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Biochar application rate affects biological nitrogen fixation in red clover conditional on potassium availability

S. Mia[a], J.W. van Groenigen[a], T.F.J. van de Voorde[b], N.J. Oram[a,b], T.M. Bezemer[c], L. Mommer[b], S. Jeffery[a,*]

[a] Department of Soil Quality, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands
[b] Nature Conservation and Plant Ecology Group, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands
[c] Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 50, 6700 AB Wageningen, The Netherlands

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ABSTRACT

Increased biological nitrogen fixation (BNF) by legumes has been reported following biochar application to soils, but the mechanisms behind this phenomenon remain poorly elucidated. We investigated the effects of different biochar application rates on BNF in red clover (*Trifolium pratense* L.). Red clover was grown in mono or mixed cultures with red fescue grass (*Festuca rubra* L.) and plantain (*Plantago lanceolata* L.) at a range of different biochar application rates (0, 10, 50 and 120 t ha^{-1}). In a separate experiment, nutrient effects of biochar on BNF were investigated using nitrogen, phosphorous and potassium (N, P and K) and micronutrient fertilization using the same plant species.

Biochar addition increased BNF and biochar applied at a rate of 10 t ha^{-1} led to the highest rate of BNF. Total biomass also showed the greatest increase at this application rate. An application rate of 120 t ha^{-1} significantly decreased biomass production in both single and mixed cultures when compared to the control, with the greatest reduction occurring in red clover. Furthermore, BNF was significantly higher in pots in which red clover was grown in mixed cultures compared to monocultures. In the absence of biochar, K fertilization caused a significant increase in BNF. For N, P, and micronutrient fertilization, BNF did not significantly differ between treatments with and without biochar addition.

We conclude that different biochar application rates lead to different effects in terms of BNF and biomass production. However, due to the high variety of biochar properties, different application rates should be investigated on a case specific basis to determine the optimum biochar application strategies.

1. Introduction

Biochar is charcoal which is made with the intention of applying it to soil. Biochar is often claimed to have several potential benefits, including carbon sequestration (Laird, 2008; Zimmermann et al., 2012); bioenergy generation (Laird, 2008; Lehmann, 2007); adsorbing organic and inorganic pollutants (Hale et al., 2011; Jiang et al., 2012) as well as improving soil fertility (Jeffery et al., 2011; Spokas et al., 2012).

Soil fertility effects have been explained in terms of inherent nutrient addition with biochar (Parvage et al., 2013) as well as by biochar-induced changes in soil physical, chemical or biological properties (Kookana et al., 2011; Oguntunde et al., 2008; Thies and Rilling, 2009). However, the mechanisms behind the observed yield effects remain unclear. Hypotheses for these effects include improved fertilizer use efficiency by reducing loss of nutrients through leaching (Blackwell et al., 2010; Laird et al., 2010) or increased nutrient availability due to increased microbial activity, such as arbuscular mycorrhizal fungi (AMF) (Warnock et al., 2007). Some studies also suggest that biochar addition to soil can enhance soil fertility through increased biological nitrogen fixation (BNF) when legumes are present (Nishio, 1996; Rondon et al., 2007). However, the mechanisms behind this effect also remain unclear.

Biological nitrogen fixation is estimated to contribute approximately 17.2×10^7 t of nitrogen to soils globally each year (Ishizuka, 1992). Leguminous crops have been estimated to contribute approximately half of the global symbiotic BNF at an estimated 21.5×10^6 t (Herridge et al., 2008). This demonstrates that BNF is an important ecosystem service for global agriculture and as such understanding the possible impacts of biochar application on this service is vital.

* Corresponding author. Tel.: +31 317 85749.
E-mail addresses: simon.jeffery@wur.nl, drsjeffery@googlemail.com (S. Jeffery).

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Different mechanisms for the observed effect of biochar on symbiotic BNF have been proposed. These include:

- Immobilisation of inorganic N, which is known to stimulate BNF (Rondon et al., 2007; Bruun et al., 2011; Nelissen et al., 2012).
- Increased nodulation, which has been observed in white clover (*Trifolium repens*) (Rillig et al., 2010), soybean (*Glycine max*) (Ogawa and Okimori, 2010; Tagoe et al., 2008) and alfalfa (*Medicago sativa*) (George et al., 2012).
- Increased P bioavailability (Brewer et al., 2012; Nelson et al., 2011) which has been correlated with increased BNF in several legumes: soybean (Tagoe et al., 2008), common bean (*Phaseolus vulgaris*) (Rondon et al., 2007) and alfalfa (Nishio and Okano, 1991).
- Interactions between biochar and signalling for nodulation through adsorption of flavonoids and Nod-factors (Thies and Rilling, 2009).
- Increased pH, as claimed for the case of soybean (Ogawa and Okimori, 2010).
- Introduction of macro and micro nutrients (Brewer et al., 2012; Major et al., 2010; Rondon et al., 2007), which may be beneficial to legumes. Further, as biochar application generally raises soil pH (Hass et al., 2012) micronutrient availability (e.g. Fe and Mn) can also be affected.

There is still a dearth of data on the effects of biochar application to soil on BNF in temperate regions. Furthermore, different effects have been reported with different application rates. For example, increasing biochar application rates has been found to increase BNF (Ogawa and Okimori, 2010; Rondon et al., 2007; Tagoe et al., 2008). However, reduced nodulation has also been reported with elevated application rates, even though nitrogenase activity remained unchanged (Quilliam et al., 2013). The mechanisms behind these changes in BNF remain largely hypothetical. Therefore, there is an urgent need to better understand the mechanisms by which biochar application affects BNF in order to allow robust predictions to be made. Natural abundance ^{15}N analysis (Unkovich et al., 1994) provides an effective means of quantifying BNF and as such is a useful tool for investigating such mechanisms.

We formulated five hypotheses regarding the mechanism behind the effects of biochar on BNF in red clover (*T. pratense* L.):

H1: Increasing application rates of biochar will increase BNF.
H2: N application will negate the effect of biochar on BNF.
H3, H4 and H5: Fertilization with K (H3), P (H4), or micronutrient fertilizer (H5) will increase BNF to the same level as with biochar.

Two separate microcosm experiments were conducted to test these hypotheses by examining how biochar and fertilization affected plant growth of leguminous and non-leguminous plants.

2. Materials and methods

2.1. Soil and biochar

The biochar was produced from aboveground plant biomass collected from a species-rich grassland in the nature reserve area De Mossel at Planken Wambuis, Ede, the Netherlands (+52°3′34.03″, +5°45′2.81″) via pyrolysis at 400 °C. Soil was collected from the same location, air dried, homogenized by sieving through an 8 mm sieve and mixed thoroughly. Selected soil and biochar properties are presented in Tables 1 and 2.

The homogenized soil was split between two experiments, described below. Pots filled with soil were incubated for 14 days under a polyethylene sheet to germinate weeds and to allow equilibration of the biochar–soil mixture. All seedlings that emerged from the seed bank were removed (Tables 3 and 4).

Table 1
Selected soil characteristics of the base soil used for both Experiments I and II.

Soil characteristics	
pH	5.24
N-NH_4 (mg N kg^{-1})	2.5
N-NO_3 + NO_2 (mg N kg^{-1})	20.0
N-DON[1] (mg N kg^{-1})	4.0
P-PO_4 (mg P kg^{-1})	3.28
K^+ (mg K kg^{-1})	29.1
EC (dS m^{-1})	0.08

Table 2
The different factors, biochar application rates and fertilizer treatments used in the two experiments.

Factors	Exp. I	Exp. II
A. Soil treatment	1. 0 (control) 2. 1 t ha^{-1} biochar 3. 10 t ha^{-1} biochar 4. 50 t ha^{-1} biochar 5. 120 t ha^{-1} biochar	1. biochar (10 t ha^{-1}) 2. N fertilizer (50 kg ha^{-1}) 3. P fertilizer (30 kg ha^{-1}) 4. K fertilizer (50 kg ha^{-1}) 5. Micronutrient fertilizer (B, 794 g; Cu, 32.4 g; Mo, 17.0 g; Mn, 803 g; Zn, 79.7 g ha^{-1})
B. Plant species composition	1. Red clover (*Trifolium pratense*) 2. Grass (*Festuca rubra*) 3. Clover and grass mixed stands	1. Red clover (*Trifolium pratense*), Grass (*Festuca rubra*) and plantain (*Plantago lanceolata*) mixed stands

2.2. Experimental set-up

Two experiments were conducted to test the five hypotheses stated in the introduction (H1–H5). Experiment I investigated the effects of increasing biochar application rate on BNF to test H1. Experiment II investigated the effect of individual fertilizer (N, P, K and micronutrients) in presence and absence of biochar on BNF to test H2–5. Both experiments were set up in a randomized complete block design with five replicates for each treatment.

2.2.1. Experiment I

The experiment was carried out from 23rd May to 18th July, 2012 in a greenhouse at Unifarm of Wageningen University, Wageningen, the Netherlands. The experiment included two factors, biochar application rate and plant species composition.

Five biochar application rates were used: 0 (control), 1, 10, 50 and 120 t ha^{-1} (w/w equivalents) of biochar. The biochar was incorporated into the top 10 cm. The rates were calculated as a soil:biochar ratio using an assumed bulk density of 1.3 g cm^{-3} and assuming that the biochar would have been mixed homogenously through the top 10 cm of the soil. This allowed the calculation of a

Table 3
Selected characteristics of the biochar produced at 400 °C through slow pyrolysis from combined various grassland species.

Biochar characteristics	
Volatile matter content	32.1% (S.E. = 1.89)
Ash	25.22% (S.E. = 5.01)
N	1.91% (S.E. = 0.09)
C	59.02% (S.E. = 1.35)
H	3.81 (S.E. = 0.06)
S	0.00% (S.E. = 0.0)
H/C	0.77 (S.E. = 0.03)
Mineral N	0.8 mg kg^{-1} (S.E. = 0.03)
K	1620.8 mg kg^{-1} (S.E. = 24.4)
P-PO_4	1.9 mg kg^{-1} (S.E. = 0.02)