Nitrous Oxide-Novel Mitigation Methodologies

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Nitrous Oxide-Novel Mitigation Methodologies:
Objective 2 - Biochar effects on urinary-N N$_2$O emissions.

to

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Prepared by

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IDENTIFICATION	
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Project Title	**Nitrous Oxide-Novel Mitigation Methodologies**
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Executive Summary

- To determine the effects of biochar (derived from *Pinus radiata*), with and without urine amendment, on N_2O emissions from urine, an experiment was performed over 29 days that included six treatments as follows:

  - Control (soil + water).
  - Soil + urine.
  - Soil + urine + biochar (10 t ha^{-1})
  - Soil + urine + biochar (20 t ha^{-1})
  - Soil + urine + biochar (30 t ha^{-1})
  - Soil + biochar + water (20 t ha^{-1})

- As the biochar rate was increased by 10, 20 or 30 t ha^{-1} the cumulative urinary N_2O emissions were reduced by 15, 52 and 74% respectively. The percentage reduction in N_2O emissions were positively related to biochar rate ($y = 2.44x$, $r^2 = 0.97$).

- No differences in NH_3-N emissions were observed between treatments.

- Soil gravimetric moisture content decreased with increasing rates of biochar addition and this may have been a factor influencing the N_2O production mechanisms.

- Biochar incorporation altered soil inorganic-N dynamics by lowering soil NH_4^+-N concentrations. These lower concentrations occurred with increasing rates of biochar. This was presumably due to biochar enhancing the cation exchange capacity.

- Biochar, in the absence of urine-N produced a liming effect, raising the soil pH by approximately 0.5 pH units when biochar was applied at the equivalent rate of 20 t ha^{-1}.

- Future work is urgently required to assess the effect(s) of biochar amendment on N_2O emissions when applied to pasture soils under field conditions, effects on plant N uptake, and N leaching along with other N transformations which occur in grazed pasture systems.

1. Rationale:

<u>Information gap:</u> What effect, if any, does biochar have on N_2O emissions when urine is applied to soil?

<u>Reasons for the gap</u>: While the effect of adding chemo-physical constituents such as relatively inert charcoal (e.g. biochar) is promoted as a carbon sequestration option the effects of such a practice, for synergistically mitigating N_2O emissions in pastoral systems, have not been examined.
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<u>In the context of known research</u>: In laboratory petri dish studies the addition of charcoal to soils has been postulated to stimulate N_2O reducing activity thus reducing N_2O emissions (Yanai et al. 2007)[1]. While the only other study to examine N_2O fluxes from biochar affected soils found that the incorporation of biochar reduced N_2O emissions by 80% when applied to grass ecosystems (Rondon et al. 2005)[2].

<u>Work programme</u>: A short term laboratory study was performed with varying rates of biochar to assess the mitigation potential of biochar incorporation as an N_2O mitigation tool following urine application to pasture. Urine contained ^{15}N to enable tracing of the N_2O gas source.

2. Objectives

2.1 Aims:
  (i) To compare the N_2O emissions from bovine urine applied to a repacked pasture soil containing biochar.
  (ii) To elucidate the effect (if any) of soil biochar amendment on N_2O emissions in the presence of urinary-N.

2.2 Outcomes:
  (i) A laboratory assessment of the effectiveness of biochar in mitigating bovine urinary N_2O emissions.

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[1] Yanai Y., Toyota K., Ozaki M. (2007) Effects of charcoal addition on N_2O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. *Soil Science and Plant Nutrition* 53: 181-188.
[2] Rondon M., Ramirez J.A., Lehmann J. 2005 Charcoal additions reduce net emissions of greenhouse gases to the atmosphere. Proceedings of the 3rd USDA Symposium on Greenhouse Gases and Carbon Sequestration, Baltimore, USA, March 21-24 2005, p. 208.

3. Approach

A laboratory study was performed with repacked, freshly sieved, pasture soil.

To determine the effects of biochar, with and without urine amendment, on N_2O emissions from urine, an experiment was performed that included six treatments as follows:

- Control (soil + water).
- Soil + urine.
- Soil + urine + biochar (10 t ha^{-1})
- Soil + urine + biochar (20 t ha^{-1})
- Soil + urine + biochar (30 t ha^{-1})
- Soil + biochar + water (20 t ha^{-1})

To enable N_2O determinations these treatments were replicated 5 times, and arranged in a randomized complete block design, 30 jars in total.

To enable destructive soil sampling over time a further set of jars were set up with four destructive sampling times (7, 14, 21, and 28 days) and the same six treatments, and these were replicated three times (72 jars).

The urine-N application rate was the equivalent of 750 kg N ha^{-1}. Urine was collected from Friesian cows at the Lincoln University Dairy Farm that had been grazing ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture. Collected urine contained 4.8 g N l^{-1}. It was further amended with urea (both at natural abundance and enriched with ^{15}N) to bring the N concentration up to 10.0 g N l^{-1}. A concentration typically found in bovine urine.

Biochar, manufactured from *Pinus radiata*, was sieved and the 0-10 mm fraction was retained and incorporated into the treatments as described below. The pasture soil (Wakanui silt loam) was collected from the 0-10 cm depth. Plant roots, earthworms and any obvious organic material were removed during sieving (≤4 mm). Fresh soil was placed into Mason jars (equivalent of 120 g dry soil).

The biochar was then incorporated into the appropriate treatments. This was performed by weighing out the required mass of biochar with 10, 20 and 30 t ha^{-1} being the equivalent of 5, 10 and 20 g of biochar per Mason jar. The jar was then capped and the biochar and soil were mixed by tumbling the jar for 1 – 2 minutes. Treatment solutions were (urine or water (38 ml)) were then applied by pipette to the soil surface.

For all sets of jars, described above, a sub-set of jars were weighed every second day and soil moisture was maintained and adjusted by adding any water lost due to evaporation.

4. Measurements

4.1 Nitrous Oxide flux determinations and ^{15}N enrichments

Nitrous oxide determinations were made on 16 occasions over the 29 days of the study (days 0, 1, 2, 3, 6, 7, 8, 10, 13, 15, 17, 20, 22, 24, 27, 29). These were achieved by

applying a screw-tight lid to the jar which had a rubber septum fitted to enable gas sampling. Gas samples were taken of ambient air and from the headspace of the jars at 15 and 30 minute intervals. To take the gas samples a glass syringe, equipped with a 3-way stopcock and 0.5 mm gauge needle, was used. The syringe was flushed with ambient air and injected into the headspace where upon the syringe was flushed twice and a 10 mL gas sample taken and injected into a pre-evacuated Exetainer® (6 mL volume). Lids were removed when gas sampling was not being performed.

Gas samples were analysed using a gas chromatograph (8610, SRI Instruments, CA.) interfaced to a liquid autosampler (Gilson 222XL, Middleton, WI.). The autosampler had been specially modified for gas analysis by substituting a purpose-built (PDZ-Europa, Crewe, UK) double concentric injection needle for the usual liquid level detector and needle. This enabled the entire gas sample to be flushed rapidly from its septum-sealed container (6 mL Exetainer®) into the GC.

The GC configuration was similar to that used by Mosier and Mack (1980) and included two 0.3 cm OD stainless steel columns packed with Haysep Q connected in series, oxygen-free dry nitrogen carrier gas (40 mL min^{-1}), and a ^{63}Ni electron capture detector at 320°C. Gas samples were analysed within 1 to 2 days of sampling. Immediately prior to analysis the over-pressurised samples were all brought to ambient atmospheric pressure, using a double-ended hypodermic needle. One end of the needle was placed at a constant depth (0.5 cm) just below the surface of some water in a small beaker while the other end pierced the Exetainer® septum. A brief flow of bubbles resulted and when these ceased, the gas in the Exetainer® was at ambient air pressure. Dissipating the excess gas pressure through the water medium not only gave a visual indication of when the samples were at ambient air pressure, it also avoided any potential contamination of the sample with ambient air. Reference gases were prepared following the same over pressure-equilibration procedure as described above.

4.2   NH$_3$-N determinations

Ammonia (NH$_3$) volatilisation was also measured at the same time as the N$_2$O sampling periods by placing a piece of Whatman No. 42 filter paper impregnated with 20 µl of 14.6 M orthophosphoric acid in the headspace of the Mason jar. These acid traps were removed after 1 h and extracted with 10 ml of deionised water, with the extract analysed for ammonium-N (NH$_4$-N) as described below. The hourly NH$_3$ fluxes were then integrated to yield the total NH$_3$-N emission over the 10 days following urine application.

4.3   Soil inorganic-N determinations

As noted above a further set of jars had been set up in order to determine changes in soil inorganic-N, pH and gravimetric moisture contents.

At 7, 14, 21 and 28 days a set of jars (18; 6 treatments by 3 replicates) were analysed for inorganic-N (NH$_4^+$-N, NO$_2^-$-N, and NO$_3^-$-N) by thoroughly mixing the soil and biochar in the soil and taking a sub-sample of the soil only (biochar removed). This was extracted with 2M KCl, filtered through Whatman 42 filter paper with the resulting filtrate analysed on a flow injection analyser for inorganic-N concentration.

A further sub-sample of soil was taken for gravimetric soil moisture determination (θ_g). This was oven dried for 24 h and the gravimetric moisture content determined according to standard methodologies. Finally, another sub-sample was taken and air-dried for determination of the soil pH, again using standard methodology.

4.4   Statistical analyses

Statistics included one-way analysis of variance with treatment as a factor and the appropriate level of replication which depended on the variable being analysed.

5. Results

5.1   Nitrous oxide fluxes, emission factors, and N_2O-^{15}N enrichments

Nitrous oxide fluxes were the lowest in the absence of urine, in the control and [soil + biochar] treatments with no significant difference between these two treatments over time. For the first 27 days these two treatments had lower N_2O fluxes than any of the other treatments but by day 28 the urine treatment with the highest rate of biochar [soil + urine + biochar (30 t ha^{-1})] had N_2O fluxes that were not significantly different from the control and [soil + biochar] treatments.

The highest N_2O fluxes occurred in the [soil + urine] treatment (Figure 1a) with a mean maximum flux of 54,573 μg m^{-2} d^{-1} immediately the treatments were applied. In the other urine treatments where biochar was applied the maximum fluxes also ensued following treatment application (Figure 1a), but the maximum fluxes decreased with increasing rates of biochar addition.

After day 0 the N_2O fluxes decreased dramatically and were sporadic in nature until day 8, and over this time the influence of increasing the rate of biochar was not significantly consistent. However after this time, until the end of the experiment, the N_2O fluxes decreased to be <3000 μg m^{-2} d^{-1}, and consistent statistically significant differences between the treatments occurred (Figure 1b) with N_2O fluxes decreasing with increasing rates of biochar amendment (P <0.001).

Cumulative N_2O emissions reflected the daily fluxes with maximum emissions from the [soil + urine] treatment and significantly lower cumulative emissions occurring at biochar amendment rates ≥ 20 t ha^{-1} (Figure 2a). As a percentage of urine-N applied the cumulative emissions equated to 27 ±2, 23 ±2, 13 ±2, and 7 ±4% (± SEM). It must be noted that the EFs derived here in no way resemble normal field practice and they CANNOT be compared to EFs derived under field conditions, Figure 2b. When plotted against the biochar amendment rate there was a significant negative correlation with increasing rates of biochar and the urinary-N EFs (Figure 3).

What is significant here is that as the biochar rate was increased by 10, 20 or 30 t ha^{-1} the N_2O emissions were reduced by 15, 52 and 74% respectively. The percentage reduction in N_2O emissions were positively related to biochar rate (y = 2.44x, r^2 =0.97).
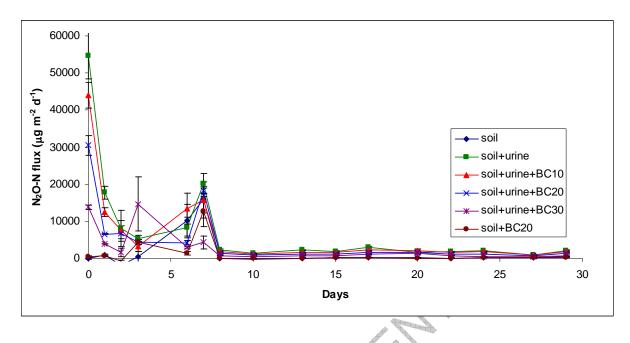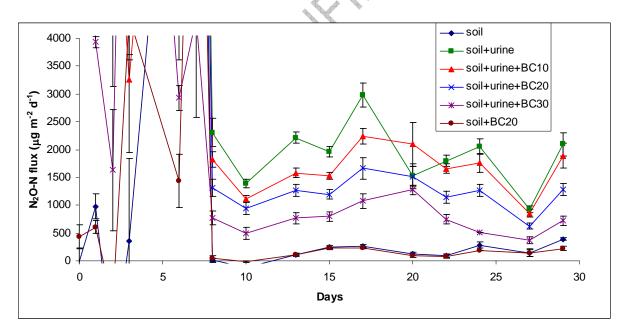
Figure 1a. N_2O-N flux over time (error bars ± SEM, n =5).

Figure 1b. N_2O-N flux over time with an expanded y-axis limited from 0 to 4000 µg m^{-2} d^{-1} to demonstrate the difference in N_2O fluxes after day 8, (error bars ± SEM, n =5).
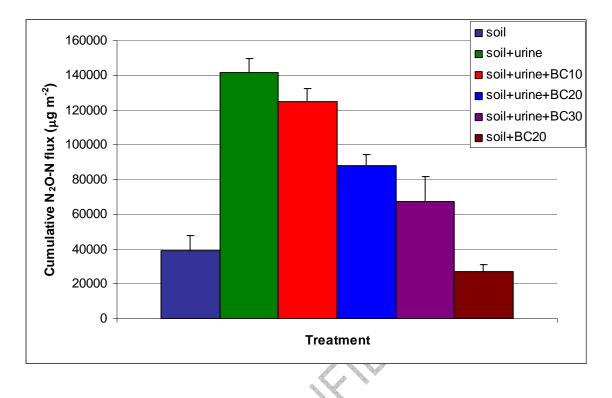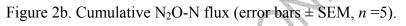
Figure 2a. Cumulative N$_2$O-N flux (error bars ± SEM, n =5).
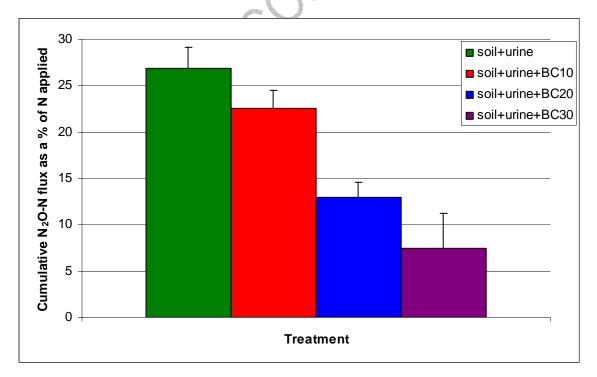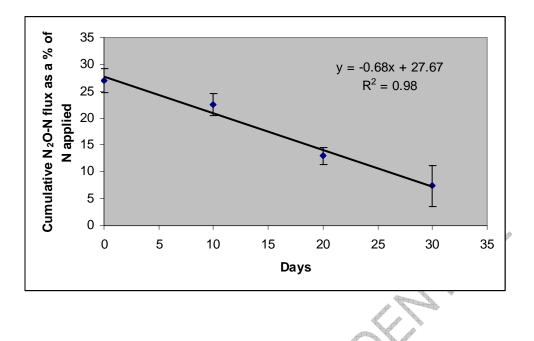

Figure 2b. Cumulative N$_2$O-N flux (error bars ± SEM, n =5).

Figure 3. Cumulative N_2O-N flux (error bars $\pm$ SEM, n =5).

The ^{15}N enrichment of the N_2O fluxes differed significantly due to biochar addition with enrichment decreasing with biochar addition (P <0.01) indicating less of the N_2O came from the urine-N pool as the biochar rate increased.
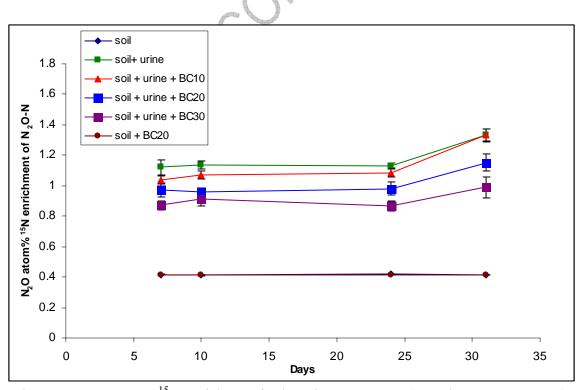

Figure 4. N_2O-N ^{15}N enrichment in the urine treatments (error bars $\pm$ SEM, n =5).

5.2   Soil Inorganic-N concentrations

Concentrations of NH_4^+-N were lowest in the treatments without urine addition (< 6 μg the soil NH_4^+-N concentrations). The addition of biochar at 20 t ha^{-1}, in the absence of urine, did no cause a change in the soil NH_4^+-N concentrations. As expected applying urine increased soil NH_4^+-N concentrations and they were at a maximum on day 7 in the [soil + urine] treatment (1709 μg NH_4^+-N g^{-1} soil). At day 7 the addition of biochar produced lower NH_4^+-N concentrations as rates of biochar addition increased (P <0.001) with mean concentrations ranging from 1709 with nil biochar, to 1191 μg NH_4^+-N g^{-1} soil at 30 t ha^{-1} of biochar, Figure 5a. This trend was less significant on subsequent days.

Concentrations of soil NO_2-N and NO_3-N were not affected by increasing rates of biochar addition with no significant differences between urine treatments (Figure 5b, 5c).
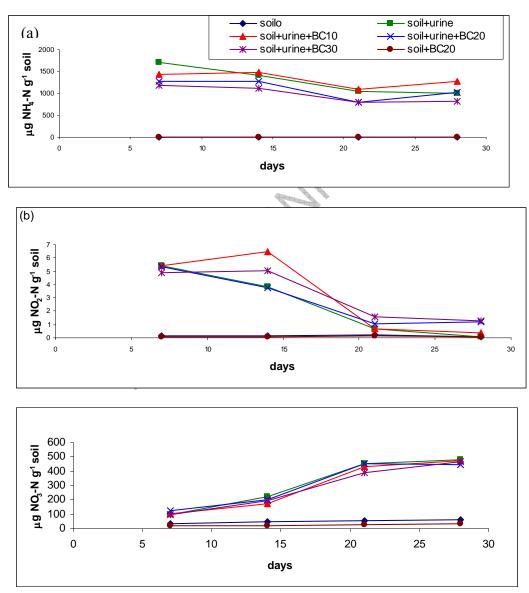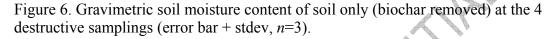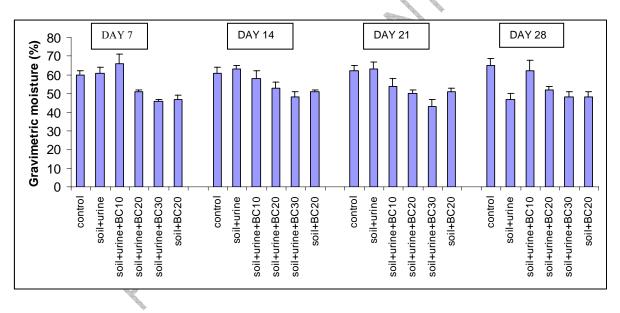
Figure 5   Soil inorganic-N concentrations over time.

5.3 Soil gravimetric water contents (θ g)

The moisture content of the soil only fraction – the biochar was removed - varied with treatment. There was no difference between the gravimetric water contents in the control and [soil + urine] treatments. However as the biochar rate was increased the gravimetric water content of the soil fraction decreased (P <0.01), Figure 6.

The gravimetric water content of the biochar removed from the soil was significantly higher than that of the surrounding soil averaging 134 (126-226), 155 (117-229), 120 (113-246), and 130 (95-208)% on days 7, 14, 21, and 28 respectively but with large variation in these data (range in brackets). But clearly the biochar was wetter – on a mass basis than the surrounding soil.

Figure 6. Gravimetric soil moisture content of soil only (biochar removed) at the 4 destructive samplings (error bar + stdev, *n*=3).

5.4 Soil pH

On day 7 the soil pH did not differ between the urine treatments but these were higher than in the non-urine treatments. There was a liming effect of biochar in the [soil + biochar 20 t ha^{-1}] treatment when compared with the control (Figure 7). This liming effect was maintained at days 14 and 21 with soil pH increasing by approximately 0.5 pH units. In the [soil + urine] treatment the soil pH decreased over time as expected following urine application. At day 7 there were no significant differences between the [soil + urine] treatment and any of the biochar plus urine treatments but by day 14 the soil pH in the [soil + urine] treatment had decreased and continued to decrease at a faster rate than in the urine plus biochar treatments (Figure 7).
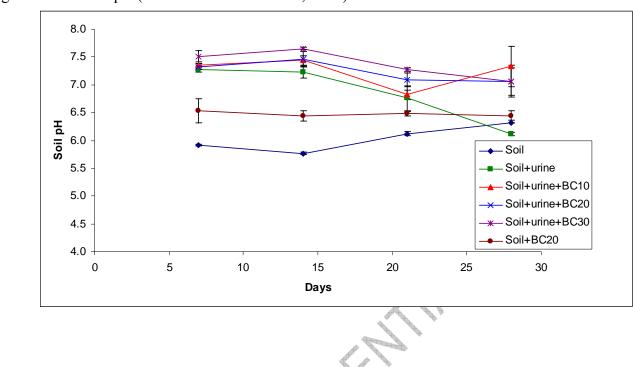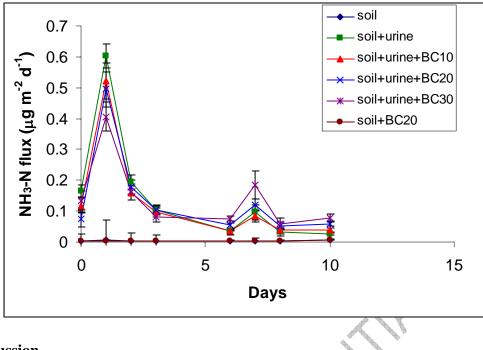
Figure 7   Soil pH (error bars are ±one SEM, *n* = 3).

5.5   *Ammonia fluxes from urinary-N*

There was no difference in the cumulative NH_3-N losses from the urine treatments (P =0.81) with the cumulative NH_3-N losses ranging from 3.1 to 3.5% of the urinary-N applied. Treatment effects on NH3-N fluxes only occurred on day 1 (P <0.05) where NH3-n fluxes were lower with increasing rates of biochar incorporation, and on day 10 (P <0.01) where the biochar treated soils had higher fluxes (Figure 8).

Figure 8   Ammonia-N fluxes from the biochar and urine treated soils (error bars are ±SEM, *n* = 5)

6. Discussion

As expected the addition of urine increased the N_2O fluxes. The addition of biochar on its own did not contribute to any enhanced N_2O flux indicating that the biochar on its own did not stimulate N_2O-N fluxes. The fluxes on day zero were almost certainly a result of soil being wetted up by the treatment solutions. The reason for the lower fluxes at the higher rates of biochar may have been due to the biochar affecting the aerobic status of the soil and thus reducing anaerobic conditions and N_2O fluxes. Evidence for biochar affecting soil moisture status is seen in the soil gravimetric water data where values of θg decreased with increasing biochar content. All things being equal this would have reduced the potential for denitrification and may explain the lower N_2O fluxes that occurred with elevated biochar rates. Exactly how biochar influenced the soil moisture content is a question for future research. Did the biochar absorb the applied liquid treatments and prevent the soil wetting up? Or did the biochar slowly absorb moisture from the soil? The soils were being regularly rewetted and it seems that the soil-moisture equilibrium was being maintained. The physical characteristics of water absorption and release of by biochar must be investigated in the field.

Biochar incorporation may have also reduced N_2O fluxes by altering the inorganic-N supply. The soil NH_4^+-N concentrations were affected, with lower concentrations at higher rates of biochar. Had nitrification been the source of the N_2O flux this may have been a reason for the altered N_2O fluxes. However, from day 7 onwards concentrations of both NO_2^--N and NO_3^--N were not affected by the biochar treatment which indicates adequate and unvarying rates of nitrification between the biochar treatments. This suggests denitrification of NO_2^--N and/or NO_3^--N was affected by the biochar incorporation; again this was possibly a factor of the biochar's effect on soil moisture.

The decreasing ^{15}N enrichment of the N_2O-N with increasing levels of biochar incorporation indicate less of the N_2O-N was derived from the urine-N, and more from native soil-N, as biochar rates increased. This could be due to absorption of urinary-N by the biochar-possible making less urinary-N available for denitrifiers or an increase in the inorganic-N pool with increasing rates of biochar. The soil NH_4^+-N concentrations may have

been lower due to increased cation exchange in the biochar treatments and this may have taken some of the urinary-N out of the soil inorganic-N pool, resulting in greater dilution of ^{15}N by any native inorganic-N that was present. Interestingly a liming effect was observed in the [Soil + biochar 20 t ha^{-1}] treatment. If this effect occurred in all the urine plus biochar treatments, in proportion to the amount of biochar applied, it may have been sufficient to mobilise more native inorganic-N and thus reduce the ^{15}N enrichment of the inorganic-N pool and any subsequent N$_2$O-N flux. The slower rate of pH decrease in the biochar treated soils indicates differences in the amounts or rates of nitrification i.e. slower rates of nitrification. This would be consistent with a higher cation exchange capacity (CEC) in the biochar treated soils. A higher CEC in the biochar treated soils would also explain the liming effect if acidic cations were adsorbed by the biochar on an enhanced CEC complex.

The cumulative reductions in N$_2$O-N of 15 to 74% were highly significant when biochar was incorporated but it must be remembered that this was a 'bench-top' study in the absence of plants, rainfall, leaching and all the associated effects these factors have on N transformations in the soil. However, the results appear highly promising and it is imperative that we start field trails as soon as possible in order to gauge the effect of biochar on N$_2$O fluxes under realistic pasture conditions.

Of further note was the fact that the changes in the N$_2$O-N fluxes did not result in changes in NH$_3$-N volatilisation. Thus 'pollution swapping' in as far as gas fluxes are concerned, did not occur. Also of note was the dramatic difference in moisture (θ_g) between the biochar and soil if this occurs in the field to the same extent then there are potential implications for plant and microbial activity which may have beneficial effects on plant growth and health. Likewise the liming effect seen here would also be of benefit for agricultural soils where biochar was incorporated.