Supplementary Information

How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar

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The supplementary information included as supporting information for this paper comprises:

Contents

Text S1 Biochar application in research and commercial agriculture

The majority of biochar studies are laboratory and pot trials, using biochar mixed through the soil volume. Field trials have commonly used biochar broadcast and ploughed into the topsoil, although some (e.g. Blackwell et al. (2015) and Graves (2013)) have placed biochar in a concentrated band adjacent to seeds at sowing, or as a discrete dose beside tree seedlings at planting (e.g. Joseph et al., 2020) (Figure S2, S3). Most biochars are not suitable as replacements for fertilizers, as they generally do not provide sufficient nutrients. Researchers have applied biochar alone or with nutrients (added during pyrolysis or through post-production incubation, or as organic or synthetic fertilizer applied together with biochar) (Kim et al., 2014; Kumar et al., 2018). The form of application includes powders, pellets or granules of biochar, or granules/pellets of biochar- mineral-fertiliser mix (biochar compound fertiliser – BCF) (Kim et al., 2014).
Researchers have applied biochar at 0.1 t ha^{-1} to over 50 t ha^{-1}, with the most common rates in the range 5-20 t ha^{-1} when broadcast and less than 1 t ha^{-1} when banded. High rates are commonly applied where low-nutrient biochar is used alone as a soil conditioner to improve bulk soil properties, such as to address heavy metal contamination, (Bian et al., 2014) while low rates are used for biochar-fertilizer mixes aimed at supplying nutrients to a crop. Positive effects on plant yield have been recorded at rates of 5-20 t ha^{-1}, (Dai et al., 2020; Ye et al., 2020) while growth responses have been observed at much lower rates when biochar-nutrient mixes are applied in a band near the seed/plant (Qian et al., 2014; Schmidt et al., 2015; Yao et al., 2015; Zheng et al., 2017).

While biochar is not commonly applied in commercial agriculture, its use is growing, particularly in China (Ren et al., 2019) and also in North America, Australia and Europe (Robb et al., 2020). Three approaches to applying biochar can be distinguished, that are being utilized by farmers around the world:

- as a biochar compound fertilizer, often applied as a band using a seed drill, with each crop sown, at rates less than 1 t ha^{-1} of product, where biochar comprises up to 25% of the pellet or granule (Joseph et al., 2013; Zheng et al., 2017)
- applied in conjunction with organic or synthetic fertilizer, at rates up to 1-2 t ha^{-1} (e.g., 10% biochar in a 20 t ha^{-1} application of compost,(Schmidt et al., 2015) usually at planting, placed in furrows or wells)
- applied at a high rate, of 10 t ha^{-1} or more, either in bands or broadcast and incorporated, applied infrequently such as every ten years.

The first approach is suitable for row crops in soils with moderate nutritional limitations, and is likely to be the most affordable and practical method for biochar utilization in mechanized cropping(Robb et al., 2020). This approach is commonly applied in China, where over 50 plants are producing in excess of 300,000 tonnes of biochar per year (Ren et al., 2019) that is used in compound fertilizers. Similar products are emerging in Australia and North America (Robb & Joseph, 2020). Extensive measurements of farm-based plots are being undertaken across China to document long-term effects of BCF (G. Pan, pers. comm.).

The second approach is applicable in small-scale farming, and is typical of traditional usage of biochar for soil fertility management by indigenous people in the Amazon Basin (Steiner et al., 2009). Application rates vary. This approach has been shown to maintain soil fertility over decades and centuries (Steiner et al., 2009).

The third approach is used in high value horticulture crops (Joseph et al., 2020) or for turf and golf course applications (Robb & Joseph, 2020) for soils with severe physical and/or chemical limitations, such as in rehabilitation of degraded soils (mine spoil, contaminated sites), where rates in excess of 50 t ha^{-1} may be beneficial (Forján et al., 2017) or for strongly acidic and infertile soils, such as Ferrosols commonly found in the tropics, or sandy soils with very low organic matter levels, or when the primary goal is carbon sequestration.

An emerging alternative approach is indirect: biochar fed to grazing cattle is activated in the rumen, mixed with manure in the intestine and incorporated into the soil, such as by the action of dung beetles and worms (Joseph et al., 2015).

When biochar is used as a single large application it is commonly applied several weeks in advance of planting. When it is used as a nutrient carrier, it is applied up to a week ahead of planting.

Text S2 General properties of biochar

Biochars are heterogenous materials with complex bulk and surface properties that can vary at micron and nano-scale within a single particle. This variation leads to a range of different reactions occurring simultaneously, which contribute to the observed macro effects on soils and plants. Biochars generally have higher porosity, surface area and surface-active properties and greater reducing capacity than the feedstocks from which they are produced. There is a complex relationship between application rate, particle size distribution, pore volume, average pore diameter and surface area of a specific biochar, and its effects on soil physical (including aggregate stability), chemical and microbial properties (Alghamdi et al., 2018; Herath et al., 2013), as described in Section 2 of the main manuscript. Reaction rate is largely dependent on surface area to volume ratio of biochar, so smaller particles (especially micron and nanoscale) generally have greater effects (Liao & Thomas, 2019).

Text S3 Effects of biochar on priming of soil organic matter: Biochar-root-microbial interactions induce rhizosphere priming

Biochar amendment to soil decreases organic matter mineralization, CO_2 efflux to the atmosphere and hence sequesters carbon (C) for long periods. It is not just adding stable carbon from biochar per se, it can also increase new carbon input from plants and protect the soil, making it more fertile. The ability of biochar to increase total soil C beyond the addition of persistent biochar-C is both a function of the properties of the biochar and those of the soil. Biochar can adsorb and stabilize rhizodeposits, soil organic matter and microbial metabolites as well as microbial necromass. The understanding of the effects of biochar on the mineralization of soil organic carbon (SOC) has been improved over the last decade. However, there is a knowledge gap regarding the plant-biochar-soil interactions on the direction, magnitude and duration of SOC priming in the field over the longer term.

Definition of priming

In this review, we adopt the terminology from Kuzyakov et al. (2000) who use the term priming effect to describe changes in the mineralization of SOM induced by comparatively moderate treatments of the soil, with biochar. Improved understanding of biochar-induced priming of soil organic matter aids prediction of effects of biochar on:

- soil organic C stocks;
- water holding capacity and aggregation; and

- soil fertility and cycling of nutrients.

Recent meta-analyses of biochar-induced priming

Three meta-analyses summarize the factors influencing biochar-induced priming (Ding et al., 2018; Maestrini et al., 2015; Wang et al., 2016). Based on 650 data points from 18 studies published between 2008 and 2014, of which half the data points were measured within less than three months after biochar addition, Maestrini et al. (2015) found a broad range of mineralization rates from 0.04 mg CO_2-C g^{-1} soil day^{-1} by a sugarcane bagasse biochar (350 °C) over 14 days (Cross and Sohi, 2011) to -0.02 mg CO_2-C g^{-1} soil day^{-1} between 250 and 500 days by a hardwood biochar (Zimmerman et al., 2011) (650 °C). The short-term studies showed only the effect of water-soluble compounds on the biochar (Section 2.1). The meta-analysis of Wang et al. (2016) consisting of 116 observations from 21 studies, showed that biochar slowed the mineralization of SOM by an average of 3.8% compared with the unamended control. Ding et al. (2018) collected 1170 groups of data from 27 incubation studies published up to August 2017.

The nature of biochar-induced priming was summarized as positive priming (increased mineralization of non-biochar C) within the first 20 days followed by negative priming (decreased mineralization of non-biochar C) (Maestrini et al., 2015). Biochar with a low C content (<40% of mass) tends to induce positive priming in the short term. Maestrini et al. (2015) modelled the priming effect over time and indicated that biochar generally caused a cumulative increase of native SOM mineralization by 0.3 mg C g^{-1} soil over 12 months and no net priming over 600 days (*i.e.* the initial positive priming was counteracted by the later negative priming). There was no correlation between priming effect and the soil C content, soil pH, rate of biochar application, biochar feedstock and soil texture. Wang et al. (2016) concluded that the biochar-induced negative priming was 9% in the study period < 6 months, 20% with crop residue biochars, 19% with fast pyrolysis, 19% with low pyrolysis temperature (200-375 °C), and 12% with low biochar dose (0.1-1 % of application rate). In contrast, biochar increased SOM decomposition by 21% in sandy soils (<10% clay content). Ding et al. (2014) found that the magnitude of negative priming increased with increasing C/N ratio of biochar, pyrolysis time and soil clay content (> 50%) but decreased with increasing C/N ratio of soil (> 12%). Correlation with the biochar C/N ratio is probably a secondary effect: high C/N materials tend to have low ash and high C contents (*e.g.* wood), and have high surface area and adsorption capacity for DOC. Ding et al. (2014) also concluded that incubation length dictates the biochar-induced priming effect which explained 27.1% of the variation. The authors determined that biochar-induced positive priming tends to occur within the first two years of amendment followed by negative priming.

Mechanisms of biochar-induced priming of SOM decomposition

Positive priming effects are associated with microbial activity increase by the addition of some easily available organic compounds present within biochar. Mechanisms for the biochar-induced positive priming are proposed as the direct effects from: (1) greater microbial activity and enzyme production fueled by the addition of the easily-mineralizable C from biochar (Luo et al., 2013; Singh & Cowie, 2014) (Section 2.1) and (2) microbial nutrient mining (e.g. N and P); and indirect effects such as: (1) amelioration of acidity by biochar that promote microbial activities,(Luo et al., 2011) (2) amelioration of nutrient constraints (Mukherjee and Zimmerman, 2013); 3) enhanced microbial habitat conditions(Luo et al., 2013; Pokharel et al., 2020) and soil faunal activity.

Biochar can cause negative priming directly by (1) substrate switching where the easily-mineralizable C from biochar may be preferentially consumed by microbes to temporarily replace the use of SOC (DeCiucies et al., 2018; Kuzyakov et al., 2000) and (2) dilution effect of substrates where there is just temporarily more total easily-mineralizable C in soil (both from biochar and SOC) (Whitman et al., 2014) and indirectly from (1) the sorption of organic compounds by biochar (DeCiucies et al., 2018; Kasozi et al., 2010), (2) improved organo-mineral protection and stable aggregation slowing down the mineralization of SOC within the organo-mineral complexes (Fang et al., 2018; Weng et al., 2017; Weng et al., 2018); (3) inhibition of microbial activity because of some polyaromatic toxic compounds (Zhang et al., 2018). A meta-analysis revealed that biochar amendments reduced the soil enzyme activities associated with C cycling by 6% (Zhang et al., 2019). The greater reduction of C enzyme activities was observed with low (< 1% w/w) or high (> 5% w/w) doses of biochar produced from herb and lignocellulose materials at high pyrolysis temperatures (> 600 °C) in high pH (> 7.5), fine-textured forest soils. The changes of enzyme activities were associated with the adsorption/ inhibition of fungi and enzymes through liming, high biochar porosity and aromatic C content (*e.g.* toxic substances such as phenols and polyphenols) of biochar (Zhang et al., 2019).

The initial CO_2 emission interpreted as positive priming could be partly due to CO_2 release from ash by biochar addition to acid soils.

A recent meta-analysis by Li et al. (2020) showed that biochar produced an overall increase in soil microbial biomass, with the effect dependent on biochar properties (Section 2.2). Li et al. (2020) highlighted the importance of analytical methods (*i.e.* greater microbial biomass by fumigation extraction compared with phospholipid fatty acid analysis). In an earlier meta-analysis by Zhou et al. (2017) biochar addition to agricultural soils generally lowered the metabolic quotient by 12-21% (*i.e.* respiration rate CO_2-C per unit of microbial biomass C) compared with the unamended soils. The metabolic quotient was reduced by less than 20% in crop residue and manure biochars at a pyrolysis temperature of 500 °C compared with other biochars. Soil conditions play a bigger role in metabolic quotient which was lowered by over 30% in pH neutral clay soils and by 15% in soils with low SOC content.

This suggests that biochar can increase the microbial C use efficiency leading to decreased microbial respiration and negative priming under N addition (Liu et al., 2018).

Biochar addition can affect microbial community composition (Whitman et al., 2016; Yu et al., 2018) (Section 2.2), but it is the soil source determining the community composition (Woolet & Whitman, 2020; Yu et al., 2020). In a 9-year biochar field experiment, bacterial diversity and the relative abundance of bacteria consuming pyrogenic C were increased in the soil one year after biochar incorporation compared with the unamended control but there was no effect after 9 years (Nguyen et al., 2018). Mechanisms involved in longer term biochar responses are described in Section 2.3.

Biochar effects on new input of organic matter from amendments

To investigate the impact of biochar on the mineralization of organic matter input, it is necessary to partition the soil, amendments and rhizodeposits (Section 2).

Using a dual-isotope approach using ^{13}C and ^{12}C to distinguish three C sources (SOC, biochar and root respiration), Whitman and Lehmann (2015) reported 23% increase of SOC mineralization over 66 days in biochar-amended soil in the presence of roots. Based on this approach, a dual ^{13}C and ^{15}N isotope three source-partitioning approach was developed to separate C sources from root respiration, biochar (two levels of ^{13}C enrichment) and native SOM (Weng et al., 2020). Biochar addition reduced the mineralization of native SOC by 29% over 84 days compared with the unamended control in the presence of root.

Luo et al. (2017) combined ^{14}C labelling with ^{13}C natural abundance to trace total CO_2 from C_3 SOC, ^{14}C-labelled glucose and C_4 biochar which reported the control soil increased the glucose-induced priming of native SOC by 140% compared with the biochar addition. Cui et al. (2017) using a C_3-vegetation-derived SOM, C_4-vegetation-derived litter and ^{14}C-labelled rice residue biochar (400 °C), showed that SOM mineralization was decreased by 19% in the combined biochar-litter amended soil compared with the litter-amended soil.

Biochar effects on new input of organic matter from plants

Biochar can affect plant-derived C at the same time rhizodeposits can also prime and act as a source of SOC. All three meta-analyses of biochar-induced priming are based on plant-free laboratory incubations. Because of the limited number of field or greenhouse experiments focusing on the biochar induced priming (e.g. using stable isotope techniques), there is yet to be a meta-analysis on biochar-induced SOC priming in the field. In a subtropical pasture, a ^{13}C-depleted hardwood biochar (450 °C) initiated positive priming up to 15 g CO_2-C m^{-2} over 62 days and switching to negative priming after 188 days in the presence of plants on a rhodic ferralsol (Weng et al., 2015). Biochar builds soil organic carbon not only as a result of the persistent nature of biochar C itself, but also through soil aggregation processes that stabilize new C (*i.e.* rhizodeposits) in one experiment by 6% (Weng et al., 2017), as well as by reducing priming by plant OC input (Whitman et al., 2014). In a three-year field experiment with short rotation coppiced poplar, a maize silage biochar at 30 t ha^{-1} lowered the mineralization of native SOM by 16% in the absence of roots compared with the control (Ventura et al., 2019). Substrate switching has been suggested as a potential mechanism for the observed negative priming (Ventura et al., 2019). In a six-year field experiment with a woody biochar applied to corn and dedicated bioenergy crops, soil C increase was double the amount of C added in biochar, as a result of negative priming (Blanco-Canqui et al., 2020).

In summary, biochar amendment can decrease mineralization of soil organic matter (Wang et al., 2016) and can protect new carbon input from plants, increasing soil carbon stocks and soil fertility, through adsorption and protection mechanisms described in Section 2.2.
The capacity of biochar to increase total soil organic C beyond the addition of persistent biochar-C is both a function of the properties of the biochar and those of the soil.

Text S4 Energy co-products from pyrolysis

The pyrolysis process produces three energy-rich fractions: pyrolysis solids (biochar), liquids (bio-oils) and gases. In many commercial pyrolysis units, the pyrolysis vapours (liquids and gases) are combusted to generate heat to commence the pyrolysis process. With low-moisture biomass, only a fraction of energy in the biomass is needed to kick-start the (exothermic) reaction. Theoretically, it is possible to sustain the pyrolysis process based on the energy in the pyrolysis gases alone (at least when pyrolysing straw or woody feedstocks) (Cong et al., 2018; Crombie et al., 2015) or using solar energy (Saxe et al., 2019). The pyrolysis liquids and/or the excess heat from the pyrolysis unit can be used to dry feedstock material, for district heating or to generate electricity (Azzi et al., 2019; Kung & Mu, 2019; Matuštík et al., 2020). Alternatively, the pyrolysis liquids can be upgraded to fuels or chemicals (Zhang et al., 2007).

Table S1 Examples of biochar formulations and application methods

Production System	Application Method	Formulation	Pyrolysis feedstock	Rate of Application	Productivity gain	References
Rice paddy	Broadcast and ploughed into topsoil	B+F	Wheat straw	10-40 t ha^{-1} biochar	10%	Lu et al. (2020)
Pumpkin	In a concentrated band adjacent to seeds at sowing	BCF+ F (cow urine)	Invasive forest shrub Eupatorium adenophorum	750 $kg \cdot ha^{-1}$ BCF	300%+	Schmidt et al. (2015)
Wheat and Sorghum	Deep banding at sowing, below seed	BMC+F (mineral fertilizer+ microbes)	*Acacia saligna*, Simcoa jarrah and Wundowie jarrah	300 kg ha^{-1} BMC	20% Cf unfertilized control	Blackwell et al. (2015)
Maize	Surface spreading	BCF		450 kg ha^{-1} BCF	10.7%	Zheng et al. (2017)
			Wheat straw			
Avocado	Discrete dose beside tree seedlings at planting	B+F	Mallee wood	5-20% v/v	18-26%	Joseph et al. (2020)
Pastures	In livestock excrement, conveyed into soil profile by dung beetles (40cm depth)	Mixed with molasses and fed directly to cows	Jarrah wood	0.1kg day^{-1} in cow feed	25%	Joseph et al. (2015)
Green Pepper	Discrete dose beside tree seedlings at planting	BCF	Wheat straw	670 kg ha^{-1}	11.5%	Yao et al. (2015)
Rice paddy	Basal fertilization	BCF	Manure, compost, maize straw, peanut husk and municipal waste	450 kg ha^{-1}	20%	Qian et al. (2014)
Subtropical dairy pasture	Broadcast and ploughed into topsoil	B+F	Greenwaste and manure	100 kg ha^{-1}	11% (manure only)	Slavich et al. (2013)

B+F Biochar co-applied with mineral or organic fertilizer

BCF Biochar compound fertilizer

BMC Biochar mineral complex

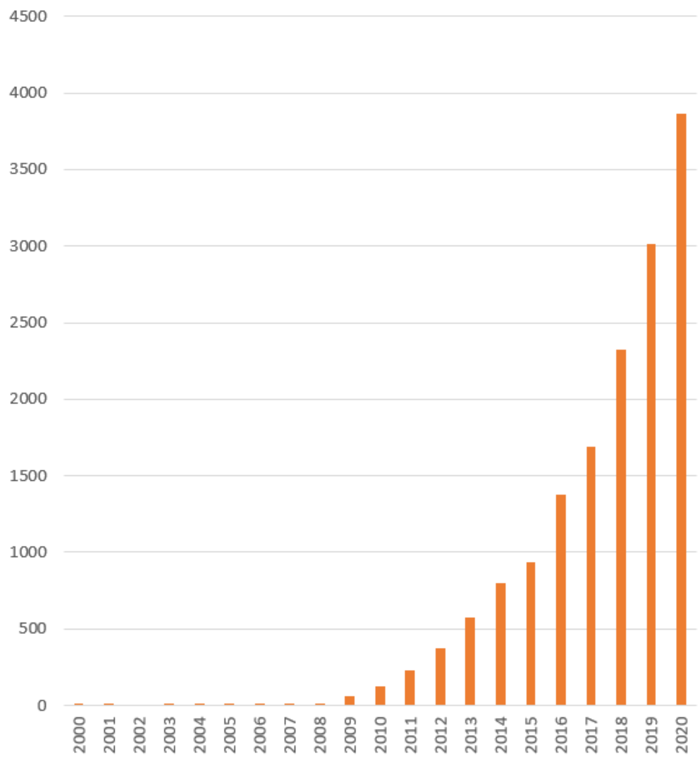

Figure S1 Publications on biochar ("biochar" in title, keywords or abstract) recorded in Scopus 12 December 2020

Figure S2 Biochar banded as a slurry
(Credit: Adam O'Toole)

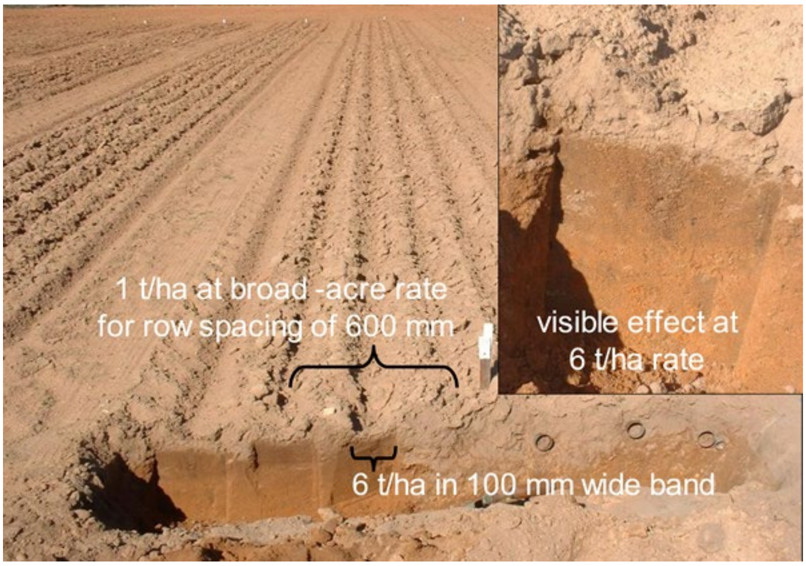

Figure S3 Deep banding of biochar
(Credit: Paul Blackwell)

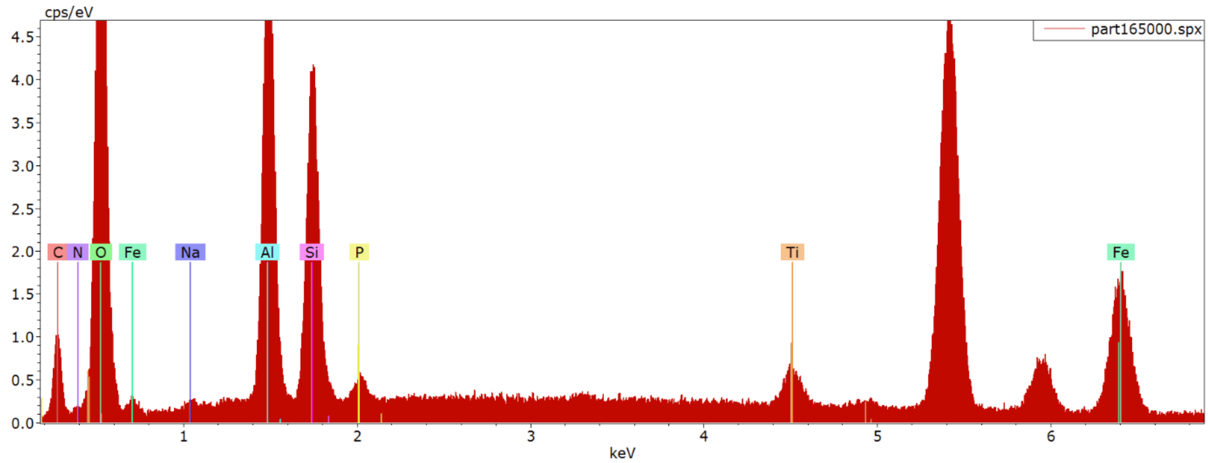

Figure S4 Energy dispersive X-ray spectroscopy (EDS) analysis of biochar

This figure show EDS analysis of the complete area of the image in Figure 3, that includes an aged biochar particle and soil microaggregates. The unmarked large peak is the chromium coating applied to make the sample conductive.

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