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# C mineralization and microbial activity in four biochar field experiments several years after incorporation

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ABSTRACT

Most studies looking into the effect of amendment of biochar on soil microbial functioning employ short-term laboratory studies and probably describe relatively transient phenomena. Multi-year experiments, spanning beyond initial degradation of biologically labile biochar constituents, on the other hand are more scarce, although these are much needed to establish the medium-term effect of biochar on soil organisms. In the present study, soil was sampled from biochar-amended and control plots of four biochar field trials at Lincoln (UK), Rivignano, Rocca Bernarda and Beano in Italy. Air-dried pre-incubated soil samples were incubated at 15 °C for 8–9 weeks to follow-up carbon dioxide ($CO_2$) emissions. We then determined soil β-glucosidase and dehydrogenase enzyme activity, and used PLFA analysis to quantify the total soil microbial biomass and community structure. The analysis indicated that soil microbial activity was either not affected or inhibited to different extents in the biochar-amended plots. At Lincoln, with the highest application rate (49 t $ha^{-1}$), an overall inhibition of all investigated measures of microbial activity, a lower sum of extracted PLFAs and lower fungal abundance were observed. On the other end at Beano, depth dispersion of biochar by deep tillage and a lower application rate (20 t $ha^{-1}$) probably explain the absence of any significant effect on microbial activity in that experiment. At Rivignano and Rocca Bernarda, dehydrogenase activity was lower in the biochar amended soil and C-mineralization was lower as well for Rivignano. Interestingly, however, β-glucosidase activity and the sum of extracted PLFAs was not affected by biochar treatment. Several mechanisms could reconcile the different effect of biochar application on overall microbial activity on the one hand and microbial abundance and rate of cellulose degradation on the other. Biochar amendment led to a lowered or equal soil microbial activity and abundance in most field sites. In contrast to many short-term laboratory studies, it therefore seems unlikely that biochar would still function as a substrate 1–4 years after incorporation in the field.



## 1. Introduction

The effect of biochar on soil biological properties has been reviewed by Lehmann et al. (2011) and more recently by Ameloot et al. (2013b) with a specific focus on biochar stability. Both direct and indirect interactive effects exist between biochar and soil organisms. Although biochar was found not to provide a suitable habitat for soil microorganisms (Quilliam et al., 2013), the authors state that soil microbial activity may be indirectly influenced by changes in the physicochemical properties, e.g. soil porosity, pH, cation exchange capacity (CEC) and adsorption properties. In a direct way, microorganisms can utilize a number of labile biochar constituents as an energy source (Cross and Sohi, 2011). These are presumably either relatively untransformed biomass components that have not been subjected to volatilization during pyrolysis (Ronsse et al., 2013) or volatilized compounds that have recondensed in the biochar matrix during pyrolysis (Imam and Capareda, 2012; Kloss et al., 2012). Ameloot et al. (2013a) found that the volatile matter content of biochar was positively correlated to the short-term carbon dioxide ($CO_2$) emissions from soils amended with biochar. Indeed, 1.7 years after biochar incorporation Kuzyakov et al. (2014) found substantial proportions of biochar-C in

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microbial biomass, while this proportion dropped to one third of its original value 3.5 years after biochar incorporation. In most studies the effects on soil biota are assessed during laboratory incubations, with fresh biochars added to soil in mesocosms (Novak et al., 2010; Zavalloni et al., 2011; Ameloot et al., 2013a). The duration of these experiments ranges from several weeks (Cross and Sohi, 2011) to several years (Kuzyakov et al., 2009, 2014), allowing for understanding biochar stability and/or its interaction with soil organisms under controlled laboratory conditions with a possible overestimation of the multi-year effect of biochar on soil microbial activity. By contrast, field conditions are more complex and dynamic, due to tillage practices, exposure to freezing/thawing and drying/wetting cycles and the cultivation of crops. Hence, the question remains if and how microbial activity is affected in biochar-amended soil several years after application and under field conditions. After its field incorporation, some biochar constituents may be degraded (Cross and Sohi, 2011; Ameloot et al., 2013b) as several authors found specific labile biochar compounds to be very rapidly mineralized, i.e. within some days (Smith et al., 2010) or several months (Kuzyakov et al., 2009). Additionally, after soil incorporation biochar may ‘age’ as a result of biotically and abiotically mediated oxidation (Cheng et al., 2006; Hale et al., 2011). This ageing process may alter the biochar pH, cation exchange capacity (CEC), surface area and oxygen-to-carbon ratio (Hale et al., 2011), which is likely to influence the microbial community in biochar-amended soils.

In recent years, a number of field experiments have been established to assess the medium to long term effects of biochar addition on plant productivity (Jeffery et al., 2011; Hammond et al., 2013), physical soil properties (Major et al., 2010), nutrient dynamics (Major et al., 2012), greenhouse gas emissions (Castaldi et al., 2011; Afeng et al., 2012; Liu et al., 2012; Case et al., 2013) and soil microbial community (Rutigliano et al., 2014). Case et al. (2013) studied $CO_2$ emissions via *in-situ* field measurements and concluded that $CO_2$ emissions decreased in the biochar-amended plots compared to unamended plots. The mechanisms underlying this suppression were uncertain, but it indicates that stabilization of soil organic matter (SOM) might occur in the presence of biochar. Stabilization of SOM against microbial decay has been postulated by Zimmerman et al. (2011). Due to oxidation of the biochar surface oxygen containing functional groups can be formed (Cheng et al., 2008; Calvelo Pereira et al., 2014), which may sorb dissolved OM particles. Additionally, SOM-derived organic constituents might be physically protected from microbial degradation in the fine biochar pores (Pignatello et al., 2006). Both pathways warrant the assumption that after degradation of volatile biochar compounds a longer lasting effect of biochar on SOM decomposition is the resultant of biochar's indirect control on soil microbial activity, i.e. either through sorption or sterical entrapment of native SOM components. Very little is known, however, on the existence of such a lasting, i.e. multi-year effect of biochar on soil microbial activity. In this study, we investigated microbial biomass, activity and community structure in the different treatments of four biochar-amendment field experiments (United Kingdom and Italy). We hypothesized that biochar-induced changes of physicochemical soil properties continue to influence soil microorganisms even after the readily-available biochar compounds have been degraded.

## 2. Material and methods

### 2.1. Soil characteristics

Soil was collected in September 2012 from one experimental site in the UK (Lincoln) and from three sites in Italy (Rocca Bernarda, Rivignano and Beano). All soils had a sandy loam to silt loam texture. The applied biochars were produced from woody feedstocks at 400 °C or 500 °C (Table 1). The application rates varied between 20 and 49 t ha$^{-1}$. With a range in application depth from 10 to 35 cm, this created large variation in the soil biochar concentration. The Rivignano site is a recently established experiment (February 2012), while the Lincoln, Rocca Bernarda, and Beano trials were established in 2010, 2010 and 2008 respectively. In September 2012, around twenty five soil samples were randomly taken from each of the three control and three biochar-amended plots at the Rocca Bernarda and Beano sites, from each of the four control and four biochar-amended plots at Rivignano and from each of the five control and five biochar-amended plots at the Lincoln site (Fig. S1). Soil samples were taken with a soil auger to the depth corresponding to the biochar incorporation depth. Thereafter soil samples from each plot were bulked and

**Table 1**
Coordinates, soil texture, last crop, biochar application rate, concentration, depth and date, incorporation time and biochar characteristics (feedstock, pyrolysis circumstances, and C & N content) at each site.

| | Lincoln | Rivignano | Rocca Bernarda | Beano |
|---|---|---|---|---|
| Coordinates | 53°14′ 13.92″ N<br>0°32′ 15.58″ W | 45°53′ 16.59″ N<br>13°02′ 03.85″ E | 46°1′ 22.96″ N<br>13°25′ 40.23″ E | 46°00′ 00″ N<br>13°01′ 00″ E |
| Mean air temperature | 9.4 °C | 13.2 °C | 13 °C | 13 °C |
| Sand % | 49 | 31 | 18 | 27 |
| Silt % | 23 | 38 | 57 | 58 |
| Clay % | 28 | 31 | 24 | 15 |
| Soil texture | Sandy clay loam | Clay loam | Silt loam | Silt loam |
| Crop | Miscanthus -not harvested at sampling | Maize- harvested at sampling | Grapes (vineyard)- no undercover | Maize-not harvested at sampling |
| Biochar application rate | 49 t ha$^{-1}$ | 30 t ha$^{-1}$ | 30 t ha$^{-1}$ | 2 × 10 t ha$^{-1}$ |
| Application depth | 10 cm | 15 cm | 15 cm | 35 cm |
| Biochar concentration | 2.92% | 1.67% | 1.67% | 0.48% |
| Application date | 2010 | February 2012 | July 2010 | 2008 (10 t ha$^{-1}$)<br>2009 (10 t ha$^{-1}$) |
| Biochar incorporation time | 2 years | 7 months | 2 years | 4 years |
| Biochar feedstock | Thinnings of hardwood trees (oak, cherry and ash greater than 50 mm diameter) | Woody: Pruning orchard | Woody: Pruning orchard | Coppiced woodlands (beech, hazel, oak, birch) |
| Pyrolysis method | To 180 °C to release volatile gases, then 400 °C (24 h) | Retort kiln at atmospheric pressure at 500 °C | Retort kiln at atmospheric pressure at 500 °C | Charcoal kiln at 500 °C |
| Biochar C | 72.3% | 42.5% | 42.5% | 81% |
| Biochar N | 0.172% | 0.62% | 0.62% | 0.37% |