on its feedstock and production temperature, which determines key biochar characteristics such as surface area, porosity and pH. Biochars derived from manure or crop residue feedstocks tend to promote microbial abundance more than wood-derived biochars. Biochars derived from wood and other lignocellulosic-rich feedstocks tend to exhibit beneficial effects on soil microbial abundance later ($\geq$60 days) than biochars from manure or crop residue feedstocks. Coarse textured soils tend to have less aggregation, lower microbial biomass and lower enzyme activities when amended with slow pyrolyzed biochars produced at high temperatures (>600 °C), but these biochars did not affect the physico-chemical and biological properties of clayey soils. Further research is needed to evaluate the magnitude of biochar influence on soil microbial abundance and activities considering (1) the biochar particle size, surface area, porosity, nutrient content and pH, and (2) the soil organic matter (SOM) content and microbial abundance of the soil matrix. Once the microbial activities in the biochar–soil system are understood, they can be manipulated through organic and inorganic fertilizer applications to sustain or improve agricultural crop production.

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1. Introduction

Biochar is a solid carbonaceous residue made by burning biomass under oxygen-free to oxygen-deficient conditions. Wood chips, crop residues, nut shells, seed mill screenings, algae, animal manure and sewage sludge are some of the many feedstocks used in biochar production. Biochar is highly resistant to decomposition when applied to soil, its residence time ranges from tens of years to millennia (Preston and Schmidt, 2006; Verheijen et al., 2010). The persistent nature of biochar-C in soil indicates that it will contribute to soil C sequestration (Ennis et al., 2012; Lai et al., 2013; Malghani et al., 2013) and reduce greenhouse gas emissions (Stewart et al., 2013), resulting in a negative carbon balance for bioenergy generation systems that produce biochar (Lehmann, 2007).

Historically, biochar was used as a soil amendment for at least 2000 years in the Amazon basin. The “Terra Preta” soils that were regularly amended with biochar and other organic materials (e.g., fish and animal bones, plant tissues, animal feces) have higher pH, are richer in nutrients and have larger microbial populations and more diverse microbial community structure than unamended Oxisols, which are generally acidic and infertile (Liang et al., 2008; Germano et al., 2012; Taketani et al., 2013; Table 1). The higher productivity of Terra Preta soils than their unamended Oxisol counterparts led to world-wide interest in applying biochar to agricultural soils and is creating new markets for the biochar produced as a co-product from the thermochemical conversion of biomass via pyrolysis. Soil microbial communities are responsive to biochar amendment because it increases microbial abundance and activities (Lehmann et al., 2011; Chan et al., 2008; Ameloot et al., 2013a) by providing an environment with ample aeration, water and nutrients (Ameloot et al., 2013b; McCormack et al., 2013). A diverse microbial community structure is implicated in efficient nutrient transfer to crops and greater nutrient retention in soil (e.g., Gul et al., 2014a,b), which is beneficial in reducing nutrient loss from agricultural soil to the environment.

Table 1
Characteristics of Tera Preta soils of various land use types (i.e., secondary forest, grassland and agricultural land, compared to nearby unamended Oxisols (compiled from Liang et al., 2008; Germano et al., 2012; Taketani et al., 2013).

Soil chemical characteristics	Terra Preta	Unamended Oxisol
pH	4.1–5.5*	2.6–3.8
Organic C content (g kg^{-1})	15.7–31.5*	10.2–21.8
Total nitrogen (mg kg^{-1})	10–18	4–16
Total phosphorus (mg kg^{-1})	5026–9064*	139–273
Total calcium (mg kg^{-1})	40–17545*	50–165
Soil biological characteristics: microbial diversity indices		
Shannon–Weiner	6.08–6.38	5.59–5.66
Simpson	0.004	0.006–0.007
ACE (abundance-based coverage estimators)	1834.0–3523.3	1559.6–1684.5
S_{obs}	941–1696	820–852
Chao1	1551.1–2736.4	1214.4–1379.9
Singletons	10–17	11–13

Values with an asterisk (*) were significantly different ($P < 0.05$) the referenced papers.

The thermochemical conversion processes generating renewable fuels such as combustible gas (syngas) and bio-oil, leaving biochar as a byproduct, include slow and fast pyrolysis, gasification and hydrothermal carbonization. Due to the cost and scale of production that is commercially feasible, the slow and fast pyrolysis pathways are most commonly employed in making biochar to be used as a soil amendment for agriculture. Slow pyrolysis biochar is a product of traditional heating of feedstocks under oxygen-limiting conditions, for cooking and house-warming purposes and it is achieved by heating the feedstocks at temperatures from 300 to 800 °C at atmospheric pressure for hours to days (Brewer and Brown, 2012). Fast pyrolysis aims to maximize the production of bio-oil by rapid quenching of vapor produced from burning biomass at higher temperatures (400–1000 °C) with a fast heating rates i.e., >300 °C s^{-1}, for few hours (i.e., 1–2 h; Brewer and Brown, 2012; Mohanty et al., 2013).

The physico-chemical characteristics of slow and fast pyrolysis biochars depend on the feedstocks and production temperature used. Higher production temperatures yield biochars with greater surface area and porosity (Mukherjee et al., 2011; Brewer and Brown, 2012; Mohanty et al., 2013), more alkaline pH, higher carbon:nitrogen (C:N) ratio (Singh and Cowie, 2010; Cantrell et al., 2012; Novak et al., 2013; Ronsse et al., 2013) and lower dissolved organic carbon (DOC) concentrations (Uchimiya et al., 2013; Budai et al., 2014; Rajapaksha et al., 2014). These variations in biochar characteristics have implications when biochar is applied as a soil amendment. Depending on the native soil properties (e.g., texture and SOM content), biochar inputs can cause negligible to significant alteration of soil physico-chemical and biological properties.

The objective of this review is to document how biochar produced from slow and fast pyrolysis creates new habitats and changes the soil physico-chemical environment for microorganisms, which may lead to changes in microbial abundance, community structure and activities. Specifically, this review seeks to answer the following questions: (1) how does biochar type, based on its feedstock, production temperature and characteristics such as surface area, porosity and pH, affect soil physico-chemical and biological properties? and (2) will soil attributes (e.g., texture) buffer or resist biochar-induced changes in physico-chemical properties and microbial processes?

2. Biochar properties as function of feedstock and production temperature

Each biochar has distinct physico-chemical properties such as surface area, pH, concentration of various elements/nutrients (e.g., carbon (C), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca)). These biochar properties are a function of the feedstock and biochar production temperature, as shown in Table 2. Generally, biochars produced from seaweeds, manures and crop residues are richer in nutrients, have higher pH and less stable carbon than lignocellulosic rich feedstocks such as wood (Bird et al., 2011; Brewer and Brown, 2012; Novak et al., 2013; Table 2). In general, the nutrients such as P, K, Ca, surface area, pH, carbon:nitrogen (C:N) and carbon:oxygen (C:O) ratio of biochar increases, while DOC and dissolved organic matter concentration decreases when biochar production temperature increases (Huff et al., 2014; Crombie et al., 2013; Uchimiya et al., 2013; Table 2). Fast pyrolysis tends to have no effect on the biochar C:O ratio, relative to slow pyrolysis, however, it increases the surface area of biochar (Mohanty et al., 2013; Chintala et al., 2014a; Table 2).

3. Biochar-mediated changes in soil physico-chemical properties

There is ample evidence from the scientific literature that biochar improves soil physical qualities of importance for crop production. Greater aeration and water holding capacity is reported in biochar-amended soils, due to the fact that biochar inputs reduced bulk density, enhance porosity and reduce evapotranspiration (e.g., Busscher et al., 2010; Githinji, 2014; Herath et al., 2013; Ibrahim et al., 2013; Lashari et al., 2013; Mukherjee and Lal, 2013; Schulz et al., 2014). Of interest in this review is how biochar mediates changes in soil physico-chemical properties of importance to microorganisms. Clearly, this involves both physical changes, such as in the soil pores where microorganisms live at the water–air interface, and chemical changes in soil solution that microorganisms rely upon to obtain substrates and energy as well as on organo-mineral surfaces where biofilms and fungal hyphae bind to the soil matrix. The following sections describe how biochar amendment impacts several key physico-chemical parameters of importance for soil microbial communities. It should be noted that the empirical evidence comes from research carried out with fine particle sized (<2 mm) biochars mixed thoroughly in soils for incubation- and pot-based studies. This could describe short-term changes in soil physico-chemical properties following biochar addition. For field-based studies, the biochars were mostly broadcast on the soil surface then incorporated by plowing/harrowing in the topsoil (to a maximum depth of15 cm). Although, field-based studies include factors such as climate, fertilizer applications, and tillage practices that can alter soil physico-chemical and biological properties and might exaggerate/mask the biochar-induced results on these soil parameters, field studies are important to be evaluate the consistency of results obtained from controlled versus field-based studies.

Table 2
Physico-chemical properties of biochars obtained from various feedstocks that underwent slow and fast pyrolysis at various temperatures (°C). Values with an asterisk (*) were significantly different ($P < 0.05$) between the biochar production methods, for a given biomass feedstock.

Biomass feedstock	Biochar production temperature (°C)	Porosity (surface area m^2/g)	pH	C (%)	O (%)	N (%)	P ($g\,kg^{-1}$)	K ($g\,kg^{-1}$)	Ca ($g\,kg^{-1}$)	Reference
Corn stover	350	[a]–	–	67.5		0.93	–	1.04	0.27	Nguyen and Lehmann (2009)
	600	–	–	79.0		0.92	–	0.67	0.31	
Eucalyptus saligna wood	400	–	6.9	69.4	–	0.21	0.13	1.43	11.24	Singh and Cowie (2010)
	550	–	8.82	83.6	–	0.26	0.22	2.36	21.26	
Eucalyptus saligna leaf	400	–	9.17	66.2	–	1.64	2.08	12.82	17.14	
	550	–	9.88	71.9	–	1.7	2.67	14.92	20.52	
Poultry litter	400	–	9.2	43.1	–	5.18	5.76	24.85	33.35	
	550	–	10.2	41.3	–	3.8	6.04	22.98	39.85	
Cow manure	400	–	9.03	17.5	–	1.35	4.36	26.43	17.52	
	550	–	8.94	16.5	–	1.1	4.93	23.08	18.81	
Oak wood	400	252	6.7	–		–	–	–	–	Mukherjee et al. (2011)
	650	528	9.3	–		–	–	–	–	
Pine wood	400	361	–	–		–	–	–	–	
	650	643	–	–		–	–	–	–	
Grass	400	164	–	–		–	–	–	–	
	650	427	–	–		–	–	–	–	
Paved-feedlot manure	350	–	9.1	53.3	–	3.64	11.26	32	22.7	Cantrell et al. (2012)
	700	–	10.3	52.4	–	1.70	17.9	49.1	35.0	
Dairy manure	350	–	9.2	55.8	–	2.60	10.38	14.3	26.7	
	700	–	9.9	56.6	–	1.51	17.0	23.1	44.8	
Poultry manure	350	–	8.7	51.07	–	4.45	21.23	48.5	26.6	
	700	–	10.3	45.91	–	2.07	32.1	74.0	40.2	
Turkey manure	350	–	8.0	47.28	–	4.07	27.2	40.1	40.4	
	700	–	9.9	44.77	–	1.94	38.4	55.9	56.1	
Conocarpus waste	200	–	7.37	64.19	–	0.69	0.84	0.38	43.4	Al-Wabel et al. (2013)
	400	–	9.7	76.83	–	0.87	0.88	0.54	51.8	
	600	–	12.2	82.93	–	0.71	1.11	0.90	64.7	
	800	–	12.4	84.97	–	0.90	1.34	1.15	67.5	
Wheat straw	[c]SP (400 °C)	178	–	65.2	31.2	0.9	3.51	75.5	10.8	Mohanty et al. (2013)
	[d]FP	184	–	64.8	31.5	0.8	3.62	76.5	11.5	
Timothy grass	SP (400 °C)	179	–	67.5	30.8	1.9	4.93	48.3	99.0	
	FP	203	–	63.7	28.2	1.9	4.69	46.4	84.0	
Pine wood	SP (400 °C)	166	–	81.4	20.5	0.3	0.57	29.0	58.0	
	FP	185	–	75.5	15.3	0.2	0.46	19.0	47.0	
Sugarcane Bagasse	350	–	–	75.2	–	0.66	0.50	3.78	2.04	Novak et al. (2013)
	500	–	–	85.4	–	0.79	0.63	[b]5.01*	3.28*	
Peanut hull	400	–	–	74.8	–	2.7	2.58	18.55	5.21	
	500	–	–	81.8	–	2.7	2.61	19.09	6.22*	
Pecan shell	350	–	–	64.5	–	0.3	0.25	2.34	11.0	
	700	–	–	91.2	–	0.26	0.46	4.56*	23.3*	
Pine chip	350	–	–	74.7	–	0.45	0.21	1.93	3.32	

Table 2 (*Continued*)

Biomass feedstock	Biochar production temperature (°C)	Porosity (surface area m^2/g)	pH	C (%)	O (%)	N (%)	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Reference
	500	–	–	87.2	–	0.43	0.28	2.70*	0.05*	
Poultry litter	350	–	–	46.1	–	5.0	29.43	58.86	44.3	
	700	–	–	44.0	–	2.8	42.79	86.64*	62.8*	
Switchgrass	250	–	–	55.3	–	0.43	1.01	4.87	1.12	
	500	–	–	84.4	–	1.1	2.39*	11.59*	5.12*	
Pine wood	450	23	6.7	86.3	–	–	–	–	–	Ronsse et al. (2013)
	750	–	10.4	92.5	–	–	–	–	–	
Straw	450	–	10.1	86.4	–	–	–	–	–	
	750	–	11.9	93.7	–	–	–	–	–	
Green waste	450	17	10.0	82.9	–	–	–	–	–	
	750	–	11.6	93.2	–	–	–	–	–	
Dry algae	450	14	9.3	78.8	–	–	–	–	–	
	750	–	12.5	90.6	–	–	–	–	–	
Corncob	377	–	–	74.1	20.6	0.6	–	–	–	Budai et al. (2014)
	562	–	–	86.6	9.1	0.8	–	–	–	
	693	–	–	89.4	5.6	0.9	–	–	–	
Miscanthus	369	–	–	66.6	22.3	0.4	–	–	–	
	503	–	–	64.5	11.2	0.5	–	–	–	
	693	–	–	84.6	6.1	0.7	–	–	–	
Corn stover	SP	38	11	73.6	0.04	0.14	–	–	–	Chintala et al. (2014a)
	FP	241	9.8	60.6	0.10	0.42	–	–	–	
Pine wood	SP	48	5.8	82.1	0.04	0.12	–	–	–	
	FP	190	8.5	54.0	0.05	0.15	–	–	–	
Pine wood	300	–	6.4	74.17	14.54	–	–	–	–	Huff et al. (2014)
	400	–	8.4	81.64	5.26	–	–	–	–	
	500	–	8.2	83.2	4.05	–	–	–	–	
Tea waste	300	2.28	7.93	70.5	19.62	4.97	–	–	–	Rajapaksha et al. (2014)
	700	342.22	11.05	85.11	8.88	3.92	–	–	–	

[a] Represents “no data”.
[b] Represents values significant at $P < 0.05$.
[c] Represents slow pyrolysis.
[d] Represents fast pyrolysis.

3.1. pH and cation exchange capacity

An increase in soil pH following biochar application is frequently reported for across many soil types (e.g., Glaser et al., 2002; Ameloot et al., 2013a; Farrell et al., 2013; Masto et al., 2013; Stewart et al., 2013; Chintala et al., 2014b; Xu et al., 2014). This is due to the alkaline pH of biochar, which is positively related to its production temperature and type of feedstock (i.e., wood-based biochar tends to have higher pH than biochar made from crop residue and manure; Table 2). Another reason for pH increase in biochar-amended soils is the presence of negatively charged phenolic, carboxyl and hydroxyl groups on biochar surfaces (Brewer and Brown, 2012; Chintala et al., 2014b) that bind H$^+$ ions from the soil solution, thereby reducing the H$^+$ ion concentration in the soil solution and increasing the soil pH value. Moreover, the silicates, carbonates and bicarbonates originating from biochar can bind to H$^+$ ions and thereby remove them from soil solution, also contributing to an increase in soil pH. The positive influence of biochar on increasing soil pH is more profound in acidic soils and soils with low SOM content (e.g., Stewart et al., 2013), probably because SOM content is linked to the pH buffering capacity of soil (Curtin and Rostad, 1997; Curtin and Trolove, 2013; Kogel-Knabner and Amelung, 2014).

As biochar increases the pH-dependent charge of soil, this contributes to an increase in cation exchange capacity (CEC) (Liang et al., 2006; Chan et al., 2007; Nelissen et al., 2012; Masto et al., 2013; Mukherjee and Lal, 2013; Taketani et al., 2013; Ducey et al., 2013) by reducing the leaching of base cations in competition with H$^+$ ions via enhanced binding to negatively charged functional sites of organic matter (OM), biochar and organo-mineral complexes. Consequently, the precipitation of cations and formation of OH—H bonds on functional sites of organo-mineral complexes (and biochar) allows cations to make weak hydrogen bonds with OH—H bonds (e.g., Brady and Weil, 2008). The high surface area and high pH of biochars produced at higher temperatures (>600 °C) may compensate for the low biochar CEC due to low O:C atomic mass ratio (Huff et al., 2014; Wan et al., 2014) to offer greater CEC provision to soil. However, the magnitude of this effect may depends on the SOM content, which is the primary determinant of soil CEC (Sylvia et al., 2005; Brady and Weil, 2008). For instance, there was no change in the CEC of a sandy soil following application of 3% and 6% (w/w) of hardwood-derived fast pyrolysis biochar during a 91 days laboratory incubation (Basso et al., 2013), possibly due to the low SOM content and low CEC of the soil prior to biochar amendment.

Biochar properties change with its aging in soil, most notably due to its oxidization and accumulation of H$^+$ from the soil solution in the first weeks and months after it is added as a soil amendment. This degree to which biochar properties change with time depends on the biochar source (Heitkotter and Marschner, 2015), soil and climatic conditions (Cheng et al., 2008). A decrease in pH as ΔpH (subtraction of values for aged minus fresh biochars) −2.27 to −3.56 for slow pyrolyzed pine chip corn digested biochars produced at 400 °C and 600 °C in silt loam soil during 100 days of incubation in the laboratory was reported ($P < 0.05$, Heitkotter and Marschner 2015), while ΔpH −1 to −4.4 was reported for two wood derived and one macadamia nut shell biochars slow pyrolyzed at 500–550 °C during a 3 years period after they were buried in loamy soil under field conditions (Spokas, 2013). The natural fire produced wood biochar buried in clay loam soil in field for four months, when incubated in same soil for 70 days, showed 10% significantly reduced CEC of soil as compared to fresh biochar produced from the same feedstock at 450 °C. Although the pH of aged and fresh biochars was same, the surface area of aged biochar was ~2 times lower than fresh biochar (Zhao et al., 2015). This implies that the magnitude of the biochar-induced changes in soil physico-chemical and biological properties are dynamic, such that short-term changes may not be indicative of longer-term conditions in biochar-amended soils.

3.2. Soil aggregation

The positive influence of slow pyrolysis biochars (production temperatures 400–600 °C) in promoting soil aggregation is reported for soils ranging in texture from sandy loam to clay loam (Ibrahim et al., 2013; Jien and Wang, 2013; Mukherjee and Lal, 2013; Sachdeva, 2013; Demisie et al., 2014; Soinne et al., 2014; Khademalrasoul et al., 2014), in both field and incubation studies. The increase in soil aggregation with concomitant increase in SOM and microbial biomass in response to amendments of bamboo-600 and oak wood-600 biochars in clay loam soils during 372 days incubation period was also reported (Demisie et al., 2014). While SOM content and clay content are the primary determinants of aggregation in biochar-amended soil (Khademalrasoul et al., 2014), biochar properties such as surface area and O:C ratio are important to describe the binding of biochar to organo-mineral complexes as a preliminary step in the aggregate formation and stabilization process. Quinone groups in biochar as the main electron shuttling, redox-active moieties (Klupfel et al., 2014), are responsible for two-way direct linkage between organic or mineral surfaces or three-way indirect bindings via non-biochar organic matter-cross-linking agent, which bind biochar to mineral surfaces (Solomon et al., 2012; Joseph and Taylor, 2014; Kleber et al., 2014). Still, biochar generated under high production temperatures (700 °C) with low O:C ratio (42.13) did not change aggregation in a coarse-textured soil (Busscher et al., 2010, 2011), possibly due to the low OM and clay content of the soil. This has led several authors to propose that coarse-textured soils (e.g., sandy to sandy loam) with low SOM contents need to be co-amended with biochar and organic residues to promote soil aggregation (Busscher et al., 2010, 2011; see also Awad et al., 2013; Khademalrasoul et al., 2014).

3.3. Retention of low-molecular weight substances

At the time of production, biochar possesses reactive surfaces characterized by high surface area, the presence of pores and the negative charges from hydroxyl (—OH), carboxylic acids (—COOH) and small alkyl chains such as methane groups (—CH_3) (Brewer and Brown, 2012; Kameyama et al., 2012). These attributes are expected to increase nutrient retention in biochar-amended soil including negatively charged ions such as NO_3^- and $HPO_4^2/H_2PO_4^-$ (Major et al., 2009; Kameyama et al., 2012; Prommer et al., 2014) and DOC (Pietikainen et al., 2000; Abit et al., 2012; Lu et al., 2014; Ventura et al., 2014).

The ability of biochar to induce greater retention of ions and low molecular weight organic compounds, is related to (1) the biochar properties, such as surface area and O:C ratio, and (2) the alteration of charge/chemistry of the native soil organo-mineral surfaces mediated by biochar application. With regards to the biochar properties, these can be controlled by careful selection of feedstock and production temperature; however, the retention capacity of biochar will decline as it "ages" in the soil environment due to weathering, loss of reactive surface due to irreversible binding with soil substances, decrease in its pH (Spokas 2013), and decrease in its bulk density (Feng et al., 2014). Therefore, the physico-chemical properties of fresh biochar are useful in predicting its reactivity in the short-term (e.g., for periods of a few months) but probably not for field studies of longer duration. Furthermore, any alteration of charge/chemistry of the native soil organo-mineral surfaces mediated by biochar application depends on the quantity of biochar added and how often, the biochar's physiochemical properties and the response of the native soil that may be of short or long duration, depending on factors such as its SOM content and soil texture. Considering Terra Preta soils as a long-term case study, it is possible to permanently alter the soil's ability to induce greater retention of ions and low molecular weight organic compounds when regular biochar amendments are included as part of the agricultural regime. The assumption is that these alterations are a direct consequence of biochar application, and needs to be verified for other soils around the world.

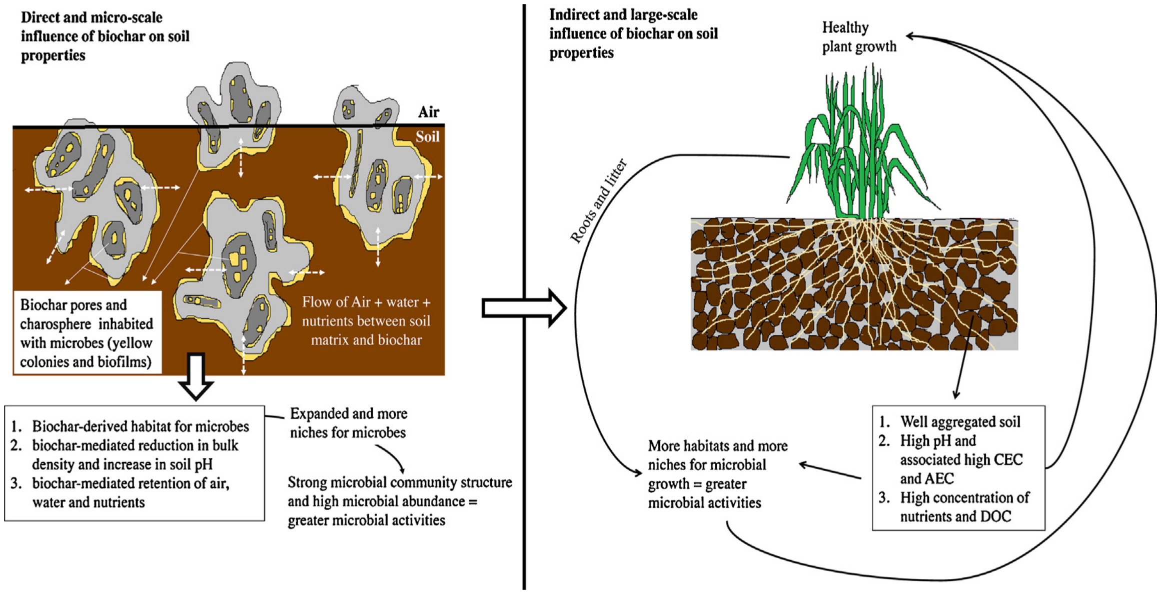

Fig. 1. A conceptual model illustrating the direct micro-scale and indirect large-scale influence of biochar on microbial activites by altering soil properties and providing them with more habitat and extended niches.

Table 3

Influence of biochar application, considering the source, production temperature (PT) and application rate (Appl. rate), soil characteristics (texture and pH) and study period (in field or lab) on microbial biomass carbon (MBC), F:B ratio and operational taxonomic units (OUTs), colony forming units (CFUs) and and community structure diversity units. All microbial parameters are given as % increase (positive value) or % reduction (negative value) compared to the control without biochar application.

Biochar			Soil	Study period		Microbial parameters		References
Source	PT (°C)	Appl. rate	Texture	Field	Lab.	% MBC or total microbial PLFA[a] (compared with control)	OUTs, CFUs, other diversity indices, community structure, F:B ratio	
Bull and dairy manure	500	1%	Silt loam		96	51 MBC	–	Kolb et al. (2009), biochar × soil interaction was significant for MBC ($P < 0.01$)
		1%	Loamy sand		96	83 MBC	–	
		1%	Sandy loam		96	48 MBC	–	
		1%	Clay loam		96	51 MBC	–	
Liter from coppice woodland	500	~1.75%	Silty loam		92	−38 MBC	Simpson index same	Rutigliano et al. (2011) Note MBC non-significant
	500	~1.75%			426	−39 MBC	Simpson index same	
	500	~3.5%	Silty loam		92	−10 MBC	Simpson index same	
	500	~3.5%			426	20 MBC	Simpson index same	
Commercial biochar from coppice woodlands	500	5%	Silty loam		21 (pot exp.)	1.4% MBC	–	Zavalloni et al. (2011)
Commercial biochar from coppice woodlands + wheat straw residue	500	5%				20% MBC	–	
Eucalyptus wood	600	2.27%	Sandy		70 (pot exp.)	−28* MBC	–	Dempster et al. (2012)
Swine manure	350	~1.5%	Sandy loam		117 (pot exp.)	31* MBC	(1) Gram +ve and gram −ve bacteria more abundant in soil amended with 350 biochars than control and other treatments (values not specified)	Ameloot et al. (2013a)
Swine manure	700					19 MBC		
Willow wood	350					29.5* MBC		
Willow wood	700					29* MBC		
Wheat shoot	450	0.5%	Aridic arenosol (coarse textured)		74	28* tPLFAs	^{13}C biochar incorporated into PLFA (%): 0.020 in gram +ve bacteria, 0.024 fungi, 0.002 in actinomycetes	Farrell et al. (2013)
Eucalyptus shoot	450	0.5%			74	30* tPLFAs	^{13}C biochar incorporated into PLFA (%): 0.04 in gram +ve bacteria, 0.012 in fungi, 0.001 in actinomycetes	
Waste wood of *Leucaena leucocephala*	700	5%	Clay loam		105	34* MBC	–	Jien and Wang (2013)
Miscanthus giganteus straw	350	5%	Clay loam		90	57* MBC	–	Luo et al. (2013)
Miscanthus giganteus straw	700	5%			90	−18 MBC	–	
Miscanthus giganteus staw + rye grass residue	350	5%			90	75* MBC	–	
Miscanthus giganteus straw + rye grass residue	700	5%			90	62* MBC	–	
Eichornia crassipes residue	300	10%	Ustorthents		20 (pot exp.)	~2.5 times* MBC	–	Masto et al. (2013)
Ryegrass residue	450	1.3%	Loam		4	37 MBC*		Maestrini et al. (2014)
					88	11.4 MBC*		
Corncob biochar pellets	–	10%	–			high MBC (quantity not specified)	CFU: 30% higher bacteria in charosphere* and 82% lower in biochar pellet*, 55% higher fungi in charosphere* and 92% higher in biochar pellet*,	Sun et al. (2013)

Table 3 (*Continued*)

Biochar			Soil	Study period		Microbial parameters		References
					98 (pot exp.)		25% higher actinobacteria in charosphere* and 79% higher in biochar pellet*. Shannon–Weiner index for bacteria $\sim$3 times higher in charosphere and $\sim$2.5 times lower in biochar pellet	
Woody feedstocks	400	3%	Sandy clay loam	730		−22% tPLFA*	–	Ameloot et al. (2014)
	500	1.7%	Clay loam	730		7% tPLFA	–	
	500	0.5%	Silt loam	730		−20% tPLFA	–	
Bamboo wood	600	0.5%	Clay loam		372	12%* MBC		Demisie et al. (2014)
Bamboo wood	600	2%			372	−1% MBC		
Oak wood	600	0.5%			372	15%* MBC		
Oak wood	600	2%			372	−2% MBC		
Bamboo + inorganic N	600	0.35%	Loam		1095		59%* higher bacterial abundance, 17% non-significant higher viruses Shannon index (H): control = 1.14, biochar + N amended soil = 1.41*, Richness (S): control = 14, biochar + N amended soil = 25.3*	Doan et al. (2014)
Bamboo + vermicompost	600	0.35%	Loam		1095		Same bacterial abundance, 83%* higher viruses than control, Shannon index (H): control = 1.14, biochar + vermicompost amended soil = 1.52*, Richness (S): control = 14, biochar + vermicompost amended soil = 33.7*	
Corn stover	600	$\sim$0.9%	Sandy loam	1095		−27 MBC	–	Domene et al. (2014)
		$\sim$2.3%				49* MBC	–	
forest litter layer	400	5%	Loam		96	–	(1) 25% higher number of bacterial and 27% higher fungal genera in biochar amended soil than control (2) 33% higher bacterial and 28% lower fungal OTUs in biochar amended soil than control (3) 12%, 30% and 37% higher bacterial diversity and 17%, 40% and 23% lower fungal diversity as Shannon–Wiener, Simpsons and Chao indices, respectively in biochar amended soil than control	Hu et al. (2014)
Swine manure	500	1%	Sandy loam		90	20%* tPLFA	F:B ratio: −11%* lower than control	Muhammad et al. (2014)
Swine manure		3%				8.5% tPLFAs	5%	
fruit peels		1%				25%* tPLFA	Same as control	
fruit peels		3%				45%* tPLFAs	27%* higher than control	
Phragmites australis		1%				12.5%* tPLFA	10%	
Phragmites australis		3%				17* tPLFAs	10%	
Brassica rapa		1%				11% tPLFA	Same as control	
Brassica rapa		3%				−1 tPLFAs	16%	
Jarrah wood	600	0.1–0.5%	Loamy (red ferrosol)	305		24.4 MBC ($P < 0.06$)	2–4 times high OUTs of acidobacteria:acidobacteria and acidobacteria: verrucomicrobia associations at 1 t ha^{-1} amendment	Nielsen et al. (2014)
Willow wood	470	2%	Clay loam		30	8 tPLFA	PLFA: bacteria 9%, actinobacteria same, gram −ve bacteria 19%, fungi 12%*	Prayogo et al. (2014)
Willow wood biochar + fresh forest leaf litter (1% w/w)		2%				18 tPLFA	PLFA: bacteria 13%, actinobacteria 25%, gram −ve bacteria 14%*, fungi 40%*	
Eucalyptus saligna wood	400	0.8%	Clay loam		195	6 MBC	CFU: 37% bacteria, 57% fungi, −46% actinomycetes	Singh and Cowie (2014) Note Treatment *P* value significant ($P < 0.001$) except for fungi at 720 days study period
Eucalyptus saligna leaves	400	0.8%				15 MBC	CFU: 63% bacteria, 43% fungi, 14% actinomycetes	
Poultry manure	400	0.8%				25 MBC	CFU: 81% bacteria, 76% fungi, 19% actinomycetes	
Cow manure	400	0.8%				6 MBC	CFU: 36% bacteria, 36% fungi, 2% actinomycetes	
Eucalyptus saligna wood	400	0.8%			720	Same MBC	CFU: −10% bacteria, 43% fungi, −52% actinomycetes	
Eucalyptus saligna leaves	400	0.8%				−15 MBC	CFU: 13% bacteria, 58% fungi, −39% actinomycetes	
Poultry manure	400	0.8%				25 MBC	CFU: 4% bacteria, 65% fungi, −16% actinomycetes	
Cow manure	400	0.8%				7 MBC	CFU: −7% bacteria, 51% fungi, −10% actinomycetes	
Eucalyptus saligna wood	550	0.8%			195	8 MBC	CFU: 45% bacteria, 68% fungi, −44% actinomycetes	
Eucalyptus saligna leaves	550	0.8%				8 MBC	CFU: −12% bacteria, 33% fungi, −49% actinomycetes	
Poultry manure	550	0.8%				8 MBC	CFU: 50% bacteria, 47% fungi, −28% actinomycetes	
Cow manure	550	0.8%				Same MBC	CFU: 12% bacteria, 57% fungi, 10% actinomycetes	
Eucalyptus saligna wood	550	0.8%			720	Same MBC	CFU: −36% bacteria, 56% fungi, −43% actinomycetes	

Eucalyptus saligna leaves	550	0.8%				−18 MBC	CFU: 1% bacteria, 51% fungi, −62% actinomycetes	
Poultry manure	550	0.8%				8 MBC	CFU: −10% bacteria, 41% fungi, −36% actinomycetes	
Cow manure	550	0.8%				8 MBC	CFU: −18% bacteria, 54% fungi, −45% actinomycetes	
Bamboo leaf	500	~0.25%	Loamy (ferralsol)	30	–	~14%* MBC	–	Wang et al. (2014)
				90	–	~21% MBC	–	
				365	–	~11% MBC	–	
Wheat husk	525	3%	Sandy loam		100	12.5% high tPLFAs		Watzinger et al. (2014)
		3%	Silt loam		100	−9.5% tPLFAs		
Sugar maple wood	500	10%	Sandy to sandy loam		28	Lower tPLFAs (value not mentioned)	Reduction (non-significant) in gram −ve bacteria by 54%, gram +ve bacteria 37%, actinomycetes 50%, fungi 54% as compared to control	Mitchell et al. (2015)
		20%			28	Lower tPLFAs (value not mentioned)	Reduction (non-significant) in gram −ve bacteria by 56%, gram +ve bacteria 50%, actinomycetes 55%, fungi 63% as compared to control	
		10%			168	Higher tPLFAs (value not mentioned)	Increase (non-significant) in gram −ve bacteria by 19%, gram +ve bacteria 31%%, actinomycetes 5% and fungal abundance reduced by 18% (non-significant) as compared to control	
		20%			168	Higher tPLFAs (value not mentioned)	Increase (significant at $P < 0.05$) in gram −ve bacteria by 43%, gram +ve bacteria 59%, actinomycetes 34% and fungal abundance non-significantly higher by 51% as compared to control	
Pinus massoniana	450	2%	Clay loam		42	41% MBC*		Zhao et al. (2015)
		5%				79% MBC*		

Values followed by an asterisk (*) were significantly different ($P < 0.05$) in biochar-amended than control soils.

[a] Represents no data.

4. Microbial responses in biochar amended soils

The physico-chemical properties of biochar, as well as the biochar-induced changes in soil physico-chemical properties can alter the activities of soil microorganisms. As illustrated in Fig. 1, the biochar surfaces and pores provide habitat to microorganisms and its amendment concomitantly improves bulk density, pH and the movement of air, water and nutrients within the soil matrix. These alterations in soil physico-chemical properties help promote microbial abundance and activities by providing them with space and an environment that contains many diverse and expanded niches. This direct beneficial influence of biochar on soil quality and microorganisms can results in the indirect provision of more habitats and niches to microorganisms as litter and roots through improved plant growth. This section deals mainly with the direct influence of biochar on microbial responses such as microbial abundance, community structure, enzyme activity and microbial signaling in biochar amended soils.

4.1. Microbial habitats in biochar amended soils

Biochar pores serve as a habitat (Zackrisson et al., 1996; Pietikainen et al., 2000; Warnock et al., 2007; Quilliam et al., 2013; Jaafar et al., 2014) and refuge to soil microorganisms such as bacteria (size range from 0.3 to 3 μm), fungi (2–80 μm), and protozoa (7–30 μm), which protect them from predatory soil microarthropods (Zackrisson et al., 1996; Warnock et al., 2007). Biochar macropores (>200 nm) probably represent most of the protected microbial habitats since they are the right size to accommodate bacteria (see Quilliam et al., 2013), although biochar also contains micropores (<2 nm) and mesopores (2–50 nm) that could store water and dissolved substances that are needed for microbial metabolism (Brewer and Brown, 2012). The fraction and size of these pores depends on the production temperature of the biochar, where higher temperatures result in more water and organic matter volatilization, creating larger pores (Brewer and Brown, 2012). Moreover, the biochar feedstock also determines the size and abundance of pores. In a study with biochars produced from five feedstocks at 500 °C, Lee et al. (2013) reported that in 600 × 500 μm SEM image, sugarcane bagasse, paddy straw and umbrella tree wood biochars had mostly 10–50 μm, 20–100 μm and 50–70 μm diameter pore sizes, occupying ~70%, 80% and 30% of biochar surface, respectively. The 60 × 50 μm SEM image showed that cocopeat husk and palm kernel biochars had 5–10 μm and 1–3 μm diameter pore sizes, occupying ~15% and ~10% of biochar surface, respectively. Cross section of beech wood biochar (500 °C) showed perforations of 10–40 μm while longitudinal section had 125 μm to immeasurable long pores (full length did not come in 900 × 700 μm SEM image), while the plasmodesmata were up to 100 nm diameter (Prommer et al., 2014). The width, length and number of pores of vessels and tracheids in biochar can also depends on the part of plant residue used for its production (Carlquist and Schneider, 2007) as the size and diameter of vessels increase and their density decrease from leaves to roots along plant axis (e.g., Aloni and Zimmermann, 1983 and references therein). Other physical properties of biochar that are important for soil microorganisms include its surface area, where greater surface area leads to more opportunity for microbial colonization and its black color, which attracts more heat and thus may speed microbial growth and enzyme activity.

The chemical properties of biochar that can account for microbial growth on biochar surfaces and within its pores are (1) its surface charge, which binds microbial cells, chemical compounds and ions, and (2) the concentration of nutrients and

DOC that are desorbed or solubilized from the biochar. The volatile fraction of biochar, being low molecular weight DOC, is reported to be a preferred C source for microorganisms that are primary colonizers of freshly-applied biochar (Stewart et al., 2013). Using fluorescence excitation emission spectrophotometry, Uchimiya et al. (2013) reported that biochar extracts from different sources (almond shell, broiler litter, cottonseed hull and peacon shell) contained fulvic-like and humic-like structures, similar to those found in SOC, and thermally stable lignin-like DOC. Deenik et al. (2010) reported the presence of butyrolactone, mequinol, phenol, syringol, *p*-ethyl, guaiacol, cresol and ethyl phenol compounds in the volatile fraction of macademia nut shell biochar. The nature of the DOC and other metabolisable C compounds, as well as the pH of biochars are expected to be important controllers of microorganisms growing on biochars. Gram positive bacteria preferentially utilize biochar-derived C, suggesting that this material lacks appreciable quantities of easily degradable organic substances such as dissolved carbohydrates, amino acids, small polypeptides etc., that promote the growth of gram negative bacteria (Santos et al., 2012; Farrell et al., 2013). Moreover, the alkaline pH of most biochar may be more favorable for gram positive than gram negative bacteria. However, as the age of biochar proceeds, its pH declines, which can promote fungal growth
within biochar pores as reported by Zimmermann et al. (2012).

Despite its direct influence on microbial growth, the rate of biochar mineralization and its input into microbial biomass is much lower than the native SOC. For instance, based on ^{13}C isotope labeling short term (<200 days) incubation studies reveal less than 3% utilization of slow pyrolized biochars (350 °C–700 °C) from ligno-cellulosic feed stocks (Luo et al., 2011; Zavalloni et al., 2011; Santos et al., 2012; Farrell et al., 2013; Singh et al., 2014). Kuzyakov et al. (2009) reported that biochar obtained from combustion of ^{14}C labeled perennial rye grass (400 °C) had 0.5% loss as CO_2 per year when incubated in a silt loam soil for 1181 days at 20 °C and 70% water holding capacity, which suggested its residence time in soil would be 2000 years. Moreover, the biochar derived ^{14}C input into soil via microbial biomass during 624 days was only 2.6%. Likewise, the ^{13}C labeled slow pyrolyzed (450 °C) ponderosa pine wood biochar amended at 2 cm depth as 397 g cm^{-2} in a loamy soil of beach dominated temperate forest decomposed <1% and its ^{13}C incorporation in microbial biomass was only 0.01% during ten months while the ^{13}C labeled wood of same species mineralized by 52% and contributed ^{13}C in microbial biomass as 0.22% during that period (Singh et al., 2014).

The low nutrient contents in biochar relative to bulk soil and its high sorption capacity for low molecular weight substances explains the lower colonization of microorganisms within and on biochar surfaces in soil matrix as described by Quilliam et al. (2013). They found low microbial colonization on wood derived biochar produced at 450 °C, buried in sandy clay loam soil for 3 years (particle size of biochar range from 0–2 mm to 10 mm). The average percentage of internal-surface-biota-positive fields of view through SEM image was 40.7% but microorganisms were distributed very sparsely, moreover, pores of size <1 μm, which were 17% of the total pores present, were uninhabitable for most of the microbes.

Although biochar does not provide microorganisms with as much mineralizable C and nutrient sources as the bulk soil, the size, porosity and surface area of biochar can represents a suitable niche for microbial colonization. In an ecological context, a niche provides both physical habitat and a food supply for the organisms. We suppose that biochar characteristics of surface charge and porosity that facilitate the transfer of water and nutrients from the bulk soil into biochar pores will be important for supporting microbial growth and activity (e.g., Jaafar et al., 2014; Quilliam et al., 2013). Such an assumption merits further systematic study to relate biochar characteristics to microbial colonization, growth and activities.

4.2. Microbial abundance in biochar-amended soils

Several studies reveal the positive influence of biochar on increasing microbial biomass in variety of soil textural classes. As evident from Table 3, the biochar properties and soil native characteristics such as texture exert an important influence on microbial abundance in biochar-amended soils. An interesting study was carried out by Hale et al. (2015). They inoculated *Enterobacter cloacae* UW5 strains with biochars produced from five feed stocks (i.e., stone fruit pits, palm fronds, coconut shells, pine wood and pistachio nut shells), slow pyrolyzed at 300 °C and 600 °C. The inoculation was achieved by shaking bacterial liquid cultures with known amount of dry biochar for 24 h. The biochar-bacterial mixture was further mixed with sandy loam soil and incubated for 4 weeks. The significantly higher bacteria population density (16% greater than control) was achieved in soil amended with pine wood biochar produced at 600 °C. The authors also found a significant positive relationship between inoculum population density in biochar-amended soil and the pH of biochar ($R^2 = 0.84$, $P < 0.05$) while C:N ratio of biochar had a non-significant effect in this regard ($R^2 = 0.37$, $P > 0.05$). This study suggests that biochar-amended soils favor growth of gram negative bacteria, and this is related to the pH of biochar.

Table 3 shows a trend of greater microbial biomass in soils amended with biochars produced from feedstocks with lower ligno-cellulosic contents (e.g., manure, fruit peels, leaf litter) slow pyrolyzed at >500 °C. This trend was evident across a variety of soil textural classes (i.e., clay loam, silt loam, loamy sand, sandy loam, Kolb et al., 2009; Luo et al., 2013; Ameloot et al., 2013a; Sun et al., 2013; Domene et al., 2014; Wang et al., 2014) during 90–1095 days in controlled or in field conditions with biochar amendment rates of 1–10% of soil mass (in 0–15 cm depth of soil). Some studies do not result in greater microbial biomass in biochar-amended soils (e.g., Rutigliano et al., 2011), so caution is needed in extrapolating this finding to all biochar–soil systems. Likewise, Table 3 shows no change in microbial biomass carbon (MBC) when slow pyrolyzed (470–500 °C) wood derived biochars were applied at rates of 2–20% by mass (in 0–15 cm depth of soil) during short term experiment of 20–30 days (Prayogo et al., 2014; Zavalloni et al., 2014; Mitchell et al., 2015) under controlled conditions in clay loam to sandy loam soils. This observation does not hold when considering the greater MBC concentration in biochar-amended soils (sandy to clay loam) that were sampled after 2.5 months (Farrell et al., 2013), 4 months (Ameloot et al., 2013a) or ≥1 year (Demisie et al., 2014; Nielson, 2014; Mitchell et al., 2015) following biochar amendment (0.1–10% by mass). Some studies also reported that biochars produced at high temperatures (≥600 °C) had no effect (Luo et al., 2013) or a negative influence on microbial biomass, especially in coarse-textured soils (Dempster et al., 2012; Table 3 For instance Dempster et al. (2012) reported 28% reduction ($P < 0.05$) in MBC in response to the amendment of slow pyrolyzed *Eucalyptus* wood biochar produced at 600 °C as 2.3% amendment in coarse-textured sandy soil during 70 days in controlled conditions. In contrast, Ameloot et al. (2013a) reported a 29% increase in MBC ($P < 0.05$) in sandy loam soil amended with willow wood biochar produced at 700 °C during 117 days of pot experiment. In another study, a significant 62% increase in MBC in response to amendment with *Miscanthus giganteus* residue derived biochar produced at 700 °C during three months in clay loam soil was reported (Luo et al., 2013; Table 3. In summary, high production temperature slow pyrolyzed biochars with low nutrient contents (e.g., woody feed stocks) may hinder MBC in coarse textured soils with low OM content in the first 2–3 months following its addition to soil. We have already supposed that biochar can attract and retain water and nutrients from the soil solution, but if those substances are preferentially stored in biochar micro- and meso-pores (<50 nm) that are inaccessible to microorganisms, then this could leave microorganisms nutrient-impoverished for a period of time. The short term reduction in MBC in such biochar-amended soils can be offset by co-applying organic amendments such as compost or manure to increase available substrates for microorganisms.

4.3. Microbial community structure in biochar amended soils

Biochars are frequently reported to promote the microbial community structure of soils (Table 3), which is expected to result in a shift in the bacterial and fungal community structure. Sun et al. (2012) found that fungal community structure was less dynamic than bacterial community structure in biochar-amended brown soil under field conditions. Gomez et al. (2014) reported significantly lower F:B ratio in four soils (two sandy loam, clayey and clay loam) amended with fast pyrolysis biochar after 12 months of incubation. It is notable that the F:B ratio of biochar-amended soil depends on its C:N ratio, as a result of biochar application (Brewer et al., 2011; Farrell et al., 2013; Muhammad et al., 2014) or its native C:N ratio status (Rousk et al., 2013). An interesting finding by Muhammad et al. (2014), reveals a significant, positive correlation of F:B ratio with total DOC:total N ratio and C:N ratio of sandy loam soils ($r^2 = 0.68$; $P < 0.05$) amended with slow pyrolyzed biochars (500 °C) produced from swine manure, fruit peals, *Brassica Rapa* residues and reed grass (*Phragmites australis*) after 90 days of incubation. These findings provide evidence that biochar influence on microbial community structure is similar to the changes expected after plant residues are incorporated with soil (Gul et al., 2012), since both are causing changes in microbial community structure by altering the C:N ratio of readily-metabolisable substrates in soil.

The increase in microbial biomass within the soil microbial community as a result of biochar amendment can help detect the presence of a given microbial genera or species via DNA/RNA-based techniques, due to increase in their population size and density in the soil matrix (Forney et al., 2004; see also Sheibani et al., 2013). Sun et al. (2013) found ~3 times higher Shannon–Weiner index for the bacterial DGGE profile for 16S rDNA in the charosphere of corn cob pellet biochar than bulk soil during a 96 days pot experiment. Hu et al. (2014) found 12%, 30% and 37% higher bacterial diversity and 17%, 40% and 23% lower fungal diversity as Shannon–Weiner, Simpsons and Chao indices, respectively in forest-litter-biochar amended loamy soil than the control soil during a 96 days incubation. Substantially higher microbial diversity is also reported for Terra Preta soils of Amazonian anthrosols (Table 1). This improved detection of microbial genera/species and groups due to an increase in the size and density of microbial populations in biochar amended soils gives researchers the ability to use DNA-based tools to further probe microbial processes that are affected in biochar-amended soils such as plant-residue transformation processes and production/consumption of greenhouse gases.

4.4. Enzyme activity in biochar amended soils

Soil extracellular enzymes are the proximate agents of organic matter decomposition and nutrient cycling (Burns et al., 2013). Hence the influence of biochar on activities of soil extracellular enzymes is important. Available data reveals a variable effect of biochars on extracellular enzyme activities (Bailey et al., 2011; Awad et al., 2012; Daquan et al., 2012; Paz-Ferreiro et al., 2012; Ameloot et al., 2013a; Masto et al., 2013). The influence of biochar on soil enzyme activity depends on the interaction of substrate and enzyme with biochar (i.e., sorption and desorption of substrates on biochar CEC/AEC sites, binding of extracellular enzymes to the biochar surface, e.g., Bailey et al., 2011; Lammirato et al., 2011) and is related to the porosity and surface area of biochar (e.g., Lammirato et al., 2011). Biochar with greater porosity and surface area is expected to reduce extracellular enzyme activity, since functional groups on such biochar would tend to bind substrates and extracellular enzymes, thus interfering with the rate of substrate diffusion to the active site of enzyme catalysis (e.g., Bailey et al., 2011; Lammirato et al., 2011). This point is supported by Ameloot et al. (2013a), who reported a 47% reduction in dehydrogenase activity with biochar produced at 700 °C, and a 73% increase in dehydrogenase activity with biochar produced at 350 °C during a 117 days laboratory study. Furthermore, they found no difference in microbial biomass for the soil amended with biochar of 700 °C while the MBC increased significantly in soil receiving biochar produced at 350 °C (Table 3).

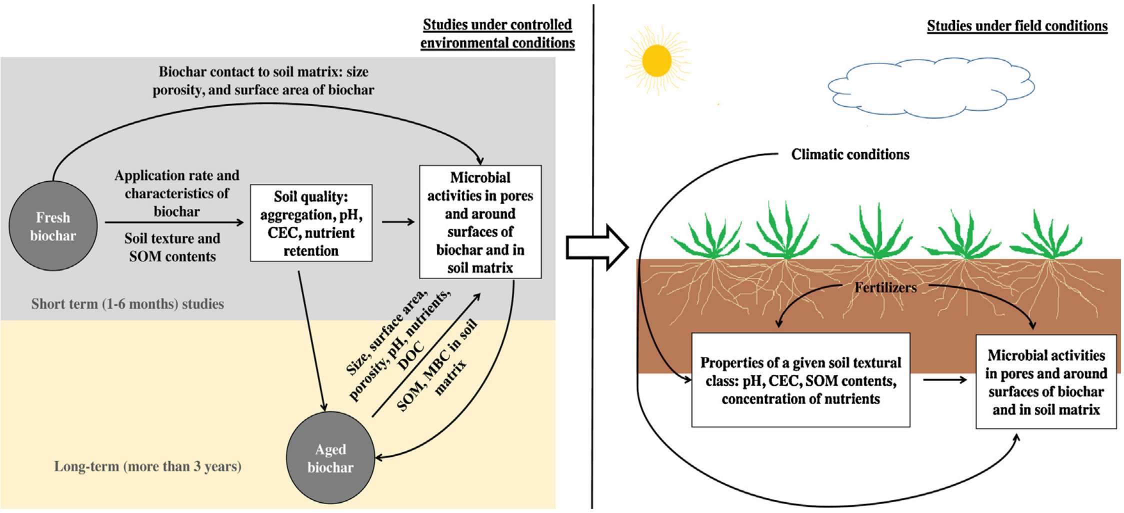

Fig. 2. A conceptual framework for exploring ideas/hypotheses about influence of biochar on soil physico-chemical properties and microbial activities as function of interactive effect of its characteristics as size, porosity, surface area, nutrient contents and pH and soil native characteristics as texture and residual SOM contents (left side). This model also indicates the need for comparing results from studies conducted in controlled conditions with the findings from field studies.

4.5. Microbial signaling biochar-amended soils

Quorum sensing is well documented for soil microorganisms and allows for cell–cell recognition, signaling and cross-talk among genera and organisms (e.g., microbe–microbe and plant–microbe interactions). Wood-derived biochar hindered *N*-(3-oxododecanoyl)-L-homoserine lactone mediated cell–cell communication between gram-negative soil bacteria in agar based growth medium, with 10-fold more hindrance for the biochar produced at 700 °C as compared to the biochar produced at 300 °C (Masiello et al., 2013). In this situation, the mechanism was probably the sorption capacity of biochar, which increases with increasing the production temperature. However, since soil-applied biochar interacts with number of organo-mineral substances, the magnitude of microbial signaling interruption may depends on the availability of free space on biochar sorptive surfaces, where signaling molecules could bet adsorbed. If the findings of Masiello et al. (2013) could be extrapolated to the soil environment, it may suggest that biochars produced at higher temperature (>600 °C) in low OM containing soils would cause microbial signaling interruption to greater magnitude than in soils with higher SOM contents. This possibility merits further study.

4.6. Microbial responses in fresh- versus aged-biochar-amended soils

The change in biochar properties associated with its aging also causes changes in microbial processes. For example, Spokas (2013) reported 27%, 27% and 81% higher CO_2 production from silt loam soils amended with 3 years field-aged biochars produced from three feedstocks; hardwood, macadamia nut shell and wood pellet (slow pyrolysed at 500–550 °C) respectively, as compared to the fresh biochars during a 100 days incubation period. The aged biochars had respectively 16%, 50% and 19% more volatile matter and 17%, 27% and 60% more ach contents than fresh biochars, which may be attributed to the higher adsorption of biochars for low molecular weight substances due to their increased oxidation with time during their burial in soil. However, comparing fire-produced char buried in oil for 10 years with freshly prepared slow pyrolysed biochar (450 °C) with the same woody feedstock, Zhao et al. (2015) found 24% lower C mineralization of clay loam soil amended with aged as compared to fresh biochar during 42 days of incubation. Moreover, they reported ~3 times higher ash contents in fresh biochar whereas the volatile organic matter in fresh biochar was 2.77 $mg\,g^{-1}$ and in aged biochar was negligible (<1 $mg\,g^{-1}$), while the concentration of ammonium was 84% and nitrate was 91% higher in aged biochar. An interesting finding of Zhao et al. (2015) was no difference in microbial abundance in soils amended with these biochars despite of higher C mineralization in fresh biochar amended soil, which indicates that the nutrient use efficiency of microorganisms was higher in aged biochar amended soil. These studies suggest that the nutrient contents in biochar, including nutrients derived from the biochar and nutrients transferred from the bulk soil to biochar, controls microbial growth and activity. Changes in soil microbial community structure and the associated changes in their activity in biochar-amended soils should consider the aging effect from the perspective of nutrient and metabolizable C adsorption/desorption reactions on biochar surfaces.

5. Future directions

Fig. 2 provides a conceptual framework of ideas/hypotheses of the future research needs to evaluate the influence of a biochar types on microbial responses in a given soil textural class, through short term studies (1–6 months) and in the longer-term (1–3 years or longer). These ideas are further explained and research recommendations are proposed below.

5.1. Laboratory incubation and pot-based studies

Studies are needed to evaluate:

- Short-term influence of biochar (1–6 months) on soil physico-chemical properties such as aggregation, pH, and CEC/AEC and to link the magnitude of this influence with the interaction of rate of application of a given biochar type regarding its source and production temperature with soil edaphic factors such as texture, pH and SOM contents.
- The long-term (>3 years) influence of biochar on soil physico-chemical properties, considering the magnitude of biochar effects in early stages following soil amendment and how this evolves through time.
- The short-term influence of biochar on microbial abundance and community structure within biochar pores, as determined by pore size (as it determines the movement of microbes and nutrients from soil matrix into biochar and vice versa), pH, concentration of nutrients, and DOC contents of biochar and in soil matrix.
- The influence of aged biochar (older than at least 1 year since its application in soil) on microbial abundance and community structure within its pores needs to be linked with its size, porosity, pH, DOC and concentration of nutrients before its amendment in soil as well as with the pH, SOM contents, microbial abundance, and microbial community structure of soil. This study will help understand (1) whether microbial colonization in biochar pores is the function of their accessibility or is controlled by the chemistry of biochar (2) how rapidly the biochar environment gets changed according to the environment of its surroundings (soil matrix) as the function of its size and porosity (3) although the chemical properties of biochar gets synchronized with the chemical properties of soil with its aging, to what extent it contributes to increase the microbial abundance via providing them with “habitat”, and (4) to what extent microbial colonization within biochar pores is related to the microbial abundance in soil matrix.
- The microbial abundance and community structure in biochar-amended soils after a certain period of time (1–3 years or more), considering the soil edaphic factors (i.e., texture, pH, C:N ratio, SOM contents) before biochar amendment, the physico-chemical properties of biochar (i.e., pH, surface area, porosity, nutrient contents) before its amendment to soil and its application rate. Such an assessment will help understand the suitability and proper application of that biochar for that soil.
- The biochar-induced inoculation of microbial strains to roots of plants/crops as affected bybiochar-induced changes in microbial abundance and community structure of soils. A more abundance and diverse community structure (greater abundance of a given microbial group) is supposed to be responsible for more inoculation of microbes to plant roots.
- The effectiveness of a given biochar type in improving soil physico-chemical properties and microbial processes in a given soil textural class, when co-applied with organic and inorganic amendments. Such studies will help understand how to manipulate biochar-induced changes in soil properties to acquire desired results such as greater SOM contents, more aggregation, higher CEC/AEC, well pH buffering capacity, higher microbial abundance, stronger microbial community structure etc. Moreover, such studies will help improve our understanding of proper application of a given biochar type in a given soil textural class.

5.2. Field-based studies

The understanding of biochar induced changes in soil properties needs to be further developed by comparing the results of laboratory and pot-based studies with the studies conducted in field. Such a comparison will help understand the role of environmental factors in controlling biochar-induced physico-chemical and biological properties of soils in relation to biochar type (i.e., crop/manure versus woody feedstock-derived biochars and biochars produced at lower (≤400 °C) versus higher production temperature (≥600 °C) and soil edaphic factors such as texture, pH, and SOM contents.

6. Conclusions

The influence of biochar on the physico-chemical properties of soils depends on the biochar characteristics as determined by its source and production temperature and soil native characteristics such as texture. Surface area, pH, O:C ratio are the important controllers for the change in pH, CEC, soil aggregation and retention of low molecular weight substances in soil. The biochars produced from ligno-cellulosic rich feedstocks at higher production temperatures (≥600 °C) tend to reduce aggregation in coarse-textured low organic matter containing soils. Such biochars possess low nutrient contents as compared to manure or crop residue based biochars, and high sorption capacity because of high temperature induced greater surface area. Consequently, they tend to reduce microbial abundance and enzyme activities in coarse-textured soils. In contrast, fine-textured soils exhibit no change in microbial abundance when amended with such biochars. This suggests that the amount of residual soil organic matter of a given soil textural class can buffer the negative influence on microbial populations and their activity following soil amendment with biochars from high temperature production that have low nutrient content. Co-amendment of organic or inorganic fertilizers with such biochars is recommended for coarse-textures soils to prevent nutrient deficiency for microbial growth and to attenuate the sorption of compounds on biochar surfaces that can reduce extracellular enzyme activities and microbial signaling. Moreover, it is recommended to evaluate the role of size, porosity and surface area of biochar in influencing microbial colonization to biochar as a consequence of accessibility of nutrients and microorganisms from soil matrix to biochar pores. The long-term influence of biochar (>3 years) on soil physico-chemical and biological properties is unlikely to be similar to the short-term effects, since the aging process results in the development of equilibrium conditions for chemical exchange and biological activity in the biochar–soil system.

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