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Effect of biochar addition on soil microbial community in a wheat crop

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ABSTRACT

Biochar is known to enhance soil fertility and C sequestration, but relatively little information is currently available about its effect on soil microbial community, a component of terrestrial ecosystems that plays a key role in nutrient cycling. This study tested the effects of soil amendment with two loads of wood-derived biochar (30 and 60 t ha^{-1}) in a wheat crop in Tuscany (Italy). Soil samples were collected 3 and 14 months after treatments over two successive growing seasons, and analysed for pH, total organic C (C_{org}), extractable C (C_{ext}), microbial biomass-C (C_{mic}), 25 specific microbial activities, mean substrate-induced respiration (mSIR) for 25 substrates, functional microbial diversity and bacterial genetic diversity. No significant effect of biochar treatment was observed on C_{org}, C_{ext}, C_{mic}, microbial quotient (C_{mic} % C_{org}) or genetic diversity. An increase in mSIR, some specific microbial activities and soil pH, and a significant change in functional diversity were observed 3 months after treatment. In contrast, no effect of biochar was detected 14 months after treatment for the parameters considered, except for a small but significant increase in pH. Our data suggest that biochar addition stimulated soil microbial activity without causing any apparent disturbance, but this positive effect was very short-lived.

1. Introduction

Biochar is produced by low-temperature (400–500 °C) pyrolysis of biomass (such as manure, organic waste, bioenergy crops, crop residues) in oxygen-free or low-oxygen environment. After pyrolysis of the biomass, about 50% of the original biomass C remains in the biochar [1]. Used as a soil amendment, biochar can enhance nutrient availability to plants and improve physical and biological properties of the soil [2–6]. Moreover, because of its molecular structure dominated by aromatic C blocks, biochar is much more resistant to microbial decomposition than uncharred organic matter [7] and can persist in the soil from 1000 to 10,000 years [8], thus increasing soil C storage [9]. In addition, biochar might improve soil fertility on the long term, mainly by indirect effects, such as increase of cation exchange capacity, surface area and water retention in soil pores, which decreases nutrient leaching, and, to a minor extent, as a consequence of direct nutrient release [2,10]. The use of biochar has been advocated not only in addressing the widespread problem of loss of soil organic matter and consequent reduction of fertility, but also as a manageable option to dispose of organic waste and favour C sequestration in soil [11].

Beneficial effects of biochar application on crop yield have been documented [12–14], but specific effects on the soil microbial community are still poorly explored. Yet, soil microorganisms play a central role in nutrient cycling and provide thus an important ecosystem service [15]. Biochar, or at least some labile biochar compounds, has been found to be a potential source of organic matter and inorganic nutrients for microorganisms [16] and can act as a refuge protecting microbes from predators [8]. In fact, biochar pores may be below 5 μm in diameter [17], thereby being accessible to bacteria and fungal hyphae but not their larger predators such as mites and collembolans or most nematodes and protozoans [18,19]. In addition, biochar can bind toxic compounds such as heavy metals, polycyclic aromatic hydrocarbons and organic pesticides, thus reducing their bioavailability [20–24]. However, the current information on the effect of biochar on microbial community is not univocal. Organic pyrolytic products, such as phenolics and polyphenolics, may be present in biochar and negatively affect soil microorganisms [25]. Negative, null or positive effects of biochar on soil microbial community have been reported depending on the biochar employed and type of soil [11,16,26–29]. Moreover, only few of these studies have been performed in the field [26,29],

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where usually a greater number of factors interacts compared to laboratory conditions. The topic clearly demands further investigation to be performed in the field considering specific biochar, soil and plant cover types. A further aspect that needs consideration is how long the impact of biochar addition, if evidence is provided of positive or negative effects, lasts in time. With the aim to address the above questions, the present study was performed in an agricultural soil in the Mediterranean area, with a focus on microbial quotient (microbial C % organic C), microbial activities, and microbial functional and genetic diversity. These parameters are known to vary in response to several environmental factors including plant cover, land use, agricultural management, pH, salinity, heavy-metal contamination and fire [30–38], and only rarely have been considered simultaneously in field studies on biochar [29]. Moreover, no data are currently available on the topic for Mediterranean crop soils. This research is a part of a larger study also including biochar effects on crop yield [14] and on greenhouse gas fluxes from soil [39].

2. Material and methods

2.1. Study site and experimental design

The study was set in Pistoia (Tuscany, Italy, lat. 43°56′ N, long. 10°54′ E, 65 m a.s.l.) in a fallow area. The soil had a silty-loam texture and had a bulk density of 1.2 Mg m^{-3}, pH 5.2, 21 g organic C kg^{-1} soil, 1.2 g N kg^{-1} soil and cation exchange capacity 18 meq/100 g [14]. The experiment was carried out over two growing seasons (2008/09 and 2009/10) of wheat (durum wheat, *Triticum durum* L., cultivar Neolatino). Total rainfall and mean air temperature for the growing seasons 2008/09 and 2009/10 were respectively 1159/1222.8 mm and 13.9/15.1 °C (data from an automatic weather station at site).

The experimental design was defined to verify the effects of two biochar loads (30 and 60 t ha^{-1}) after 3 and 14 months of exposure in the field. Twenty-four plots (25 m^2 each) were selected in an area of 0.5 ha and randomly assigned to one of three different treatments, each with 4 replicates: 1) soil incorporation of 30 t ha^{-1} of biochar (B30); 2) incorporation of 60 t ha^{-1} of biochar (B60); 3) no biochar addition (C, control). Twelve plots were treated with biochar in the season 2008/2009 and twelve in the season 2009/2010. All plots were subjected to tillage in October 2008 and 2009 and were sowed with wheat on 16th January 2009 (season 2008/09) and 14th December 2009 (season 2009/2010) in rows of about 450 seeds m^{-2}. Nitrogen-phosphate and phosphorus fertilizer were distributed at sowing (22 kg ha^{-1} of N and 50 kg ha^{-1} of P_2O_5) followed by a second N fertilization in April (100 kg ha^{-1} as ammonium nitrate) for both experiments. The plots treated with biochar in the season 2008/2009 were cultivated without further biochar application in the successive season (2009/2010). Biochar (crushed into particles < 1 cm in order to increase the area/volume ratio) was added just before sowing, in the same day, and was partially buried (up to the depth of 10–15 cm) with a rotary hoeing tillage [14]. In March 2010, a synchronic sampling was carried out in all plots so that soil was collected 3 or 14 months after biochar treatment, respectively, for 2009/10 and 2008/09 growing season.

The applied biochar, purchased from Lakeland Coppice Products (England), was obtained by a slow pyrolysis process (at 500 °C) of organic materials from coppiced woodlands (beech, hazel, oak and birch) in a transportable ring kiln of 2.15 m in diameter and holding around 2 t of hardwood. After setting fire, the kiln was closed, letting pyrolysis proceed for two days. Biochar had total C, total N and available N contents respectively of 840 g kg^{-1}, 12 g kg^{-1} and 0.03 g kg^{-1}, pH (1:2.5 H_2O) 7.2, maximum water absorption 4.5 g g^{-1} of d.m., bulk density 0.42 Mg m^{-3} [14].

2.2. Soil sampling and analysis

Twenty four samples (4 replicates × 3 treatments × 2 wheat growing seasons) were examined, each sample being obtained by mixing together four soil cores (0–10 cm depth, 5 cm diameter) from each plot. The soil was sieved (mesh size: 2 mm) in order to obtain homogeneous samples free of stones, larger roots and other coarse fragments. The part excluded from analysis is the less reactive soil fraction [40] but it strongly influences the ratio between microbially active soil and total soil weight. Fine-earth (<2 mm) is conventionally used for chemical [40,41] and microbial analysis of soil [42,43].

Soil samples were analysed for the following parameters: pH, total organic C (C_{org}), extractable C (C_{ext}), microbial biomass C (C_{mic}), bacterial diversity and respiratory response to 25 specific substrates, the latter being used to evaluate both the mean respiratory response and microbial functional diversity. Soil pH and total organic C was determined on samples dried at 75 °C. Bacterial genetic diversity was determined on soil stored at −20 °C. All other parameters were determined on fresh soil (stored at 4 °C until to analysis).

Soil pH was determined on soil water suspension (1:2.5 soil:water ratio) by a pH-meter (Hanna HI8424). Total organic C (C_{org}) was determined by humid digestion in 0.33 M $K_2Cr_2O_7$ [44,45]. Microbial biomass carbon (C_{mic}) was measured by fumigation-extraction [46]. Fresh soil (4 g-equivalent dry weight), either fumigated with chloroform or non-fumigated, was extracted with 0.5 M K_2SO_4 and the organic C content was determined on filtered extracts (Whatman 42 filters) by humid digestion with 0.066 M $K_2Cr_2O_7$ at 160 °C. The 0.5 M K_2SO_4 concentration has been demonstrated to extract organic C effectively in biochar-treated soils [47]. The microbial quotient, *i.e.* the percentage of soil organic C present as microbial biomass C (C_{mic} % C_{org}), was calculated from microbial biomass C and total soil organic C. By expressing the proportion of the C_{org} that can be readily metabolized, this is a useful parameter for monitoring soil organic matter [48]. The organic C measured in non-fumigated soil samples corresponds to the extractable C (C_{ext}).

Specific microbial activities were determined as substrate-induced respiration (SIR) by measuring short-term respiratory responses of fresh soil to addition of each of the following readily available 25 organic compounds [49]: 15 carboxylic acids (L-ascorbic acid, citric acid, fumaric acid, gluconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-malic acid, malonic acid, pantothenic acid, quinic acid, succinic acid, tartaric acid, uric acid and urocanic acid), 7 amino acids (L-arginine, L-asparagine, L-glutamic acid, L-glutamine, L-histidine, L-lysine, L-serine), 3 carbohydrates (D-glucose, D-mannose, D-glucosamine). These compounds have been reported as the most sensitive substrates to different types of land management among 83 organic molecules tested [31]. Soil respiration responses were measured as the increase in CO_2 evolution from fresh soil samples (1 g equivalent dry weight) amended with each substrate, compared to soil without substrate, after incubation in standard conditions (4 h, 25 °C, in the dark) [37]. The mean response to the substrates tested was calculated to obtain the mean substrate-induced respiration (mSIR). Microbial functional diversity of each soil sample was evaluated in terms of catabolic response profile (or catabolic fingerprint) to all 25 substrates and as catabolic evenness (E). The last was calculated by Simpson-Yule index [50] as $E = 1/\Sigma p_i^2$, where "p_i" is the respiratory response to each substrate expressed as a proportion of total response to addition of all substrates.

Bacterial genetic diversity was determined by 16S rDNA analysis, after elimination of extracellular DNA by alkaline soil washings [42]. The extracellular DNA fraction, secreted by live microorganisms or released after death, can persist over time due to interaction with soil