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Life in the ‘charosphere’ – Does biochar in agricultural soil provide a significant habitat for microorganisms?

Q2 Richard S. Quilliam [a], *, Helen C. Glanville [b], Stephen C. Wade [c], Davey L. Jones [b]

[a] Biological and Environmental Sciences, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, UK
[b] School of Environment, Natural Resources & Geography, Bangor University, Bangor, Gwynedd LL57 2UW, UK
[c] Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Ceredigion SY23 3DA, UK

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ABSTRACT

Biochar application has become a novel and emergent technology for sequestering C, improving soil quality and crop production, and is a potential win–win strategy for ecosystem service delivery. Biochar addition can also stimulate soil microbial activity, and although it is unclear exactly why biochar should benefit soil microorganisms, it is thought that the large surface area and volume of pores provide a significant habitat for microbes. The aim of this study was to determine the level of microbial colonisation of wood-derived biochar that had been buried in an agricultural soil for three years. We have examined the level of colonisation on the internal and external surfaces of field-aged biochar by scanning electron microscopy, and used ^{14}C-labelled glucose to quantify the rates of microbial activity in different spatial niches of the biochar and the surrounding soil. Microbial colonisation of field-aged biochar was very sparse, with no obvious differences between the external and internal surfaces. At the high field application rate of 50 t ha^{-1}, biochar contributed only 6.52 ± 0.11% of the total soil pore space and 7.35 ± 0.81% of the total soil surface area of the topsoil (0–30 cm). Further, 17.46 ± 0.02% of the biochar pores were effectively uninhabitable for most microbes, being <1 μm in diameter. The initial rate of microbial mineralization of ^{14}C-labelled glucose was significantly greater in the control bulk soil and the soil immediately surrounding the biochar than on the biochar external and internal surfaces. However, lower C use efficiency values of microbes on, or within, the biochar also suggested lower available C status or differences in the structure of the microbial community in the biochar relative to the surrounding soil. This study suggests that, at least in the short term ($\leq$3 y), biochar does not provide a significant habitat for soil microbes. While biochar is extremely recalcitrant and largely unavailable to soil microbes, changes in soil physicochemical properties and the introduction of metabolically available labile compounds into the surrounding soil (the ‘charosphere’) may significantly alter soil microbial activity and structure, which could ultimately affect soil–plant–microbe interactions. Therefore, before the wide-scale application of biochar to agricultural land is exploited, it is important that we understand further how the properties of biochar positively or negatively affect soil microbial communities, and in turn, how they interact with, and colonise biochar.

1. Introduction

Biochar is produced from the pyrolysis of organic materials and when buried in soil can act as a long term recalcitrant source of soil organic carbon (C), i.e. remaining over centuries (Glaser et al., 2002). Consequently, using biochar as a soil amendment for sequestering C is a powerfully simple tool to offset the C emissions associated with the burning of fossil fuels (Atkinson et al., 2010; Powlson et al., 2011). In addition to acting as a long term soil C store, biochar application to agricultural soil can increase crop yields, reduce the leaching of nutrients from soil, reduce N_2O emissions from agricultural soil, and stimulate soil microbial activity (Kolb et al., 2009; Jeffery et al., 2011; Singh et al., 2010). Further benefits of applying biochar to agricultural soil include the sorption or stabilisation of pesticides and nutrient ions, improved soil structure and the retention of soil moisture (Brodowski et al., 2006; Clough and Condron, 2010; Jones et al., 2011a; Laird et al., 2010).

Changes in soil properties are mediated by the intrinsic properties of the biochar, e.g. the surface charge, density and pore size distribution, which are dependent on the feedstock and pyrolysis conditions during the production process. Biochar can affect both physical

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* Corresponding author. Tel.: +44 (0)1786 467769.
E-mail address: Richard.Quilliam@stir.ac.uk (R.S. Quilliam).

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and chemical parameters of soil, e.g. pH, soil structure, release of soluble C and the availability of micronutrients, which in turn differentially influence microbial activity and community structure (Kolb et al., 2009; Anderson et al., 2011; Jones et al., 2011b; Lehmann et al., 2011). Therefore, the soil surrounding the biochar, which is directly influenced by the chemical and physical properties of the biochar (the ‘charosphere’, analogous to the rhizosphere surrounding plant roots) may ultimately affect soil–plant–microbe interactions. Subsequent alterations in soil microbial communities can impact upon nutrient cycling and thus affect crop growth (Graber et al., 2010; Kolton et al., 2011). Despite a growing body of evidence for biochar having a positive effect on soil microbial activity, it is still unclear exactly how biochar benefits soil microorganisms, especially as positive responses observed in the laboratory are not always reflected in the field (Jones et al., 2011a,b). Proposed explanations include, increased water availability (Lehmann et al., 2011); decreased leaching of nutrients from soils (Pietikäinen et al., 2000); increased aeration of the soil (Jones et al., 2011b); increased sorption of toxic compounds (Kasozi et al., 2010) and changes in pH (Rillig et al., 2010); however, one of the most commonly proposed reasons is that the numerous pores within the biochar provide additional habitat for microbes and provide refuge from their grazers (Saito, 1990; Pietikäinen et al., 2000; Ezawa et al., 2002; Thies and Rillig, 2009).

Although little is known about the mechanisms by which biochar influences soil microbial communities it is clear that biochar application can have positive effects on beneficial soil microorganisms, e.g. increased levels of mycorrhizal root colonisation, biological N_2 fixation by rhizobia in legumes and elevated activity of plant-growth promoting organisms in the rhizosphere (Rondon et al., 2007; Graber et al., 2010; Solaiman et al., 2010; Quilliam et al., 2013a). In addition to enhancing beneficial plant–microbe interactions, there are reports of biochar mediating increased resistance to plant pathogens, either directly through induced disease resistance or by promoting the activity of antagonistic microbes in the rhizosphere (Elad et al., 2010; Meller Harel et al., 2012). However, there are also reports of decreased arbuscular mycorrhizal fungi (AMF) root colonisation (Warnock et al., 2010) and decreased biological N_2 fixation above a certain threshold of biochar application (Rondon et al., 2007), which is probably driven by the increased levels of available P associated with the biochar and a lower N availability (Hale et al., 2013; Mukherjee and Zimmerman, 2013). Our previous work has also suggested that, like the initial flush of nutrients following the addition of biochar, the positive effects on soil microorganisms can also be transient (e.g. saprophytic bacteria and fungi; Jones et al., 2012; Quilliam et al., 2012). The addition of biochar to soil is effectively irreversible; therefore, before the wide-scale application of biochar to agricultural land is exploited, it is important that we have a comprehensive understanding of how biochar properties affect soil microbial communities, and in turn, how they interact with, and colonise biochar.

The aim of this study was to determine the level of microbial colonisation of field-aged wood-derived biochar. We hypothesised that after being in the soil for three years, biochar would be heavily colonised by soil microorganisms. To test this hypothesis we have estimated the level of colonisation on the internal and external surfaces of the biochar by scanning electron microscopy, and used ^{14}C-labelled glucose to quantify the rates of microbial mineralization in different spatial niches of the biochar and the surrounding soil.

2. Methods and materials

2.1. Biochar field site and experimental setup

The soil and biochar used in this study were taken from a field trial, established in 2009 at Abergwyngregyn, Wales (53°14′N, 4°01′W). The soil is classified as a Eutric Cambisol, has a sandy clay loam texture and is derived from mixed glacial till of Ordovician origin deposited approximately 10,000 years ago. Replicated ($n = 4$) trial plots (6 m × 3 m) were laid out in a randomized block design in an existing flat agricultural field, with biochar incorporated into the soil surface layers (0–20 cm) at rates of either 0 (control), 25 or 50 t ha^{-1}. Biochar was derived from mechanically chipped trunks and large branches of *Fraxinus excelsior* L., *Fagus sylvatica* L. and *Quercus robur* L. pyrolysed at 450 °C for 48 h (BioRegional Home-Grown®; BioRegional Charcoal Company Ltd, Wallington, Surrey, UK). The biochar chip size distribution was 17 ± 1% 0–2 mm, 19 ± 2% 2–5 mm, 32 ± 1% 5–7.5 mm, 32 ± 2% 7.5–10 mm and had a dry bulk density of 0.20 ± 0.01 g cm^{-3} whilst the pH was 8.81 (±0.1), and the electrical conductivity (EC) was 310 (±34) μS cm^{-1} in a 1:1 v/v extract with distilled water Further physicochemical details of the biochar and experimental field trial are provided in Jones et al. (2012) and Quilliam et al. (2012). In the summer of 2012 (three years after biochar addition), soil samples were collected from the 50 t ha^{-1} plots at a depth of 0–20 cm and biochar pieces between ca. 10 and 20 mm wide were hand-picked from the soil; soil samples from the control (0 t ha^{-1}) plots were collected simultaneously. The soil had a pH of 6.8 (±0.2) and an EC of 94 (±3) μS cm^{-1} (1:1 v/v soil:distilled water). The porosity of both the soil and biochar were determined by mercury intrusion porosimetry and the surface area was calculated by BET multipoint nitrogen adsorption at 77 K using a Micromeritics TriStar 3000 (MCA Services, Cambridge, UK). Total pore volume (void space) of the soil was calculated by collecting replicate 100 cm^3 soil cores from the field, drying the cores at 105 °C (24 h), weighing the dry soil and calculating total pore volume assuming a bulk density of the solid phase (organic-mineral matrix) of 2.52 g cm^{-3}.

2.2. Mineralization of ^{14}C-labelled glucose

Microbial mineralization was quantified in different spatial niches within 1 h of biochar being collected from the field by adding ^{14}C-labelled glucose to either, (i) the thin layer of soil that had been brushed from the surface (ca. 0.2–0.5 mm depth) of field-aged biochar, (ii) the surface of field-aged biochar following the removal of the soil, (iii) the internal snapped open surface of field-aged biochar, (iv) bulk soil collected from control plots having received no biochar, and (v) the internal snapped open surface of fresh biochar. Field-moist soil adhering to the biochar surface was carefully brushed away using a soft-haired paintbrush. To determine the rate of microbial activity (i.e. $^{14}CO_2$ evolution), 2 μl of uniformly ^{14}C-labelled glucose (Perkin Elmer, MA, USA; 10 μM, 7.38 kBq) was added to the soil (1 g) or biochar (0.5 g) treatments ($n = 5$) in 50 ml polypropylene vials. The added glucose concentration was chosen based on the field measurements of Boddy et al. (2007).

To capture evolved $^{14}CO_2$, a 1 M NaOH (1 ml) trap was placed inside each hermetically sealed polypropylene vial and suspended above the soil/biochar. $^{14}CO_2$ evolution was monitored by replacing the NaOH trap after 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48 and 100 h. The ^{14}C content of the 1 M NaOH traps was determined by adding Scintisafe 3® scintillation cocktail (Fisher Scientific, Loughborough, UK) and quantified using a Wallac 1404 liquid scintillation counter (Wallac EG&G, Milton Keynes, UK).

The partitioning of ^{14}C immobilized in the soil microbial biomass was modelled using first-order kinetic decay equations (Farrar et al., 2012). The choice of equation was based on a critical assessment of the inter-dependency between the different model parameters and the fit of the model to the experimental data (r^2 value) (Wolfe and Chinkes, 2005). The selected model was chosen based on a dependency value <0.98, above this the model was

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deemed too complex for the data and a lower-order model was selected instead (Wolfe and Chinkes, 2005; Farrar et al., 2012). Finally, to validate the chosen model, a least-squares estimation (LSE) was calculated to evaluate the accuracy of the estimation of parameter values (Wolfe and Chinkes, 2005).

To examine the immobilization-to-mineralization ratio (i.e. C use efficiency) of the ^{14}C incorporated into the soil microbial biomass, a kinetic model was fitted to the experimental $^{14}CO_2$ evolution data. The results for the bulk soil, soil brushed from the surface of the field-aged biochar, and both the internal and external surfaces of the field-aged biochar fitted best to a double first-order exponential decay model which represented a biphasic pattern of mineralization.

$$f(t) = \left(a_1 \times \exp^{-k_1 t}\right) + \left(a_2 \times \exp^{-k_2 t}\right) \tag{1}$$

where t is time and $f(t)$ is the amount of ^{14}C remaining in the soil at time t. For this model a_1, and a_2 describe the initial size of each respective pool, k_1 and k_2 correspond to the exponential decay constants for each mineralization phase. For this model, the first rapid phase described by k_1 is thought to correspond to $^{14}CO_2$ efflux as substrates are immediately used for catabolic processes (i.e. respiration), mirroring to some extent the depletion of substrate from the soil solution (Jones, 1999; Jones et al., 2004). The remaining ^{14}C-substrate is immobilized in the microbial biomass (pool a_2) via anabolic processes. The second, slower mineralization phase (k_2) is thought to be attributable to the use of this C temporarily immobilized in the biomass (i.e. microbial biomass turnover or use of storage-C). The dimensionless C use efficiency ($\mathrm{Mic_{CUE}}$) is therefore defined as

$$\mathrm{Mic_{CUE}} = a_2/(a_1 + a_2) \tag{2}$$

For the fresh biochar, a first-order single exponential decay model provided the best fit to the experimental data:

$$f(t) = \left(a_3 \times \exp^{-k_3 t}\right) \tag{3}$$

where a_3 describes the size of a single very slow, mineralizable pool and k_3 represents the exponential decay constant for this phase.

The substrate half-life for the first mineralization pool (a_1 and a_3 for double and single exponential decay models respectively) can be calculated using the following equation:

$$t_{1/2} = \ln(2)/k \tag{4}$$

Calculating the half-life for the slower second phase (k_2) was not calculated due to uncertainty over the amount of isotopic pool dilution (Saggar et al., 1999; Boddy et al., 2007).

2.3. Scanning electron microscopy

Samples of biochar were frozen in super cooled liquid nitrogen and then sputter coated with approximately 10 nm of gold/palladium in an Emscope SP2000A cryo-preparation system. The samples were imaged in a Jeol 840A scanning electron microscope fitted with a cold stage. Samples were also mounted on Cambridge stubs with double sided tape and sputter coated with approximately 4 nm of platinum/palladium in an Agar High Resolution Sputter Coater and imaged in a Hitachi S4700 field emission scanning electron microscope. The preservation of the various microorganisms was compared between the two methods and as no discernible differences were seen, the quicker and more direct method of imaging with the Hitachi field emission scanning electron microscope was employed. An accelerating voltage of 1.5 kV with an emission current of 10 μA was used at a working distance of 10 mm. The lens was used in normal mode and images were obtained with the upper secondary electron detector. The presence/absence of biota across a transect of the internal surface of field-aged biochar pieces ($n = 20$) was recorded and calculated as the percentage of biota present at each location along the transect.

2.4. Statistical analysis

All radio-labelled ^{14}C-substrate experiments were carried out with five replicates per treatment and data visually inspected for normality using quantile–quantile plots (Crawley, 2007). Any data not normally distributed were log-transformed to achieve normal distribution and equal variances. Differences in substrate mineralization were compared using a one-way ANOVA performed in the statistical package 'R' v 2.15.2 (2012), with $P = 0.05$ used as the upper limit for statistical significance. Differences in physical properties between soil and biochar samples were analysed by Student's t-test (Minitab 12.0 software, Minitab Inc., PA, USA). Kinetic modelling of ^{14}C turnover was undertaken with SigmaPlot v14.0 (Systat Software, Inc., San Jose, CA).

3. Results

3.1. Pore size distribution

The total surface area of the fresh biochar was about five times greater than that of the soil (Table 1), and both the total pore volume and pore surface area were significantly higher in the biochar compared to the soil ($P < 0.001$). The average diameter of the pores in the soil was higher than in the biochar ($P < 0.05$) indicating a greater proportion of narrower pores in the biochar (Table 1). The mean pore diameters in the biochar and soil within arbitrary pore diameter ranges were not different from each other (Table 2). However, whilst the cumulative volume of the pores was far greater in the biochar, the distribution of the pore volume sizes across the diameter ranges was very similar in both the biochar and the soil. Assuming that most bacteria and fungal hyphae cannot penetrate pores <1 μm in size, then 17.5 ± 0.1% of the biochar and 29.6 ± 5.1% of the soil total pore volume is uninhabitable. Further, with the field application rate of 50 t ha^{-1}, an incorporation depth of 30 cm and a total soil porosity of 0.570 ± 0.016 (v/v), biochar contributes 6.52 ± 0.11% of the total soil pore space and 7.35 ± 0.81% of the total soil surface area.

3.2. Microbial mineralization of ^{14}C-glucose

The evolution of $^{14}CO_2$ following the addition of ^{14}C-labelled glucose over 4 d is shown for each treatment in Fig. 1a. With the exception of the fresh biochar, a double exponential kinetic model (Eqn. (1)) fitted well to the experimentally measured evolution of $^{14}CO_2$ ($r^2 = 0.989$–0.998). For the fresh biochar, a single exponential model was selected ($r^2 = 0.972$).

The short-term rate of $^{14}CO_2$ evolution (within 1 h) following the addition of ^{14}C-labelled glucose is shown in Fig. 1b. The bulk soil

Table 1
Physical properties of soil and fresh biochar. Values are the mean of 3 replicates ± standard error of the mean.

	Soil	Biochar
Total pore volume (cm^3 g^{-1})	0.41 ± 0.07	2.39 ± 0.04
Total pore area (m^2 g^{-1})	4.65 ± 0.15	37.6 ± 0.10
Average pore diameter (μm)	0.349 ± 0.051	0.256 ± 0.005
Total surface area (m^2 g^{-1})	8.19 ± 0.08	39.0 ± 4.31

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Table 2
Pore size distribution of the soil and fresh biochar.

Pore diameter range (μm)	Mean pore diameter (μm)		Cumulative pore volume (cm^3 g^{-1})	
	Soil	Biochar	Soil	Biochar
648–411	589.05	588.07	0.0388	0.8406
411–210	296.14	294.75	0.0607	1.0671
210–106	147.80	147.07	0.0907	1.1676
106–71	86.52	86.71	0.1192	1.2362
71–42	52.86	51.47	0.1385	1.2698
42–30	35.97	38.22	0.1516	1.3084
30–20	25.75	24.18	0.1648	1.3851
20–10	14.52	13.80	0.2446	1.4834
10–1	5.830	4.843	0.3186	1.5768
1–0.1	0.363	0.303	0.3281	1.9390
0.1–0.01	0.032	0.032	0.3316	2.1718
0.01–0.003	0.007	0.007	0.3349	2.3573

and soil brushed from the field-aged biochar surface showed faster $^{14}CO_2$ evolution rates when compared to both the internal and external surfaces of the field-aged biochar and the fresh biochar. Both bulk soil and soil brushed from the biochar surface showed similar $^{14}CO_2$ evolution rates (bulk soil, 6.09 ± 0.26% h^{-1}; surface soil, 6.32 ± 0.95% h^{-1}) ($P > 0.05$). Despite similar total amounts of $^{14}CO_2$ having evolved after 100 h for both the internal and external surfaces of the field-aged biochar (Fig. 1a), there were differences in the rates of evolution after 1 h (Fig. 1b). The rate of $^{14}CO_2$ evolution on the field-aged biochar external surface was significantly faster than on the internal surface (3.26 ± 0.59% h^{-1} compared to 0.82 ± 0.06% h^{-1}; $P < 0.05$). There was a nominal rate of $^{14}CO_2$ evolution of 0.03 ± 0.003% h^{-1} from the fresh biochar.

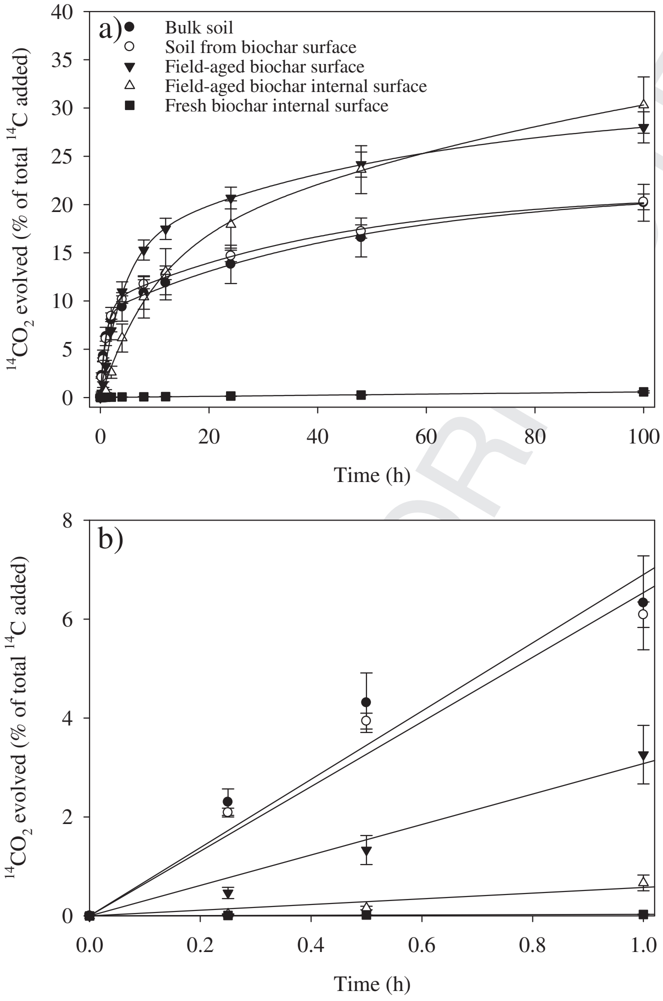

Fig. 1. Mineralization of ^{14}C-glucose added to different spatial niches within field-aged biochar, fresh biochar or bulk soil from 0 to 100 h (a) and 0–1 h (b). Values are the mean of 5 replicates ± standard error of the mean (a). The legend is the same for both panels.

These short-term differences in initial mineralization rate are also reflected in the modelled rate constant, k_1 (Table S1); these values were subsequently used to determine the relative half-life for ^{14}C-glucose in soil. The bulk soil and soil brushed from the biochar surface had similar half-lives of 0.04 ± 0.002 and 0.05 ± 0.002 d respectively ($P > 0.05$), whereas the substrate added to the external and internal surface of field-aged biochar had significantly longer half-lives of 0.13 ± 0.03 and 0.25 ± 0.03 d respectively ($P < 0.05$). The half-life of the fresh biochar (595 ± 119 d) was significantly longer than all the other spatial niches ($P < 0.05$).

After 4 d, the rate of ^{14}C-mineralization was very low indicating exhaustion of ^{14}C-glucose in the soil and the progressive turnover of ^{14}C immobilized in the microbial biomass. At this point, the amount of total $^{14}CO_2$ evolved from both the external and internal surfaces of the field-aged biochar was 30% higher than observed in the bulk soil and soil taken from the biochar surface ($P < 0.05$). Calculated microbial C use efficiencies (i.e. proportion of substrate-C immobilized; Mic_{CUE}) were significantly different ($P < 0.01$) between both the soil and biochar treatments (Table S1).

3.3. *Spatial distribution of microorganisms within biochar*

To estimate the level of microbial colonisation on the internal surfaces of the field-aged biochar, the presence/absence of biota within 50 random fields of view (100 μm^2) from 20 individual pieces of biochar was recorded. The mean percentage of biota-positive fields of view on the internal surface was 40.70% (±7.85); although representative images show how infrequent and spatially heterogeneous microbial colonisation was (Fig. 2). All of the fresh (control) pieces of biochar examined (i.e. those kept in an air-tight container since production) showed no signs of microbial colonisation. The biota inside the biochar was comprised of single-celled, filamentous and hyphal microorganisms and was most often found colonising the vascular tissue structures that had fractured longitudinally. Microbial colonisation, estimated by presence/absence in 100 μm^2 fields of view, was fairly evenly distributed over transects across the internal surface of the biochar (Fig. 3), with the percentage of biota in the middle of the biochar being no different to that at the interface between the soil and biochar.

4. Discussion

4.1. *Physical properties and colonisation of biochar*

One of the most commonly given reasons for the correlation between biochar application and the increased abundance and activity of soil microorganisms, is the considerable porous physical properties of biochar providing a significant microbial habitat niche (Pietikäinen et al., 2000). However, while extensive microbial colonisation has been demonstrated for natural forest fire biochars buried in soils for between 100 and 350 years (Zackrisson et al., 1996; Hockaday et al., 2007), and for artificially inoculated fresh biochar (Ascough et al., 2010), there is a lack of data on microbial colonisation of field-aged biochar in the relatively short term. Our study has shown that after three years burial in the soil, despite transient benefits to crop growth and soil–plant–microbe interactions (Quilliam et al., 2012, 2013a), biochar remained only sparsely colonised by soil microorganisms.

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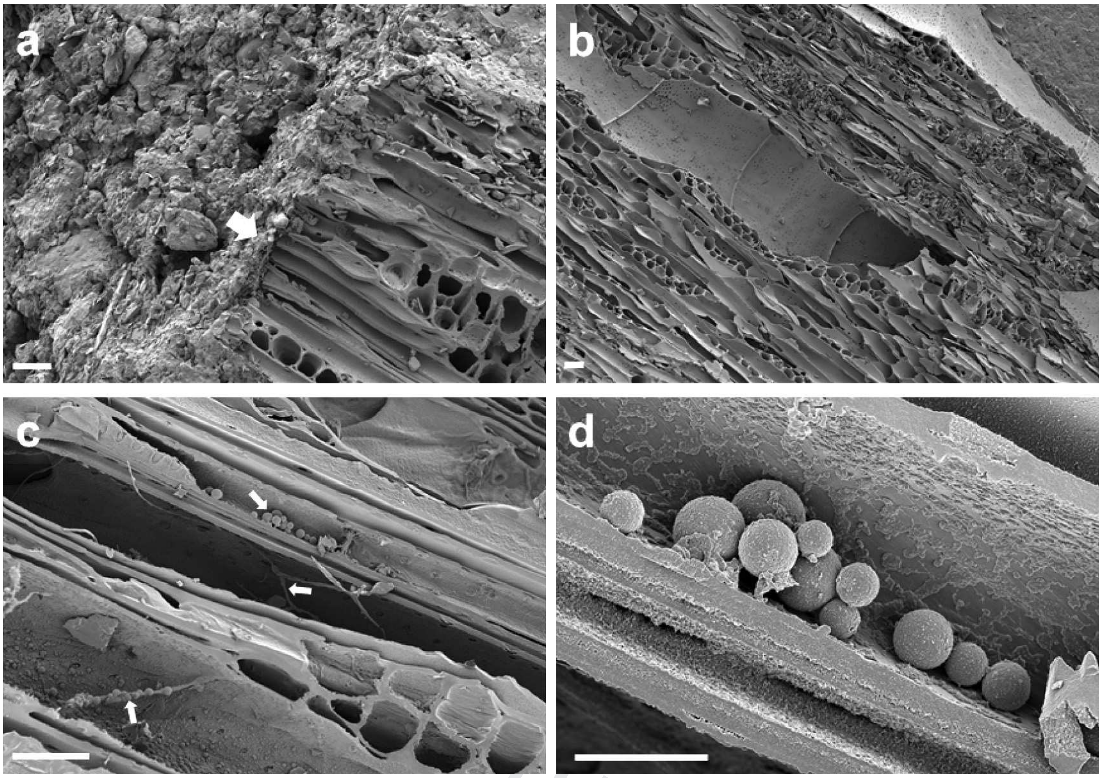

Fig. 2. Scanning electron micrographs of field-aged biochar buried in agricultural soil for 3 years. The biochar–soil interface on the outer surface, with arrow showing an example of pore blockage (a); spatial heterogeneity and sparsity of internal microbial colonisation (b); internal colonisation by hyphal and single-celled microbes (arrows) (c and d). Scale bar = 20 μm (a–c) and 5 μm (d).

Biochar porosity can influence its function in soil, e.g. larger pores can affect soil aeration and water holding capacity, while smaller pores are important for molecule adsorption and transport. Inherently, biochar contains a greater concentration of pores than soil (by nearly an order of magnitude in this study) and consequently has a far larger surface area. Despite a significant proportion of the biochar pores being <1 μm in diameter (which would provide unsuitable habitat for most soil microorganisms), the pore volume available for microbial colonisation was far greater than in the soil per unit volume. When we calculated the significance of biochar on total soil pore space and surface area on a per hectare basis (0–30 cm depth), however, its contribution was small (<10% of the total) despite the high rate of biochar application (50 t ha^{-1}). As cereal roots typically occupy a much greater soil depth (0–100 cm depth) the influence of the biochar on the soil's physical properties becomes even more diluted. However, significant levels of microbial colonisation of soil-buried biochar may take longer than the three years of our study. This could be due to the negative effects of high concentrations of mineral salts and PAHs that are often present in fresh biochar, which can have differential impacts on bacterial and fungal communities (Boonchan et al., 2000; Rajapaksha et al., 2004; Quilliam et al., 2013b). In addition, whilst probably not P and N limited, the recalcitrant nature of biochar, and its sorption of both cations and anions, can influence C and nutrient bioavailability in the charosphere. Microorganisms colonising the internal surfaces of biochar would have to rely on an external source of C diffusing in. This seems unlikely as any labile C would either be stripped out by microbes at the external surfaces or would become sorbed to the biochar preventing any further diffusion through the pores. Taken together, these conditions can make biochar a fairly nutrient poor and potentially toxic environment for microorganisms to colonise. It is therefore difficult to identify the advantages of colonising fresh or newly applied biochar, as the majority of microorganisms will be unable to utilise the limited resources thus making it a poor habitat niche.

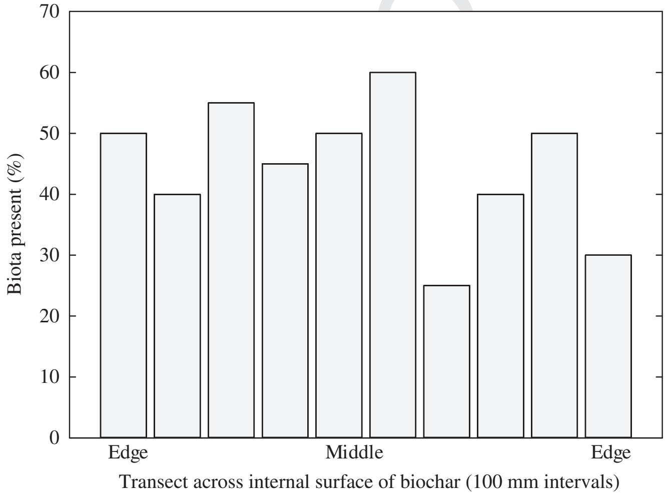

Fig. 3. Determination by scanning electron microscopy of biota presence across a transect of the internal surface of field-aged biochar pieces (n = 20) with biota absence/presence recorded in each transect.

The process of incorporating biochar into soil can decrease the available surface area and pore volume of biochar by strongly sorbing soil mineral and organic compounds, which can cause blockage of the pores (Joseph et al., 2010) and may prevent microorganisms penetrating the biochar interior. In our study, a biota presence/absence transect across the internal surface of field-aged

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biochar showed no evidence for increased presence at the soil-biochar interface compared to the centre of the biochar piece. However, this approach did not provide any information about the density, activity or heterogeneity of colonisation. Our measurements did indicate that microorganisms were most frequently observed in the longitudinal remains of the vascular tissue of the feedstock wood, suggesting that these relatively large hollow tubes could provide a pathway for microbial growth from the external surface of the biochar into the interior. Alternatively, the wicking-up of soil moisture by capillary action into the freshly applied biochar could lead to microbial cells and spores being passively sucked into biochar pores and down tubes, which would leave them isolated on the internal surfaces of the biochar. Many soil microbes form biofilms on the surfaces of soil aggregates where there is a ready supply of organic matter and an influx of nutrient resources and moisture. However, the limited planar sites available on the external surfaces of biochar, could force microbial communities onto the internal surfaces of the biochar, although the size of the biochar chunk would significantly influence gas diffusion, e.g. oxygen, to the middle of the biochar, which could limit aerobic microbial growth. If this was the case, powdered biochar may provide a more beneficial microbial habitat than larger biochar chunks.

4.2. Microbial activity in soil and biochar

An indication of microbial activity in different spatial niches of the biochar was provided by quantifying the mineralization of ^{14}C-labelled glucose. The initial lag phase of microbial activity when the glucose was applied to the internal surface of the field-aged biochar was most likely due to the smaller microbial community present and their low rates of basal activity (due to C limitation). However, following this short lag phase, the rate of glucose mineralization was much faster on both the internal and external surfaces of the biochar compared to the soil samples suggesting a switch from a C limited to a more metabolically active state (rather than due to an increase in biomass which would require longer timescales). This C limitation is also supported by the lower substrate C use efficiency values in these treatments (compared to soil).

Despite using such a small volume of radio-labelled glucose to inoculate the biochar, it is possible that the glucose could have diffused through the biochar and been mineralized by microbes away from the site of ^{14}C-glucose introduction. The average linear distance (L) of diffusive movement of glucose over time can be calculated according to the following equation (Barber, 1984):

$$L = 2(D_1 \times f \times t)^{1/2} \tag{5}$$

where D_1 is the effective diffusion coefficient of glucose in pure water, f is the soil impedance (tortuosity) factor (typically ranging from 0.1 to 0.3) and t is time in days (Barber, 1984; Jones et al., 2005). For biochar, we calculate the linear diffusive movement of glucose to be in the region 0.48 cm d^{-1}. This would indicate that our initial mineralization measurements (0–3 h) truly reflect the activity of the microbial community in the biochar interior but that at later time points (>6 h) the ^{14}C-glucose may have reached the microbial community towards the edge of the biochar.

4.3. Conclusions

The large volume of pores within biochar has been hypothesised to provide a significant habitat for microbes and provide them with refuge from their grazers. However, while this may be true in the long-term, i.e. after >100 years in the soil, we have shown that after three years in the soil biochar is only very sparsely colonised by microorganisms. Whilst evidence from short-term pot trials suggest that biochar can stimulate microbial growth and activity, the change in microbial dynamics following the addition of biochar appears to be largely due to transient changes in soil physico-chemical properties, e.g. pH and soil structure, and the introduction of metabolically available labile-C compounds into the charosphere. In the long term, microbial colonisation of biochar may be much greater as abiotically-mediated disintegration and partial microbial decomposition of the biochar will provide both a nutrient source and a habitat. Increasing this rate of physical breakdown, e.g. by applying biochar powders instead of coarse chunks, may speed up microbial colonisation, although this would be coupled with more rapid decomposition and decrease the potential for C sequestration. As soil microorganisms are vital for soil quality and sustainable agricultural production systems, improving our understanding of the microbe-charosphere-biochar dynamic under a number of difference scenarios will provide valuable information for the assessment of productive and strategic biochar application to agricultural soils.

Uncited references

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Glanville et al., 2012; Oburger and Jones, 2009.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.soilbio.2013.06.004.

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