

## Nitrogen mineralization dynamics of meat bone meal and cattle manure as affected by the application of softwood chips biochar in soil

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### Abstract

We studied the impact of added biochar on the N mineralization dynamics of two organic fertilizers by incubating sandy loam soil for 133 days in controlled conditions. Biochar made from softwood chips was added to soil at 0, 4.6, 9.1 and 13.6 g kg<sup>-1</sup> soil DM either alone, or in combination with meat bone meal (MBM) and composted cattle manure (CCM) fertilizers. Soil mineral N concentration was determined on days 0, 14, 28, 56, 84 and 133.

Net N mineralization in MBM treatment was much larger than in CCM and unfertilized treatments. Constant soil moisture during the incubation provided suitable aerobic soil conditions for nitrification: after day 14, soil mineral N was dominated by nitrate in all treatments. Biochar additions decreased the mineral N concentrations in all treatments, probably because of immobilization by microbes. In unfertilized soil, the immobilisation by biochar increased steadily with application rate and time, but in MBM and CCM it started to decrease or level off after two months, possibly due to the turnover of microbial biomass.

**Keywords:** ammonium, biochar, carbon sequestration, nitrate, nitrogen immobilization, organic fertilizers

## Introduction

Biochar (BC) is a porous carbonaceous solid produced by thermochemical conversion (pyrolysis) of biomass in a low-oxygen atmosphere. Owing to its intrinsic properties, the application of biochar to soil is expected to sequester carbon and concurrently improve soil functions in sustainable manner (Verheijen et al. 2009, Shackley and Sohi 2010). The potential of biochar to mitigate global climate change is chiefly due to its highly recalcitrant nature (Cheng et al. 2008), which slows down the rate at which photosynthetically fixed carbon is returned to the atmosphere.

Furthermore, the agricultural use of biochar is claimed to yield several potential co-benefits, such as increased crop yields (Major et al. 2010), reduction of nutrient leaching (Brockhoff et al. 2010), and reduced N<sub>2</sub>O emissions from soils (Yanai et al. 2007). Such potential co-benefits make biochar application remarkable, if not unique, among the possible strategies to remove CO<sub>2</sub> from the atmosphere.

The application of biochar to soil may increase the microbial activity of soils by providing water, nutrients and habitat for microorganisms (Lehmann et al. 2003; Warnock et al. 2007) and stimulating the decomposition of original soil organic matter (Wardle et al. 2008). Moreover, the degradation of biochar provides a partly labile C source for microbes (Cheng et al. 2008). Even though most of the biochar C is stable in aromatic C compounds, many surface functional groups are prone to abiotic and microbial mediated oxidation reactions (Cheng et al. 2008). Small amounts of wood-derived biochars (0.26 to 0.4% of total C) have been reported to be mineralized within the first two months when incubated in laboratory conditions (Cheng et al. 2008). However, the mineralization rate decreases strongly in longer-term experiments (Kuzyakov et al. 2009).

Incorporating biochar to agricultural soils does not only change the biology of soils but is also likely to have a related strong effect on the nitrogen dynamics of soils. As the C: N ratio of biochar is commonly relatively high; the initial mineralization of biochar C would result in a short-term N immobilization. This has been reported in both pot and field experiments on tropical N- limited soils (reduced plant N uptake and yields (Lehmann et al. 2003) as well as in laboratory incubations (Novak et al. 2010; Nelson et al. 2011). However, there is lack of knowledge on the duration and mechanisms responsible for the N immobilization by biochars of different quality and application rates in agricultural soils, and whether the impact depends on the chemical form of fertilizer.

The effects on soil nitrogen dynamics of the applications of BC alone or in combination with mineral N fertilizers has been the focus of few recent studies (Novak et al. 2010; Nelson et al. 2011). However, there is a gap in the understanding of longer term BC effects, as in these studies the incubations were run for less than 70 days. Furthermore, there is lack of studies that have focused on the effects of biochar application to N dynamics over time when applied together with organic fertilizers. As the importance of nutrient recycling through increased use of organic fertilizers is gradually more recognized (Römer 2009), the effects of biochar application on the nutrient dynamics of organic fertilizers in soil are of particular interest.

One of the organic N fertilizers today increasingly used in Europe is a by-product of the slaughtering industry, meat bone meal (MBM). Despite the fact MBM contains only 8% nitrogen, its low C: N ratio (about 4.5) provides a large potential for N mineralization (Jeng et al. 2004). The potential of MBM as an effective organic fertilizer was supported also by the enhancement of the biomass and activity of soil microorganisms in an incubation experiment (Mondini *et al.* 2008). In earlier studies on the fertilizer use of meat bone meal attention has been paid to a variety of characteristics including the effects on the yields of cereals and grasses (Jeng et al. 2004, 2006, Chen et al. 2011), the nutrient use efficiencies of N and P by plants (Jeng & Vagstadt 2009, Ylivainio & Turtola 2009) as well as the impact on the composition of microbial populations in soil (Mondini et al. 2008). Yet there have been no studies on the interactive effects of using biochar together with MBM. Knowledge on such interactions is especially important when biochar practices are extended to organic farming systems.

The objective of this study was to determine how biochar application to soil affects the N mineralization dynamics and if the effect is dependent on type or organic source of the N. This paper is based on manuscript submitted to Earth and Environmental Science Transactions of the Royal Society of Edinburgh.

## Materials and methods

### Biochar

Biochar was produced by pyrolyzing softwood chips made of partially debarked spruce and pine from Southern Finland in a continuously programmable pressurized carbonizer (Preseco Oy, Finland). The temperature of carbonizer was maintained constant at 550-600 °C during the whole pyrolysis process. Dried softwood chips were fed into the reactor tube through an airtight feed-in system and were then slowly moved by a screw conveyor through the hot region of reactor tube where the chips were heated for 10-15 minutes. At the termination, the biochar was cooled overnight in an airtight silo and moved by a conveyor to a roller mill for grinding. After grinding, the biochar had a particle size of less than 2 mm and was stored in a plastic bag for six months.

Prior to experiment, the biochar was first dried at 40 °C for 72 hours and sieved through a 0.2-mm mesh. The gravimetric moisture content ( $9.1 \text{ g kg}^{-1}$ ) of the sieved biochar (w/w) was determined by oven-drying a 4-g portion overnight at 105 °C. Biochar pH was measured with standard combination electrodes from 1:5 (v/v) suspension of biochar in deionised water, and it was 8.9.

For total elemental analyses, the biochar was dried and then digested according to the EPA 3052 microwave-assisted acid digestion method (USEPA, 1996). The elemental concentrations (expressed on a dry, w/w, ash-free basis) in the biochar digests were determined using an inductively coupled plasma optical emission spectroscopy (ICP-OES) (Thermo-Fisher iCAP3600 MFC Duo, Thermo Fisher Scientific, UK). The biochar contained 29.72, 25.36, 6.32 and 4.26  $\text{g kg}^{-1}$  of Ca, K, Fe and Mg, respectively, with other elements being significantly less abundant.

The total C and N contents of biochar (903 and 6.1  $\text{g kg}^{-1}$ , respectively) were determined by dry combustion with a VarioMax CN analyzer (Elementar Analysensysteme GmbH, Germany). The Brunauer-Emmett-Teller specific surface area (BET SSA) was determined by the Earth and Foundation Structures laboratory of the Tampere University of Technology with a single point (at 0.30 partial pressure) method using samples ground and sieved to pass 0.063 mm mesh and pre-heated at 300 °C for 30 minutes before analysis. The BET SSA ( $11.8 \text{ m}^2 \text{ g}^{-1}$ ) was then determined by using nitrogen adsorption techniques with a Micromeritics Flowsorb 2300 gas adsorption analyser (Micromeritics, USA).

Solid-state magic angle-spinning  $^{13}\text{C}$  NMR spectra were obtained by Bruker Avance spectrometer (Germany) operated at  $^{13}\text{C}$  frequency of 150 MHz. The direct excitation  $^{13}\text{C}$  NMR spectra of the biochar sample provided evidence that the majority of the biochar-C was in aromatic form.

### Soil

The experimental soil was taken with a shovel from the upper layer (0 to 25 cm deep) of a Gleyic Phaeozem (FAO 1998) from a field of Viikki Experimental Farm of University of Helsinki, Finland (N 60° 13' 46", E 25° 2' 33") in October 2010. Small grains (wheat and barley) had been grown in the field with conventional mouldboard ploughing and mineral fertilizer practices for the preceding six years. Prior to that, the field had been under clover cultivation for four years.

Physical and chemical soil analyses were carried out after air-drying and sieving the soil through a 2-mm mesh. According to the particle size analysis by a pipette method (Elonen, 1971), the soil had a sandy loam texture with 832, 153 and 15  $\text{g kg}^{-1}$  sand, silt, and clay, respectively. The pH of soil (6.3) was determined in a 1:2.5 (w/w) soil-to-water mixture (Vuorinen & Mäkitie 1955, MTT 1986).

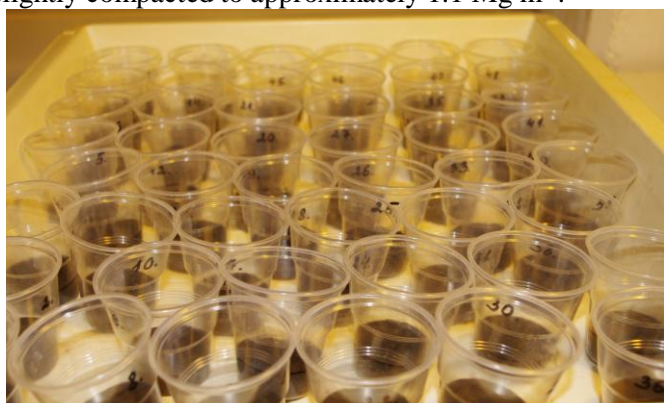
A VarioMax CN analyzer (Elementar Analysensysteme GmbH, Germany) was used for the determination of total carbon (C) and nitrogen (N) contents (28.7 and 1.9  $\text{g kg}^{-1}$ , respectively).

### Fertilizers

Two organic fertilizers were used in the experiment: a granulated meat-bone-meal-based fertilizer Aito-Viljo® (MBM) and composted cattle manure (CCM). Aito-Viljo® is a commercial fertilizer produced by Honkajoki Oy, Finland. It contains approximately 39% of C and 8% of N, which results in C/N ratio of 4.7. The composted cattle manure (N content 1.13% and C/N ratio of 19.7) was provided by an organic cattle farm, where straw bedding had been used. Prior to the experiment, the manure had been composted for six months. Both fertilizers were air-dried and ground before experimental use.

### Experimental design

The effects of biochar on the net N mineralization dynamics of two organic fertilizers were investigated in factorial laboratory incubation with time, biochar, and fertilizer as experimental factors. The incubation experiment included a total of twelve completely randomized combinations of biochar and fertilizer treatments. The biochar treatments were 0, 4.6, 9.1 and 13.6 g kg<sup>-1</sup> soil DM (corresponding to 0, 10, 20 and 30 Mg ha<sup>-1</sup> assuming a furrow slice of 2200 Mg ha<sup>-1</sup>). The fertilizer treatments were no fertilization (control), 1.7 g kg<sup>-1</sup> Aito-Viljo and 12.4 g kg<sup>-1</sup> composted cattle manure. The applied rate of both fertilizers corresponded to 139 mg N kg<sup>-1</sup> soil (306 kg N ha<sup>-1</sup>). A total of 288 conical 100-ml, open-top PVC beakers were used as incubation vessels. A portion of 24.3 g fresh soil (20.6 g dry weight) was weighed to each beaker. Each treatment was set up in four replicates and six identical batches to be analysed during 133-day incubation, so that a set of 48 vessels with all treatment combinations of biochar and fertilizers were destructively analysed on days 0, 14, 28, 56, 84 and 133 from the beginning of the incubation. The beakers for each extraction time were put randomly on plastic trays so that for each extraction time there was a separate tray (Fig. 1). Biochar and fertilizers were mixed thoroughly into soil (carefully avoiding any contamination) and the mixtures were slightly compacted to approximately 1.1 Mg m<sup>-3</sup>.



**Figure 1.** A set of 48 PVC beakers completely randomized on a plastic tray. Each sampling time corresponded to one tray.

Soils of all treatments were then wetted with sufficient deionised water to obtain the field capacity moisture content. Soil moisture was kept constant at field capacity by weighing the beakers weekly and adding water if necessary. The trays with the beakers were put in separate polyethylene bags to avoid moisture loss. Incubations were carried out in a constant temperature room at 15 ± 1°C.

The contents of soil mineral N (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) were determined at the soil samplings on days 0, 14, 28, 56, 84 and 133 of the experiment. All soil in each beaker (20.6 g soil DM) was poured to a 100-ml centrifuge tube, 50 ml of 2 M KCl was added and the tubes were closed with silicon caps. The suspensions were shaken with a low speed reciprocal shaker for two hours. After letting the suspensions to settle for 40 minutes the supernatant was filtered through an ashless filter. The ammonium and nitrate concentrations of the extracts were determined by a standard colorimetric flow injection analysis with a Lachat QuikChem 8000 (Lachat Instruments, USA).

### Calculations

Net N mineralisation in each fertilizer and biochar treatments was calculated as a difference to the mineral nitrogen content at the start of incubation according to:

$$\Delta N_{t,f,b} = N_{t,f,b} - N_{0,f,b} \quad (\text{Eq. 1})$$

where  $\Delta N_{t,f,b}$  is the amount of net N mineralisation (mg N kg<sup>-1</sup> soil), and  $N_{t,f,b}$  and  $N_{0,f,b}$  are the mineral N contents at a given measurement day  $t$  and day 0, respectively, for a given fertilizer type ( $f$ ) and biochar application rate ( $b$ ) (mg N kg<sup>-1</sup> soil DM).

Subsequently, the effect of biochar application on the net N mineralisation/immobilization at a given time ( $t$ ) was calculated for each fertilizer ( $f$ ) treatments by subtracting the corresponding amount of net N mineralisation in the soil with no added biochar ( $b = 0$ ):

$$\Delta N_{t,f,b}^{\text{eff}} = \Delta N_{t,f,b} - \Delta N_{t,f,0} = (N_{t,f,b} - N_{0,f,b}) - (N_{t,f,0} - N_{0,f,0}) \quad (\text{Eq. 2})$$

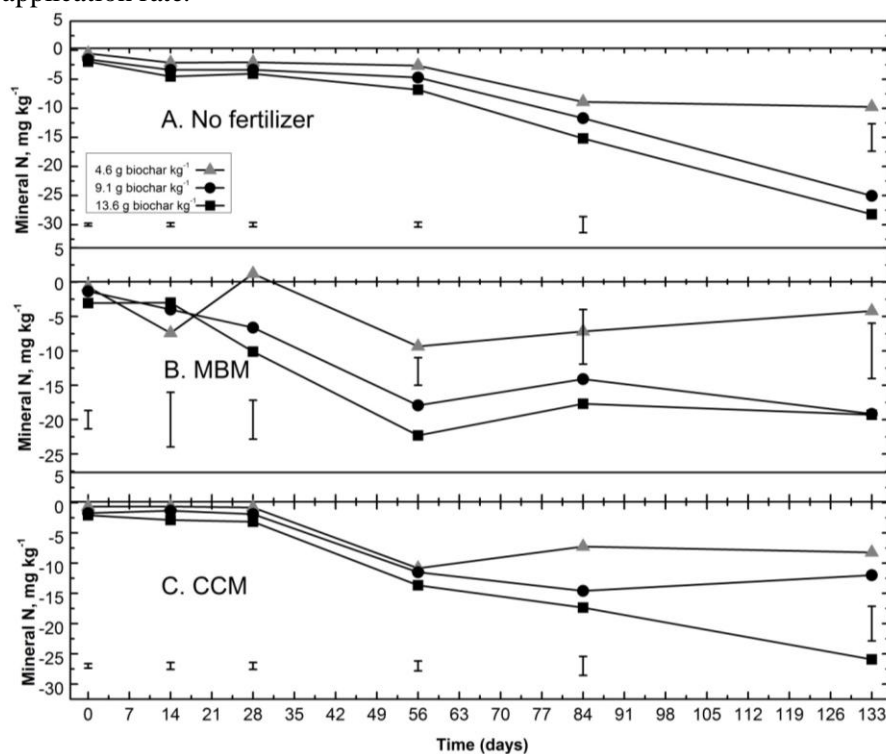
where  $\Delta N_{t,f,b}^{\text{eff}}$  is the net effect of biochar addition on the N mineralisation/immobilization in soil (mg N kg<sup>-1</sup> soil DM).

## Results and discussion

For both fertilizer treatments, the ammonia concentrations in the soils decreased over time, and most of it was obviously nitrified already by day 14. Since then the ammonium concentrations remained low and nitrate dominated the mineral N pool with 97-100 % share in all experimental treatments.

The  $\text{NO}_3^-$ -N contents increased fastest in the MBM treatments, reaching almost three times higher level by the end of the incubation than in the CCM treatment and control soil. The mineralization of nitrate from the MBM treated soil was extremely rapid: already after two weeks of incubation, the nitrate concentrations exceeded the maximum levels found in other fertilizer treatments at the end of the 133-day experiment. The nitrate content increased with time in all biochar and fertilizer treatments. However, the  $\text{NO}_3^-$  contents decreased in a progressive manner as the amount of added biochar increased in case of no fertilizer and CCM treatments at most times.

The suppressive effect of biochar was reflected in the temporal pattern of net immobilization of N in soil. For the no fertilizer treatment, added biochar decreased soil mineral N concentrations at all application rates. The net N immobilization with biochar were highest for the non-fertilized control, followed by CCM and MBM, largest reductions were 28, 26 and 19  $\text{mg N kg}^{-1}$ , respectively. The impact of biochar increased with time, and became stronger with larger additions. On the other hand, if fertilizer was added to the soil, the biochar-induced reductions in the net N mineralization from fertilizers started to decrease or level off after the day 56 (Fig. 2). The tendency was most evident at the lowest biochar application levels. Also, fertilizer types were differed in this respect. With MBM, the reductions in the net N mineralization were highest at day 56, and then levelled off at all biochar application rates, quickest at the lowest rate. With CCM, the inhibitory effect of biochar levelled off at the two lower application rates, but continued to the end of the incubation period at the highest application rate.



**Figure 2.** Reduction in soil mineral N-content caused by biochar addition at three levels, calculated as difference to treatments without biochar addition (Eq. 2 in the text. The value 0 at the ordinate axis stands for a given no-biochar treatment). A. no fertilizer, B. MBM and C. CCM treatments. Vertical bars represent the SE of means (n=4).

The principal results of the present study consist of decreased nitrate concentrations with increasing biochar application rates. This provides evidence of mechanism of short-term increase in N immobilization in temperature and soil moisture conditions corresponding to the average conditions during a growing season in Boreal climatic zone. However, the effects were different between the fertilizer treatments. The net N immobilization by biochar was highest for unfertilized control, followed by CCM and MBM. The significant differences between fertilizers for soil mineral N

( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) contents may be contributable to the different C:N ratios of fertilizers used, as this ratio is known to alter the N mineralization rate (Brady & Weil, 2002). The high straw bedding content of the CCM probably increased its C: N ratio 28% higher than that in the unfertilized control soil, and 319% higher than in the MBM.

The mechanism for reduced  $\text{NO}_3^-$  concentrations with increasing amounts of biochar added into soil are likely attributable to the N immobilization by microbes rather than denitrification. The latter is not probable, as strongly increased denitrification with biochar application has been shown at wet or moist soils (water contents more than 83% of WFPS) whereas decreased denitrification were recorded at water contents drier than 73% WFPS (Yanai et al., 2007). As the soil water content in our experiment was kept constant at about 45% WFPS and the soil air-filled porosity was more than 31% by volume during the incubation, we rule out the possibility of biochar-induced denitrification as a mechanism for the reductions in  $\text{NO}_3^-$  contents in soil and argue they are most likely explained by N immobilization to microbial biomass. As a general rule, the N immobilization after biochar incorporation is a temporary phenomenon, as part of C readily available for microbial assimilation is used up after few months leaving highly recalcitrant biochar fractions for the longer term microbial interactions (Novak et al. 2010; Nelson et al. 2011). This view was supported also by our experiment, where the reductions in the net N mineralization from fertilizers started to decrease after two months of incubation, possibly because of the turnover of microbial biomass.

### Conclusions

This study confirms that when biochar is applied together with organic fertilizers, the effects of added biochar on soil N mineralization dynamics depends greatly on the C: N ratios of fertilizers. Organic fertilizers of high C: N ratios cause short-term N-availability problems that however will start decreasing within a few months, whereas the initial N immobilization caused by biochar application is less significant when such low C: N materials as MBM are used as organic fertilizers. With suitable timing, biochar additions may effectively prevent leaching of N especially from uncropped soil, and when biochar is applied e.g. into fallowed soil well before next growing season, the risks of N availability problems during early growth could be minimized.

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