

Biochar amendment of urine-treated New Zealand pasture soil induces unique shift in active bacterial but not archaeal ammonia-oxidizer communities

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Introduction

Recent studies implicate biochar as a potential nitrous oxide mitigation agent as well as a carbon sink. The objectives of this study were to:

- (1) Investigate the ability of biochar to reduce emissions of nitrous oxide (N_2O) from bovine urine-treated cores packed with a Wakanui silt loam
- (2) Determine the extent to which shifts in total and active bacterial and archaeal ammonia oxidizer communities in these soil cores explain observed N-turnover.

The results from the first objective have recently been submitted for publication (Clough et al., *submitted*), and show that biochar amendment actually increased N_2O emissions from urine-treated soil. This poster summarizes the results from the second objective in light of the findings from the first objective.



Experimental procedures

Wakanui silt loam was collected from Lincoln University Dairy Farm (43°38.48S, 172°26.39E), sieved to 4 mm and packed into PVC cores (70 mm X 50 mm internal diameter). The experiment consisted of four treatments: control (water only), biochar control (20 t ha⁻¹ biochar + water), urine (760 kg N ha⁻¹ urine-N) and biochar plus urine (20 t ha⁻¹ biochar + 760 kg N ha⁻¹ urine-N), with four replicates per treatment. Total nucleic acids were extracted from 0.5g (wet weight) soil samples using the method of Griffiths et al. (2000). PCR was used to amplify the bacterial *amoA* gene from both DNA and cDNA using primers *amoA*-1F[GC] and *amoAR1* (Avrahami et al., 2003). PCR amplification of archaeal *amoA* utilised primers *AOA-amoA-f* and *AOA-amoA r[GC]* (Coolen et al., 2007). DGGE was performed at 60°C for 18h at 100V on 30-60% (bacterial *amoA*) and 10-40% (archaeal *amoA*) denaturing 7% polyacrylamide gels.

References

Avrahami S, Liesack W and Conrad R (2003) *Environ Microbiol* 5, 691-705.
Clough T, Bertram J, Ray J, Condon L, O'Callaghan M, Sherlock R, Wells N. (2008) Biochar incorporation stimulates N_2O and NH_4^+ emissions from bovine urine amended soil (*submitted to Soil Biology & Biochemistry*).
Coolen M, Abbas B, van Bleijswijk J, Hopmans E, Kuypers M, Wakeham S and Damsté J (2007) *Environ Microbiol* 9, 1001-1016.
Griffiths R, Whiteley A, O'Donnell A and Bailey M (2000) *Appl Environ Microbiol* 66, 5488-5491.

Results

- N_2O fluxes for the biochar plus urine treatment were significantly greater ($P < 0.001$) than for the urine treatment between days 15 to 29, peaking on day 21. (Figure 1)
- Percentage of urinary N applied, as N_2O -N, was greatest for the biochar plus urine treatment (28.6 ± 11.7), but this was not significantly different from the urine treatment (16.8 ± 5.3) ($P > 0.05$). (Data not shown)
- In the biochar plus urine treatment the ammonium-N (NH_4^+ -N) concentrations were significantly higher ($P < 0.01$) than the urine treatment on days 10 and 20, the nitrite-N (NO_2^- -N) concentration was higher than the urine treatment on day 20 (not significant, $P > 0.05$), and the nitrate-N (NO_3^- -N) concentration was higher than the urine treatment on day 55 only ($P < 0.01$). (Figure 2)
- Active bacterial *amoA* communities for the biochar plus urine treatment were significantly different from the urine, control and control plus biochar treatments ($P < 0.05$). Total bacterial *amoA* and both archaeal *amoA* communities responded to urine without a specific response to biochar amendment. (Figure 3)

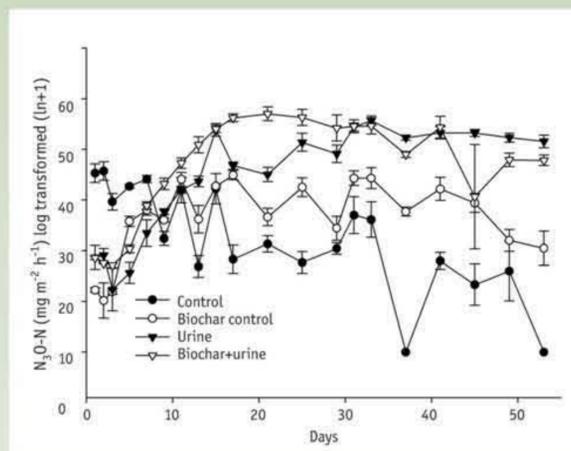


Figure 1. Nitrous oxide (N_2O) flux (log-transformed) versus time. Error bars represent standard error of the mean where $n=4$.

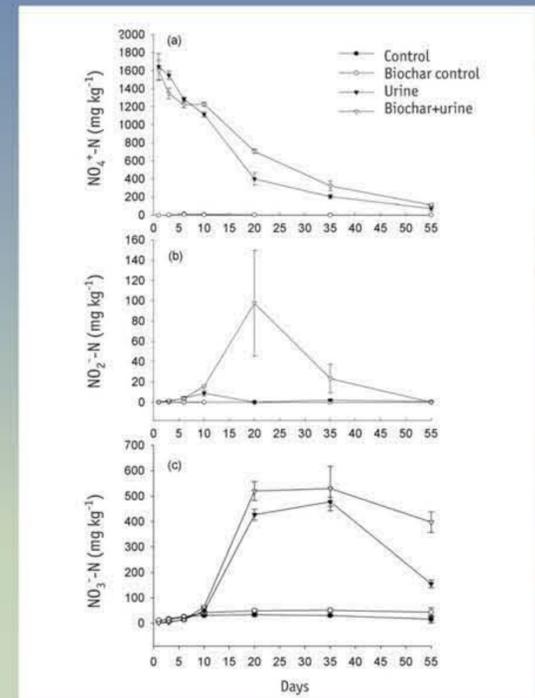


Figure 2. Soil (a) ammonium (NH_4^+), (b) nitrite (NO_2^-) and (c) nitrate (NO_3^-) concentrations versus time. Error bars represent standard error of the mean where $n=4$.

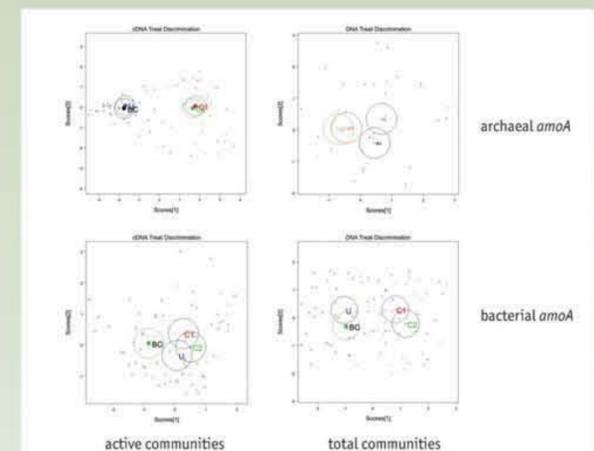


Figure 3. Principle component analysis of DGGE results for bacterial and archaeal ammonia oxidizer communities. C1, control treatment; C2, biochar control treatment; U, urine treatment; BC biochar plus urine treatment. Circles represent 95% confidence intervals where $n=4$.

Conclusions

N_2O flux and inorganic-N data suggest that inclusion of biochar in soil cores treated with bovine urine resulted in inhibition of nitrification and thereby increased nitrifier denitrification, observable as higher N_2O fluxes (Clough et al., *submitted*) and a community shift in the active contingent of bacterial ammonia-oxidizers.

Future Work

- Investigation of biochar chemistry in soil to determine if biochar sequesters NH_4^+ or NO_2^-
- Microscopic examination of biochar particles from urine treatment experiments to assess microbial colonisation
- Elucidation of nitrite oxidizing microbial community dynamics under urine patches