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Biochar does not affect soil N-transformations or microbial community structure under ruminant urine patches but does alter relative proportions of nitrogen cycling bacteria

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ABSTRACT

Nitrogen (N) cycling, especially denitrification, can be significantly altered when biochar is used as a soil conditioner. These alterations in N-cycling have been attributed to a combination of physicochemical change, alterations in microbial community ecology and pervading climatic conditions. This study investigated seasonal bacterial community change over two years in combination with a short-term winter study of N-transformations under bovine urine patches. A silt-loam pastoral soil in Canterbury, New Zealand was amended with either 0, 15 or 30 t ha^{-1} of *Pinus radiata* biochar (pyrolysed at ~450 °C) and bovine urine was added to patches within the 0 and 30 t ha^{-1} biochar amended plots (designated as 0 U and 30 U treatments, where U indicates 'urine').

No discernible differences in bacterial community structure were observed during the two year study or the short term N-transformation study when comparing non-amended and biochar-amended soil. Differences in bacterial community structure were only evident when comparing seasons, with data pertaining to each season from successive years clustering together. During the short-term N-transformation study, bacterial communities formed 3 distinct clusters corresponding to elevated levels of urine derived NH_4^+-N (days 0–10), increases in NO_3^--N and N_2O (days 10–22) and a decline in NO_3^--N and N_2O (day 20 onward). Biochar amendment did increase the relative abundance of up to 50% of individual operational taxonomic units (OTUs or 'species'), including key nitrite oxidisers and nitrate reducers. Biochar amendment did not affect the concentrations of inorganic-N compounds.

The *nir*S (nitrite reductase) gene became elevated in the 30 U treatment relative to the 0 U treatment ~10 days after the initial urine application. The *nos*Z (nitrous oxide reductase) gene became elevated in the 30 U plots during the latter part of the experiment.

Conclusions:

- Biochar did not have a significant impact on the microbial community structure in pastoral soil over the course of two years.
- The relative proportion of nitrifiers and denitrifiers increased in biochar amended soils subjected to large influxes of urine derived N.
- Differences in N-transformation dynamics in the presence of biochar during the winter months were not statistically significant.

1. Introduction

Nitrogen (N) loss from agricultural ecosystems is of environmental concern since excess nitrate (NO_3^-) pollutes aquatic systems, nitrous oxide (N_2O) emissions contribute to global warming and catalytic destruction of ozone, and nitrite (NO_2^-) is thought

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to be a source of atmospheric HONO and OH radicals (Forster et al., 2007; Ravishankara et al., 2009; Su et al., 2011). Currently, anthropogenic N application to the earth's land surface equates to approximately 170 Tg yr^{-1} with a further 125 Tg yr^{-1} of N inputs from natural N-fixation. Large amounts of this N are lost from agricultural systems with at least 15% of N-inputs unaccounted for (Schlesinger, 2009). Clearly there is a need to reduce N-inputs, reduce ammonia (NH_3) and NO_3^- leaching losses, increase N use efficiency, and maximise denitrification to N_2.

Grazed pasture receives N through a combination of biological fixation, atmospheric N inputs and fertilizer application, while most N consumed by grazing animals is returned to the soil in animal excreta (Haygrath et al., 2013). Concentrated pools of ammonium (NH_4^+) form as a result of animal urination with biological nitrification processes transforming urine-derived NH_4^+ into NO_2^- and NO_3^-. Inorganic-N concentrations in these pools are far higher than pasture plants requirements and the unused inorganic-N undergoes nitrification, nitrifier-denitrification and/or denitrification to NO_3^- and N_2O (Haynes and Williams, 1993). Under such high N loadings, the concentration of NO_3^- and N_2O exceed the capacity of the biological system to denitrify the excess NO_3^- or to reduce excess N_2O to N_2. Physicochemical constraints such as aeration and soil pH also affect the efficiency and function of biological N-transformations. The end result is leaching losses of NO_3^- and large N_2O fluxes from agricultural soils, specifically in and around animal urine patches.

Biochar is a by-product of biomass pyrolysis (Lee et al., 2010). It is a relatively stable high-carbon (C) product that is being promoted as a tool to sequester C in terrestrial systems (Laird, 2008; Lehmann and Joseph, 2009; Novak et al., 2009). When added to soil, biochar can improve agronomic properties and can increase NH_3/NH_4^+ adsorption, while reducing NO_3^- leaching, N_2O emissions, and NH_3 volatilisation (Clough et al., 2013; Singh et al., 2010; Spokas et al., 2009; Steiner et al., 2010; Taghizadeh-Toosi et al., 2011; van Zwieten et al., 2010). A recent study by Cayuela et al. (2013) suggested that biochar facilitates the transfer of electrons, produces localised liming (aiding the functionality of microbial denitrification proteins) and alters reactive surface areas. The exact mechanisms of how biochar influences N-fluxes and transformations remain largely unclear (Clough et al., 2013), but are definitely attributed to changes in soil physicochemical properties and biological functions.

Taghizadeh-Toosi et al. (2011) reported that KCl extractable NH_4^+ was about 15–20 times higher in biochar that has been exposed to an NH_3 atmosphere. This indicated that the NH_3 was sequestered by acidic functional groups in an NH_4^+ form, although NH_4^+ only accounted for a fraction of the total N increase in biochar following NH_3 exposure. Knowles et al. (2011), also showed reductions in NO_3^- leaching of 60% when biochar was added to soil/bio-solid mixtures. They suggested a variety of mechanisms were responsible for the reduction in NO_3^- leaching, including adsorption of NH_4^+, NO_2^- and NO_3^- to biochar, and potential inhibition of N-mineralisation, although Cayuela et al. (2013) discounted the possibility of NO_2^- and NO_3^- adsorption.

Reductions in N_2O emissions are closely related to the effect that biochar has on soil pH and water-filled poor space (WFPS) (Clough and Condron, 2010; Singh et al., 2010). Singh et al. (2010), reported that after successive wetting/drying cycles, biochar amended soils produced consistently lower emissions of N_2O and also noted that any N_2O produced declined rapidly at >85% WFPS, attributing this to increases in N_2O-reducatase activity in response to anoxic conditions and biochar induced increases in soil pH. In contrast, Yanai et al. (2007), suggested that biochar addition improves soil aeration at higher WFPS, precluding denitrifier activity so less N_2O is produced in the first place. Similarly, in a study by Cayuela et al. (2010), biochar treated soils produced less N_2O emissions than control soils at 80% WFPS, which was partly attributed to inhibition of nitrifier and/or denitrifier communities. Preclusion of denitrifier activity due to biochar affecting soil water distribution could also provide a partial explanation as to why Taghizadeh-Toosi et al. (2011) observed 70% reductions in N_2O flux when the maximum WFPS in their study only reached 67%.

Despite the mechanistic contrasts and unknowns, all these studies suggest that biochar affects the metabolic behaviour of N-transforming microorganisms and the physicochemical control of N bioavailability thereby altering soil biogeochemistry. Clearly, there is still a lack of definitive knowledge about the influence of biochar on biological activity and biogeochemical cycles especially under *in situ* conditions (Lehmann et al., 2011). The aims of this study were to:

- Assess the effect of biochar on long-term bacterial community structure and stability *in situ*.
- Investigate any effects that biochar amendment has on *in situ* biological N-transformations under animal urine patches.
- Investigate any effects that the presence of biochar may have on the *in situ* bacterial community structure when ruminant urine is applied.

2. Materials and methods

All aqueous solutions were prepared using ultrapure water from a MilliQ water system (18 MΩ-cm resistivity) and all chemicals used were ACS reagent grade, unless otherwise stated.

Meteorological data (air temperature and rainfall) along with soil temperature at 0.1 and 0.3 m soil depth were obtained from the Lincoln University meteorological station which is located approximately 3 km from the field site.

2.1. Pasture establishment, treatments and experimental design

For a full description of pasture establishment and field trial design please refer to Taghizadeh-Toosi et al. (2011). Field trial design, soil properties and biochar properties are presented in Fig. S1 and Tables S1 and S2, respectively. Briefly, a runout perennial ryegrass (*Lolium perenne* L.) pasture situated at Lincoln University (43°38′58″ S, 172°27′53″ E) on a Templeton silt-loam soil (Hewitt, 1998), was renovated in May (autumn) 2009 for a field trial. The pasture was cultivated to a depth of 0.30 m using a roto-cultivator, and then unweathered *Pinus radiata* biochar was added at 3 amendment rates (0, 15 and 30 t ha^{-1}) replicated 5 times by spreading the biochar on the plots and then mixing it into the first 0.1 m by making a shallow pass with the roto-cultivator. The trial area was then rolled before sowing with a forage perennial rygrass (*Lolium perenne*, cultivar 'Samson'). After ryegrass emergence, urea fertiliser was applied twice – 83 kg ha^{-1} on the 9th of September 2009, and 50 kg ha^{-1} on the 28th of October, 2009. A selective broadleaf herbicide was applied on the 21st of October 2009 and a fungicide was applied on 19th November 2009 to prevent stem rust.

Headspace chamber bases (stainless steel, diameter 0.39 m) were installed on the 13th of November 2009. These bases protruded 0.1 m into the soil and contained an annular water-filled trough. During gas sampling, insulated stainless steel headspace covers with 0.1 m high walls were placed on the bases with annular trough water creating a gas tight seal. Total headspace volume under the covers was 11.6 L. Adjacent to each gas sampling chamber was a soil sampling plot (0.37 m × 0.43 m) (see Fig. S1 for the layout of gas and soil sampling areas).