



N₂O and ammonia emissions from biochar amended urea prills.

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Executive Summary

• Three experiments were performed to determine the effects of combining urea and biochar together, in prills, on nitrous oxide (N_2O) and/or ammonia (NH_3) emissions from soil.

• Initially a binding agent had to be assessed for binding the biochar and urea materials that contained little or no additional N. These materials were gluten, starch, sugar and polyvinyl alcohol (PVA). Sugar or PVA binding agents provided urea-biochar granules that were robust enough to withstand handling.

• Experiment 1 aimed to test if the urea-biochar ratio of the granule influenced the emissions of N₂O. Treatments (replicated 5 times) comprising urea, biochar, and urea-biochar prills at four different rates of biochar, were assessed in the laboratory over 21 days for N₂O emissions after prills were surface applied. The N application rate was 70 kg N ha⁻¹. The urea prills' emission factor (EF) equaled 1.03 ± 0.26 (\pm stdev). Combining biochar and urea in prills, with biochar: urea ratios up to 4:1 (w/w) did not statistically influence cumulative N₂O emissions with EF values ranging from 0.73% ± 0.09 to 1.38% ± 0.57 .

• An *in situ* experiment was performed with ¹⁵N labeled urea and urea-biochar prills placed onto the pasture surface. The N₂O emissions, soil inorganic-N and soil water soluble carbon were followed for 21 days during a climatic period suitable for pasture growth. Pasture dry matter yields, plant N uptake and recovery of ¹⁵N did not vary as a consequence of adding biochar to the urea prills. Likewise, soil inorganic-N and EFs (range 0.42-0.89%) did not differ with treatment.

• In the third experiment both NH_3 and N_2O emissions were followed over a 16 day period *in vitro*. No treatment effects were seen on cumulative NH_3 and N_2O emissions as a result of incorporating biochar with the urea in prill form.

• Reasons for the lack of a biochar effect most likely include the fact that the biochar material sits on the surface following prill application with the diffusion of urea, and its hydrolysis products, away from the biochar. Thus negating any chemical effect the biochar might have in reducing gaseous N emissions. Secondly, any potential effect the biochar may have in reducing N₂O emissions via improving soil aeration (more oxygen inhibits denitrifier N₂O emissions), aggregation or water retention will not occur if biochar, applied at relatively low rates in fertilizer prills, remains sitting on the soil surface.

Introduction

Ammonia volatilisation from agricultural systems is the major anthropogenic source of atmospheric NH₃ and accounts for 10–30% of fertiliser- and animal excreta-N. the emitted NH₃ is ultimately deposited back onto the soil surface or water, contributing to indirect N₂O emissions (Mosier *et al.* 1998), acidification of water and biodiversity loss (Beusen *et al.* 2008). The current IPCC stipulates that 1% of the emitted NH₃ that is re-deposited onto the soil is re-emitted as N₂O. Nitrous oxide is a potent greenhouse gas and a precursor to compounds involved in stratospheric ozone depletion (Crutzen 1981; Forster *et al.* 2007; Ravishankara *et al.* 2009). Urgent research is required on mitigating the N losses by capturing NH₃ emissions and promoting better fertiliser use efficiency. Biochar has been proposed as one mitigation option and as a review of the literature shows (Appendix 1) it is capable of modifying N transformations in the soil.

Biochar is a C-rich product that is manufactured by thermal decomposition of biomass under a limited oxygen supply (pyrolysis) at relatively low temperatures (<700°C). Pyrolysis of the biomass is undertaken to generate energy and biochar occurs as a by-product. The incorporation of biochar into soil as a carbon (C) sequestration measure has created global interest along with its ability to act as a soil conditioner, mitigate N₂O emissions, and improve crop yields (Taghizadeh-Toosi *et al.* 2011). Taghizadeh-Toosi *et al.* (2011) postulated that reduced N₂O emissions following ruminant urine deposition to soil were due to the adsorption of NH₃ on the biochar, which reduced the N pool(s) available to soil microbes generating N₂O. Further research showed that the NH₃ adsorbed was subsequently bioavailable to plants (Taghizadeh-Toosi *et al.* 2012a; b).

Ammonia formation may occur in many types of agricultural system. For example, during urea hydrolysis in ruminant urine patches (Clough *et al.* 2003), in stored and spread slurries (Sherlock *et al.* 2002), from urea fertiliser prills (Black *et al.* 1987), and from confined animal feeding operations such as poultry operations. Thus there are point sources in various agricultural operations where biochar could potentially be placed to remove NH₃ that is lost to the environment directly and indirectly. No attention so far has been given to examining the potential for reducing NH₃ and/or N₂O emissions by directly placing urea alongside biochar.

The rationale for this research was therefore to directly place urea with biochar, in the form of prills, and to study the effect on NH_3 and N_2O emissions. It was hypothesised that the placement of urea-biochar prills would improve N use efficiency by reducing N losses due to NH_3 volatilisation and N_2O emissions.

Three experiments are outlined along with their results. A combined discussion of the results and their implications is then presented.

A review of the literature pertaining to biochar and the N cycle was also performed and published in the open access journal *Agronomy* 2013, 3(2), 275-293; doi:10.3390/agronomy3020275. The title and abstract are presented below while the full article is presented in Appendix 1.

A Review of Biochar and Soil Nitrogen Dynamics

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Abstract: Interest in biochar stems from its potential to sequester carbon in soils and its potential agronomic benefits. Biochar application to soil alters soil nitrogen (N) dynamics. This review focuses on the recent literature to establish emerging trends and gaps in biochar research. Biochar can retain both ammonium (NH_4^+) and nitrate (NO_3^-) . Adsorption of NO_3^- , up to 0.6 mg g⁻¹ biochar, occurs at pyrolysis temperatures $>600^{\circ}$ C with amounts adsorbed dependent on feedstock and NO₃⁻ concentration. For NH₄⁺ up to 0.8 mg g⁻¹ biochar has been shown to be adsorbed, with results dependent on feedstock, however, no pyrolysis temperature trend is apparent. However, the mechanisms and the long-term practical effectiveness of inorganic-N adsorption as a NO₃⁻ leaching mitigation option require further research. Adsorption of ammonia (NH₃) onto biochar may prevent NH₃ and NO₃⁻ losses during composting and after manure applications and provide a mechanism for developing slow release fertilisers. For example, NH₃ losses from poultry manure were shown to be reduced by >50% when biochar was added to the soil. Reductions in NH₃ loss vary with N source and biochar characteristics. Biochars derived from manures have a role as N fertilizers. Increasing pyrolysis temperatures, during biochar manufacture from manures and biosolids, results in biochars with decreasing hydrolysable organic N and increasing aromatic and heterocyclic structures. The short- and long-term implications of biochar on N immobilisation and mineralization are specific to individual soil-biochar combinations and further systematic studies are required to predict these effects in order to enhance understanding of agronomic responses and NO_3^- leaching losses. The bulk of the studies measuring nitrous oxide (N₂O) are short-term in nature and find reductions in emissions, but long-term effects are lacking as are the potential mechanisms for observed reductions. More use must be made of stable N isotopes to elucidate the role biochar plays in soil N dynamics. There still remains a dearth of information about how biochar affects soil biota and the N cycle. Biochar clearly influences the soil N cycle and it has potential roles within agroecosystems, both as a substrate able to mitigate environmentally detrimental N losses, and as a substrate able to sustainably deliver N. Future research needs to now systematically understand and optimise this potential, especially with respect to long term studies.

See Appendix 1 for the full paper.

Experiment 1 – Varying biochar: urea ratios and N₂O emissions

RATIONALE

In order to place the biochar material with the urea it was necessary to identify a binding material that could be used. Thus the objective of this experiment was to find a material to bind the biochar with the urea compound. Commercially available urea fertiliser prills are combined with a 'binder' to maintain prill shape and allow transport and application of urea. For formation of urea-biochar prills, selection of a suitable binder is required. Ideally this chemical is required to hold the urea-biochar material together, stay relatively stable under rainfall and be potentially 'inert' so that it does not serve as a C substrate to the microbes but is still biodegradable. Hence, the objectives of this research were to examine suitable binder materials for forming urea-biochar prills that had minimal effect on N_2O emissions.

MATERIALS AND METHODS

Granule preparation

Urea-biochar prills of desired sizes (2-4 mm) were prepared on the principle of inclined pan granulation (Irshad *et al.* 2009; Hoeung *et al.* 2011) using a pan (12 cm diameter, 10 cm height) inclined at 40°, attached to a shaft that allowed the rotation of the pan at 25 rpm. To provide surface friction on the pan wall, during its rotation, the inner surface of the pan was roughened. This was performed by spraying an adhesive on the pan wall and then gently sprinkling biochar powder, which adhered to the pan wall, and allowing it to dry. This provided a rough texture to the wall.

Approximately 30 g of a given urea-biochar-binder mixture that had been previously powdered and mixed in a mortar and pestle were fed into the inclined pan. Then deionised (DI) water was gradually sprayed into the rotating pan at the rate of 1 mL min⁻¹ with a total of ~20 mL applied. The pan was gently tapped at the base during the rotation to avoid the mixture sticking to the pan's base or the edges. During the rotation, as prills formed, prills >4 mm were collected with a spatula, crushed gently and fed back to the pan. The pan was rotated for 40 min and the resulting prills were passed through sieves to separate the prills by size after which they were air-dried for 5 hours. This process produced approximately 80 or 200 prills of 4 or 2 mm diameter, respectively. The final product was dried in a desiccator for 4 days following preparation and stored in air-tight containers until further use.

Testing of binder-biochar-urea mixtures

The following binding agents were selected based on a study of reports in the literature:

- Gluten (pure grade; Weston Milling^{TM,} Christchurch, New Zealand)
- Starch (CAS:9005-84-9; mol. wt. 342.3; Sigma-Aldrich[®])
- Sugar (commercially available)
- PVA (polyvinyl alcohol, 99% hydrolysed; CAS:9002-89-5; mol. wt. 89000-98000; Aldrich[®])

Urea-biochar prills were prepared using one of the above binders at a time and the prilling method described above. Prills made with the sugar and PVA binders did not disintegrate upon immediate contact with water. See Figure 1 for an example of urea-biochar prill degradation under irrigation.

Hence, for Experiment 1, urea-biochar prills were made with sugar as the binder, using a sugar: DI water ratio of 1:2, to prepare urea-biochar prills with increasing amounts of biochar at ratios of 1:1, 1:2, 1:3 and 1:4. Analar grade urea (99% purity, Ajax Finechem Pty Ltd; B/No. 1003062) was used while the biochar material was manufactured from Monterey Pine (*Pinus radiata* L.) wood chips at a pyrolysis temperature of 350°C. This was the same biochar material used in the study of Taghizadeh-Toosi et al. (2011) and its physical and chemical properties are shown in Table 2.

Experimental Design and Treatment Application

For the experimental set up, top soil (0-10 cm; Paparua sandy loam (Hewitt 1998); Table 1) from an un-grazed pasture site at the Lincoln University Dairy Research Farm (LUDRF) was collected, sieved (2 mm) and repacked into Mason jars to a depth of 7 cm (7.5 cm diameter, 13 cm total height) giving a headspace of 0.25 L. The repacked soil in the jars was rewetted to 70% of the soil's field capacity and gravimetrically checked for loss of soil moisture every alternate day throughout the 21 day experimental period. These jars were incubated at room temperature ($20 \pm 2^{\circ}$ C). Soil properties of the experimental soil are shown in Table 2. The laboratory experiment was set up in a randomised block design with 8 treatments each replicated five times. Treatments 1 to 4 were urea-biochar prills at ratios (weight/weight) of urea: biochar of 1:1, 1:2, 1:3 and 1:4 (UBC1, UBC2, UBC3 and UBC4, respectively). Treatments 5 and 6 were 'biochar only' (BC) and 'urea only' (U) prills, respectively. Treatment 7 was a control that received no treatment application and Treatment 8 was 'urea powder' (U-powder) without any binder. Treatments 1 to 6 therefore were prills made using the sugar solution as the binder. Sub-samples of the prills were analysed for total C and N by combustion using an Elementar Vario-Max CN Elemental Analyser. Prills were applied to the surface of the repacked soil at the rate of 70 kg N ha⁻¹. Biochar quantities varied with the treatment and equated to 204, 389, 457, 654 and 267 kg biochar ha⁻¹ in the UBC1, UBC2, UBC3, UBC4 and BC treatments, respectively. Soil N₂O measurements were performed on 10 occasions (1, 3, 5, 7, 10, 12, 15, 16, 17, 21 days) following treatment application using the closed chamber technique. This involved fitting a gas-tight screw on lid, equipped with septa, to the Mason jar and sampling the headspace after 0, 20 and 40 minutes (Clough et al. 2010).

Statistical analyses of gas emission data, on each gas sampling occasion, and for cumulative emissions were tested for normality using the Anderson-Darling test. If the data were skewed, they were log transformed [ln(flux+1)] to attain normality (Press *et al.* 1989). The statistical software Minitab version 15.1 (Minitab 2006) was used to perform the analysis of variance (ANOVA) on the emission data to determine if treatment means were equal. Treatment differences were tested using Tukey's test (95% confidence interval). All data presented here are mean \pm Stdev.



Figure 1. PVA-bound urea-biochar prills pre- and post-exposure to simulated irrigation at 17 mm h⁻¹.

Table 1. Chemical properties of the Paparua soil.		
Soil properties	Result	
pH (1:2)	6.2	
Total C (g kg ⁻¹)	30	
Total N (g kg ⁻¹)	2.5	
Anaerobically mineralisable N (µg g-1)	48	
Available N (kg ha-1)	76	
Olsen P (mg L ⁻¹)	19	
Potassium (cmolc kg⁻¹)	0.32	
Calcium (cmol _c kg ⁻¹)	8.0	
Magnesium (cmolc kg⁻¹)	0.38	
Sodium (cmol _c kg ⁻¹)	0.11	
Cation exchange capacity (cmolc kg-1)	12	
Total base saturation (%)	76	

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Table 2. Physical and chemical properties of the biochar used in Experiments 1, 2 and 3.

Properties	Result
Cation exchange capacity (cmolc kg ⁻¹)	8.0
Anion exchange capacity (cmolc kg ⁻¹)	4.0
рН (н20)	7.8
pH (CaCl2)	7.4
Electrical conductivity (dS m ⁻¹)	0.5
Particle density (Mg m ⁻³)	1.1
Bulk density (Mg m ⁻³)	0.4
Surface acidity (moles H ⁺ kg ⁻¹)	1.4
Specific surface area (mg g ⁻¹)	127.4
N content (mg g ⁻¹)	0.65
C content (mg g-1)	772
C:N ratio	1187
Volatile organics found	Ethanol
Biochar particle size fractions	45 to 15 mm (24.1%), 15 to 7 mm (33.8%), 7
	to 5.6 mm (1.13%), 5.6 to 4 mm (10.6%), 4
	to 2 mm (15.2%),
	2 to 1mm (4.7%), ≤1mm (10.6%)

RESULTS

The N concentrations of the prills were 189, 113, 92, 65, 1, 470 and 417 mg N/g for the UBC1, UBC2, UBC3, UBC4, BC, U, and U-powder treatments, respectively. The C concentrations of the prills were 427, 484, 461, 465, 456, 196 and 201 mg C/g for the UBC1, UBC2, UBC3, UBC4, BC, U, and U-powder treatments, respectively.

Throughout the experiment N₂O-N emissions from the BC and control treatments were lower (p < 0.05) than those treatments incorporating urea, with the exception of the U-powder treatment (Figure 2). On day one N₂O-N emissions were higher (p < 0.05) from the U treatment than treatments with biochar-urea mixtures. During days 5 to 10 the UBC3 treatment had higher (p <0.05) emissions than the 'U' treatment. By day 15 the U-powder had lower emissions. Emissions peaked at day 5 and were (\pm stdev) 263 \pm 34, 439 \pm 141, 702 \pm 356, 394 ± 81 , 3.0 ± 2.1 , 363 ± 89 , 1.4 ± 1.6 , and $153 \pm 79 \ \mu g \ N_2 O-N \ m^{-2} \ h^{-1}$ in the UBC1, UBC2, UBC3, UBC4, BC, U, control and U-powder treatments, respectively (Figure 2). Cumulative N₂O-N emissions over the 21 d experimental period were higher (p < 0.001) from the UBC3 than the UBC1, U, BC or control treatments. While cumulative emissions from the U-powder, BC and control treatments were less than from the other treatments. Emission factors for N₂O-N corresponded with cumulative N₂O-N fluxes and were highest (p <0.001) in the UBC3 treatment than in the UBC1 treatment, which in turn was higher than the U powder treatment. Emission factors averaged 0.73 ± 0.09 , 0.97 ± 0.19 , 1.38 ± 0.57 , $0.93 \pm$ $0.14, 1.03 \pm 0.26$ and $0.46 \pm 0.18\%$ in the UBC1, UBC2, UBC3, UBC4, U and U-powder treatments, respectively.





Experiment 2 – In situ N₂O emissions

RATIONALE

The objective of the field experiment was to assess the effect of the urea-biochar prills under field conditions with respect to N_2O emissions.

MATERIALS AND METHODS

Field site and experimental design

The field site was located at the Lincoln University Dairy Research Farm on a Paparua sandy loam soil (Table 1). An area of newly sown pasture was fenced off and prior to treatment application it was mown to 5 cm in height with all the harvested herbage removed to simulate grazing. The field experiment was conducted in autumn/winter 2013 (May-June). A randomised block design was laid out with five treatments each replicated four times. Plots were defined by installing headspace chamber bases (diam. 0.39 m, stainless steel), which protruded 0.10 m into the soil. These contained an annular water trough. During gas sampling events, insulated, stainless steel headspace covers with 0.10-m-high walls created an 11.6-L headspace when they were placed on the bases. The headspace cover sat on the annular waterfilled trough, creating a gas-tight seal. Prills were prepared using the procedure described above. In addition, however, ¹⁵N-enriched urea (99 atom% ¹⁵N; Isotec Inc.) was included in the urea-biochar mixtures biochar so that the final ¹⁵N enrichment of the urea-biochar prills was between 3 to 5 atom%. This permitted the contribution of the urea to the N₂O flux to be determined. Treatments 1 and 2 consisted of urea-biochar mixtures at ratios of 1:1 and 1:2, hereafter called, UBC1 and UBC2, respectively. Treatment 3 was a 'biochar only' treatment (BC). All prills were made as described above but using the PVA binder. Treatment 4 was a ¹⁵N-enriched urea-only treatment (U) while treatment 5 was an untreated 'control'. Treatments are summarised in Table 3. Prills were applied to the soil surface at an N application rate of 50 kg N ha⁻¹ (Figure 3 & 4). As a consequence the biochar quantities varied with treatment and equated to 138, 233 and 183 kg biochar ha⁻¹ in the UBC1, UBC2 and BC treatments, respectively. The N rate used was typical of an N application rate for New Zealand pastures.

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Treatment notation	Granule type	Total N content (mg g ⁻¹)	Enrichment of ¹⁵ N (atom%)
UBC1	Urea:BC in 1:1 ratio	180.8	5.67
UBC2	Urea:BC in 1:2 ratio	114.5	5.77
BC	Biochar only	1.33	0.38
U	Urea only	414.0	3.08
Control	No application	N/A	N/A

Table 3. Treatment and prill details for experiment 2 where UBC1 and UBC2 are urea-biochar mixtures at ratios of 1:1 and 1:2 by weight, BC is biochar only, U is urea only.

Values are means of three replicates.



Figure 3. Urea-biochar prills being distributed on to field plots.



Figure 4. Urea biochar prills *in situ* (red arrow) after prill distribution.

Gas sampling for N_2O emissions was performed using a closed chamber technique on 16 occasions from 15 May 2013 (Day 1) until 5 June 2013 (Day 21). During gas sampling events the chamber was placed on the annular water-filled trough, creating a gas-tight seal. On each gas sampling occasion, 10 mL samples of the enclosed headspace volume were manually drawn using glass syringes by inserting a hypodermic needle attached to a 10 mL glass syringe via a three-way tap and these were compressed into 6 mL Exetainer® vials (Labco Ltd). Gas samples were taken at 0, 20, and 40 min after positioning the headspace covers. The gas samples were analysed within 48 h for N₂O using gas chromatography (Clough et al. 2010). Three hours after gas sampling, a further 15 mL headspace gas sample was drawn and put into 12 mL Exetainer® vials for isotopic analysis of the N2O. These samples were equilibrated to atmospheric pressure immediately prior to analysis for $N_2O^{-15}N$ enrichment using a continuous flow isotope ratio mass spectrometer (PDZ Europa Ltd) as described by Stevens et al. (1993). Emission factors (expressed as a percentage of the N applied) were calculated by determining the cumulative mass of N₂O–N emitted, subtracting the cumulative mass of N₂O–N emitted from the controls, and dividing the difference by the mass of N applied to the soil in the form of urea.

Further areas, immediately beside the chambers were also set-up and treated in an identical fashion but these were used for soil inorganic N, soil water soluble C (WSC) and soil pH sampling. Field moist soil cores were collected using a soil corer (7.5 cm deep, 2.5 cm diameter) from these areas (three replicates per treatment). A subsample of each collected soil core was dried at 105°C for 24 h to determine the gravimetric soil water content (θ_g). For determination of soil inorganic N concentrations, another subsample was shaken with 2 M KCl in a 1:10 ratio (soil: KCl) on an end-over-end shaker for 1 h followed by centrifugation of the extract at 2000 rpm (480 g) for 10 min and then it was filtered (Whatman No. 41). Analyses for ammonium N (NH_4^+ –N) and nitrate N (NO_3^- –N) were performed on an Alpkem FS3000 twin-channel flow injection analyser (Alpkem, TX, USA). For WSC determinations. a subsample of soil was extracted with DI water in a 1:10 ratio (soil: water) for 30 min on an end-over-end shaker followed by centrifugation of the extractant at 750g for 20 min and then filtering (through 0.45 µm cellulose ester membrane filter) into 30 mL plastic containers with analyse for WSC on a Shimadzu Total Organic Carbon Analyzer (TOC 5000A, Shimadzu Oceania Pty Ltd) fitted with a Shimadzu ASI-5000A autosampler (Ghani et al. 2003). For soil pH, a subsample of the soil was shaken with DI water (1:5, soil: water). This was left to equilibrate overnight, followed by measurement on a pH meter (S20 Seven EasyTM, Mettler Toledo, Switzerland) (Blakemore et al. 1987). Pasture from within the treated plots was harvested to 5 cm height 30 days after application (13 June) and analysed for total N and ¹⁵N enrichment.

RESULTS

During the study air temperatures averaged 9.6°C, with minimum and maximum values of 3.7 and 16.7°C, respectively (Figure 3). Soil temperatures averaged (10 cm depth) 15.7°C, with minimum and maximum values of 11.6 and 20.0°C, respectively (Figure 5).



Figure 5. Soil and air temperatures, and rainfall during experiment 2.

Nitrous oxide emissions

Emissions of N₂O began to increase following a rainfall event on day 3 where the fluxes were higher (p <0.05) in UBC2 than the BC treatment averaging 260 ± 136 and $34 \pm 32 \mu g N_2 O-N$ $m^{-2} h^{-1}$, respectively (Figure 6). Emissions of N₂O peaked on days 5 or 7. Significant differences in fluxes due to treatment were recorded on day 7 where emissions from UBC2 and UBC1 did not differ but they higher than the control treatment. At this time N₂O-N fluxes were 202 ± 59 , 230 ± 68 , 49 ± 41 , 161 ± 50 and $32 \pm 14 \ \mu g \ N_2 O-N \ m^{-2} \ h^{-1}$ in the UBC1, UBC2, BC, U and control treatments, respectively. Fluxes did not differ due to treatments after day 10 and reached those of the control by day 18. However, following rain on days 20 and 21 the fluxes increased in the U treatment where they were higher than the BC and control treatments (Figure 6). Cumulative N₂O emissions integrated over 21 days were greater (p <0.05) in the UBC2 treatment and lowest in the BC and control treatments, which did not differ from each other. The UBC1 and U treatments had cumulative fluxes statistically no different from the UBC2 treatment. The average cumulative N₂O-N fluxes (\pm stdev) were 37 \pm 9, 61 \pm 21, 22 \pm 15, 39 \pm 9 and 16 \pm 4 mg N₂O-N m⁻² over the study period, in the UBC1, UBC2, BC, U and control treatments, respectively. Emission factors did not differ statistically with treatment (p >0.05) and ranged from 0.42 to 0.89%. The ¹⁵N enrichment of the N₂O-N from treatments UBC1 and UBC2 did not differ but was higher than the other treatments on days 4 (2.78 atom%) and 6 (2.61 atom%) after treatment application. Enrichment of N₂O-¹⁵N from BC and control treatments remained at natural abundance throughout the experimental period. Recoveries of ¹⁵N applied as N₂O-N for the UBC1, UBC2 and U only treatments were 0.27, 0.55 and 0.27%, respectively.



Figure 6. N₂O-N fluxes versus time following surface application of urea-biochar treatments. Data are mean (n=4) \pm stdev.

Soil and herbage analyses

Soil NH₄⁺-N concentrations were elevated in those treatments receiving urea on day 2 (U, UBC1, and UBC2). While soil NH₄⁺-N concentrations declined over time there was no consistent treatment effect on NH₄⁺-N concentrations (Figure 7). Conversely, soil NO₃⁻-N concentrations increased up to day 16 in those treatments receiving urea (U, UBC1, and UBC2), with no clear effect of biochar, and NO₃⁻-N concentrations in these treatments were generally higher than in the BC or control treatments (Figure 7). Maximum soil NO₃⁻-N concentrations, on day 16, averaged 80 ± 19 , 74 ± 3 , 23 ± 3 , 76 ± 9 , and 45 ± 3 mg N kg⁻¹ soil in the UBC1, UBC2, BC, U and control treatments, respectively.

Concentrations of WSC were generally higher (p < 0.05) in the BC and control treatments when compared to the UBC1 and UBC2 treatments (Figure 8). Concentrations declined over time, peaking on day 9, and were higher in the UBC1 treatment than the UBC2 treatment from day 13 (Figure 8).



Figure 7. Soil inorganic-N concentrations over time following surface application of urea-biochar prills. Data are means $(n=3) \pm$ Stdev.



Figure 8. Soil WSC concentrations over time following surface application of urea-biochar prills. Data are means $(n=3) \pm$ Stdev.

Pasture dry matter (DM) yield and N content were not affected by treatment (p = 0.397) with DM yields and pasture N contents ranging from 176 to 263 kg DM ha⁻¹ and 3.8 to 4.6% N, respectively. Atom% ¹⁵N in the DM averaged (±stdev) 2.37 ± 0.19 , 2.00 ± 0.17 , 0.37 ± 0.01 , 1.28 ± 0.06 and 0.37 ± 0.00 atom% in UBC1, UBC2, BC, U and control treatments, respectively. Recovery of ¹⁵N applied in the herbage ranged from 6.1 to 7.7% with no significant difference due to treatment.

Experiment 3 – Ammonia and N₂O emissions

RATIONALE

The rationale for this experiment was to observe how biochar-urea prill mixtures might influence the volatilisation of NH_3 from the prills while at the same time measuring the N₂O-N fluxes evolving.

EXPERIMENTAL DESIGN AND TREATMENT APPLICATION

The soil and biochar used in this experiment were the same as used in experiment 1 and their details are presented in Tables 1 and 2. Top soil was collected from the same field site as for experiment 1 and then sieved (2 mm). This soil was repacked into Mason jars and rewetted (70% FC) in an identical fashion to experiment 1. Soil moisture loss was checked gravimetrically every alternate day throughout the experimental period of 15 days. Jars were incubated at room temperature ($20 \pm 2^{\circ}$ C). The experiment was set up in a randomised block design with identical treatments to experiment 2, each replicated 4 times. Prills, prepared as described above, and with the same properties as those in experiment 2 (Table 3) were applied to the surface of the repacked soil at the rate of 70 kg N ha⁻¹. Soil NH₃ and N₂O-N flux measurements were performed on 12 occasions following treatment application till the fluxes reached control levels. Biochar addition equated to 191, 328 and 264 kg biochar ha⁻¹ in the UBC1, UBC2 and BC treatments, respectively.

Experimental set-up for NH₃ and N₂O collection

Gas-tight lids were screwed on to the jars immediately following treatment application. Each lid contained two rubber septa, each fitted with a 3-way stopcock. Two needles, $21G \times 4.0$ cm each (Precision-Glide, Becton-Dickinson, NJ, USA), were attached to the 3-way stopcocks, acting as entry and exit ports for air flow into and out of the jar headspace, respectively. The entry port needle was connected using plastic tubing (4 mm) that directed NH₃-free air into the jar and towards the soil surface. Four manifolds were employed to distribute airflow. Each manifold contained six small and two larger terminals. The small terminals were connected to the jars while the two large terminals were attached to a manifold and air compressor. Air from the compressed air reservoir was swept through the system at 220 mL min⁻¹, which equated to a flow of 12 mL min⁻¹ for each jar or approximately 2.8 headspace changes per hour. Airflow was checked and monitored continuously using a flow meter (GAP Meter, GA Platon Ltd, England). Two NH₃ scrubbing units containing a 0.3 M boric acid solution, with bromocresol green-methyl red indicator, were placed upstream of the larger terminals to scrub any NH₃ present in the compressed air. Vials containing 20 mL of 0.1 M H₂SO₄ were placed after each incubation jar to collect any NH₃ volatilised from the soil surface (Figure 7 & 8). Ammonia collection periods were 14 h long during the first 8 days following treatment application and 24 h for the following 4 days. Sub-samples of the H₂SO₄ solution were analysed for NH₄-N concentrations and were performed on an Alpkem FS3000 twin-channel flow injection analyser (Alpkem, TX, USA). Periodically inlet and outlet valves were simultaneously closed to determine N₂O fluxes, as described in experiment 1. Statistical analyses were performed as described in experiment 1.



Figure 7. Experimental set up for sampling NH₃ fluxes from incubated soils.



Figure 8. Biochar prills on soil surface (red arrow) during sampling of NH_3 and N_2O .

RESULTS

Nitrous oxide emissions

Emissions of N₂O did not follow any obvious treatment induced trend (Figure 9). Emissions peaked on day 5 where they were higher (p <0.05) in the UBC1 than the UBC2 treatment. The N₂O-N missions were often higher in the UBC2 treatment thereafter until day 17. Cumulative N₂O-N emissions averaged 1.1 ± 0.5 , 0.80 ± 0.18 , 1.26 ± 0.51 , 0.71 ± 0.34 , and 0.21 ± 0.03 g N₂O-N m⁻² in the UBC1, UBC2, BC, U and control treatments, respectively, with higher (p <0.05) cumulative emissions in the BC and UBC1 treatments.



Figure 9. Emissions of N_2O and NH_3 over time following surface application of urea-biochar prills. Data are mean \pm SD. Note the differing scale compared to Objectives 1 and 2.

Ammonia emissions

Emissions of NH₃-N were higher in the UBC1 and UBC2 treatments but did not differ from each other, while emissions from all treatments receiving urea were higher than the control and BC treatments between days 1 and 5 (Figure 9). No treatment differences were recorded after day 7 when NH₃-N emissions were equal to those of the control. Maximum emissions were observed on day 2.2 and averaged 0.35 ± 0.32 , 0.66 ± 0.11 , 0.00 ± 0.00 , 0.33 ± 0.46 , and 0.01 ± 0.00 mg NH₃-N m⁻² h⁻¹ in the UBC1, UBC2, BC, U and control treatments, respectively (Figure 9). Cumulative NH₃ emissions over 16.8 d were higher in the UBC1 and UBC2 treatments than the BC and control treatments with cumulative emissions averaging 37 \pm 7, 36 \pm 10, 0.8 \pm 0.1, 22 \pm 10, 0.7 \pm 0.2 mg NH₃-N m⁻² in the UBC1, UBC2, BC, U and control treatments, respectively. Emission factors for NH₃-N did not differ due to treatments and were 0.52 \pm 0.11, 0.51 \pm 0.14 and 0.31 \pm 0.14% in the UBC1, UBC2 and U treatments, respectively.

Soil analysis

Soil NH₄⁺-N and NO₃⁻-N concentrations prior to treatment application averaged 21 ± 7 and 7 ± 4 mg N kg⁻¹ soil, respectively. Destructive analysis of the soil samples at the end of the experimental period showed that soil NH₄⁺-N concentrations were significantly higher in the control treatment than other treatments (Table 4) while soil NO₃⁻-N concentrations were higher (p <0.001) in the UBC1, UBC2 and U treatments than the BC and control treatments (Table 4).

Treatment	Soil NH4 ⁺ -N	Soil NO ₃ N
		(mg kg ⁻¹ soil)
UBC1	3.2 ± 0.9	105.3 ± 48.4
UBC2	2.2 ± 0.2	86.6 ± 8.2
BC only	2.0 ± 0.3	3.6 ± 0.1
U only	4.1 ± 2.7	71.3 ± 7.9
Control	7.2 ± 0.3	3.0 ± 0.6

Table 4. Mean soil inorganic-N concentrations after 17 days. (\pm stdev (n = 3)).

Discussion

Binding agents tested were chosen after examining literature and on the basis they contained little or no N and were non-toxic. As a result this meant that binding agents contained bioavailable carbon. In terms of nitrification derived N₂O fluxes this is not a concern since most nitrifiers are autotrophic. However, additional carbon could have enhanced N₂O production via denitrification pathways, or alternatively, this additional carbon could have enhanced N₂O reduction. The binding agents used after testing the various compounds were chosen based on granule strength and resistance to immediate collapse upon irrigation. The rationale for this was that the slower the release of urea from the urea-biochar granule the slower would be the N supply and any unwanted emissions (N₂O and NH₃).

The optimum ratio of urea to biochar to use was unknown. Fertiliser prills cannot be excessively large, and economically the cost effectiveness and agricultural benefits of any urea-biochar prill combination needs to be maximised. In experiment 1 the laboratory study explored using a range of urea-biochar ratios (weight/weight) and followed N₂O emissions over 21 days. The lack of any consistent reduction in N₂O emissions with increasing biochar content in the prills indicated there was little benefit to increasing the biochar content above the 1:1 ratio. Of considerable interest in this laboratory study was the lower cumulative N₂O-N emission from the urea-powder treatment. Does this mean smaller urea prills would result in reduced N₂O emissions? This should be investigated further. The lack of any reduction in the urea-biochar prills may be the result of several factors. To manufacture the prills the biochar material was finely ground and this may have destroyed any potential structure or physical mechanism(s) that may lead to N₂O reductions in the presence of biochar. Grinding the biochar may possibly have changed chemical characteristics of the biochar e.g. altered its surface chemical characteristics or their prevalence. However, this seems less likely with a new biochar material. The choice to opt for a reasonably resilient prill was based on having a prill that would cope with handling and some rainfall. However, this may have meant that as the urea solubilised it diffused away from the biochar material that was still sitting on the soil surface. Other studies reporting a reduction in N₂O emissions following biochar application have incorporated biochar into the soil and the urea source e.g. ruminant urine has been in close contact with the biochar, thus potentially creating conditions more suited to enhancing the reduction of N₂O emissions. In retrospect, a prill that collapsed more readily with rainfall/moisture may have produced a different result.

In the field study, experiment 2, the urea/urea-biochar prills were enriched with ¹⁵N allowing the urea-N contribution to plant-N uptake and N₂O emissions to be determined. In this study average soil and air temperatures were adequate for grass growth. Pasture yield and N contents were not affected by treatment and neither was the N contribution from the urea/urea-biochar prills affected by treatment, with ¹⁵N recovery in the pasture in the range of 6.1-7.7%. This demonstrates that N supply was relatively equal under the urea-based treatments and that biochar had no effect. This was reflected in the soil inorganic-N concentrations, most easily seen in the NO₃⁻-N data, where NO₃⁻-N concentrations of the urea-biochar prills closely tracked those of the urea prills. Several studies have proposed or shown that biochar materials, and the compounds embodied within them, may influence the rate of nitrification (e.g. Clough et al. 2010; Spokas et al. 2010; Taghizadeh-Toosi A. et al. 2011). Had biochar influenced nitrification and slowed down the rate of NO_3^- formation this would have been reflected in the soil inorganic-N data data but this was not the case and biochar addition did not affect nitrification in situ. Similar to the laboratory study of experiment 1 the N₂O-N flux data from experiment 2 show that biochar incorporated into urea-biochar prills had no impact on the cumulative N2O flux. All urea-based treatments had statistically similar cumulative N2O-N fluxes. Possible reasons for the lack of any effect on N transformations in situ include those mentioned above; grinding of the biochar changing physical and chemical characteristics, and separation of urea from the biochar remaining on the soil surface. In studies using the same biochar, where N_2O/NH_3 emissions decreased (e.g.

(Taghizadeh-Toosi *et al.* 2011; Taghizadeh-Toosi *et al.* 2012a; b)), biochar was thoroughly incorporated into the soil potentially allowing for greater adsorption of the NH₃ resulting from the urea hydrolysis.

Experiment 3 showed that cumulative NH_3 emissions were actually higher with the presence of biochar in the prill. While not statistically significant on all days there was a trend for NH_3 emissions to be elevated when biochar was present with urea from day 2 to 5. Again the N_2O -N fluixes showed no clear treatment effects. The lack of any biochar effect on NH_3 fluxes was unexpected and may again be the result of urea diffusing away from the relatively stable biochar material as a result of surface placement, or a lack of NH_3 adsorption potential by the biochar resulting from chemical changes induced by adding a binder.

Labbe et al. (2013) investigated the nutrient release patterns from fertiliserimpregnated biochar pellets that had been prepared by mixing various ratios of biochar with water impregnated with liquid fertiliser (12: 4: 8 = N: P₂O₅: K₂O). Pellets were made by pressing material (biochar and lignin binders) together in a die. Biochar pellets prepared using starch and insoluble lignin showed higher water stability than those with soluble lignin. Labbe et al. (2013) demonstrated the rapid release of potassium (a cation) and phosphorus (an anion) during laboratory extractions of the biochar pellets. Thus it is readily conceivable that urea, that carries no net charge, was rapidly lost from the biochar prills used in the current studies.

A review of biochar effects on N₂O emission currently in preparation (Zwieten et al. pers. comm.) will show that reductions in N₂O emissions depend on biochar feedstock type and biochar rate with lower emissions resulting from woody feedstocks and high rates. In the current studies the feedstock was a wood material but the rates were < 1 tonne ha⁻¹. Increasing the rate of biochar, to one typically used where N₂O reductions are observed, may not make any difference if it is applied in prill form and biochar remains on the soils surface because no perturbation of the soil's physical properties will occur. Currently, one of the commonly held hypotheses is that biochar reduces soil N₂O emissions by affecting soil aeration, aggregation or water retention. These effects are all going to happen at higher rates of biochar application and after incorporation. This is not the case with surface applied prills.

Further hypotheses for biochar reducing N_2O emissions include: elevation of soil pH, with alkalinity in the biochar possibly promoting N_2O reductase; carbon embodied in the biochar also enabling denitrification to go to completion; toxic compounds on biochar slowing nitrification; and adsorption of N compounds on the biochar reducing microbial available N.

Our hypothesis for NH_3 reduction centred on biochar adsorbing NH_3 as previously observed (Taghizadeh-Toosi *et al.* 2011). This did not occur and future studies could examine acidifying the biochar prior to prill manufacture to enhance NH_3 adsorption potential.

Conclusion

Emissions of N_2O and NH_3 , and soil inorganic-N concentrations were not reduced when ureabiochar prills were placed on the soil surface and compared to urea prills. This was attributed to the fact that biochar was applied on the soil surface rather than being incorporated into the soil. For biochar to be used in prills further methodologies need to be trialled such as including nitrification inhibitors in the prill, however such a product has been seen already on the New Zealand market. Statistically, gaseous N emissions did not increase and as a mechanism for delivering inert carbon to the soil fertiliser prills provide a readily verifiable and accountable way for increasing soil carbon should the economics of carbon trading improve.

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Appendix 1

A REVIEW OF BIOCHAR AND SOIL NITROGEN DYNAMICS

A Review of Biochar and Soil Nitrogen Dynamics

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Abstract: Interest in biochar stems from its potential to sequester carbon in soils and its potential agronomic benefits. Biochar application to soil alters soil nitrogen (N) dynamics. This review focuses on the recent literature to establish emerging trends and gaps in biochar research. Biochar can retain both ammonium (NH₄⁺) and nitrate (NO₃⁻). Adsorption of NO₃⁻, up to 0.6 mg g⁻¹ biochar, occurs at pyrolysis temperatures >600°C with amounts adsorbed dependent on feedstock and NO₃⁻ concentration. For NH_4^+ up to 0.8 mg g⁻¹ biochar has been shown to be adsorbed, with results dependent on feedstock, however, no pyrolysis temperature trend is apparent. However, the mechanisms and the long-term practical effectiveness of inorganic-N adsorption as a NO₃⁻ leaching mitigation option require further research. Adsorption of ammonia (NH_3) onto biochar may prevent NH_3 and NO_3^{-1} losses during composting and after manure applications and provide a mechanism for developing slow release fertilisers. For example, NH₃ losses from poultry manure were shown to be reduced by >50% when biochar was added to the soil. Reductions in NH₃ loss vary with N source and biochar characteristics. Biochars derived from manures have a role as N fertilizers. Increasing pyrolysis temperatures, during biochar manufacture from manures and biosolids, results in biochars with decreasing hydrolysable organic N and increasing aromatic and heterocyclic structures. The short- and long-term implications of biochar on N immobilisation and mineralization are specific to individual soil-biochar combinations and further systematic studies are required to predict these effects in order to enhance understanding of agronomic responses and NO_3^- leaching losses. The bulk of the studies measuring nitrous oxide (N₂O) are short-term in nature and find reductions in emissions, but long-term effects are lacking as are the potential mechanisms for observed reductions. More use must be made of stable N isotopes to elucidate the role biochar plays in soil N dynamics. There still remains a dearth of information about how biochar affects soil biota and the N cycle. Biochar clearly influences the soil N cycle and it has potential roles within agroecosystems, both as a substrate able to mitigate environmentally detrimental N losses, and as a substrate able to sustainably deliver N. Future research needs to now systematically understand and optimise this potential, especially with respect to long term studies.

Keywords: biochar; immobilization; mineralization; nitrate leaching; nitrogen; nitrous oxide; ammonia volatilisation

1. Introduction

Biochar is defined by Lehmann and Joseph [1] as a carbon (C) rich product derived from the pyrolysis of organic material at relatively low temperatures (<700°C). Bioenergy production using pyrolysis creates biochar as a bi-product. There is intense interest in using this biochar as a means to sequester C in soils as a tool for offsetting anthropogenic carbon dioxide (CO₂) emissions, and as a soil amendment due to its potential agronomic benefits [1]. An analysis by Woolf et al. [2] showed that globally implementing a sustainable biochar program could potentially offset 12% of the current anthropogenic CO₂-C equivalent emissions. Besides potentially sequestering C biochar has been observed to have agronomic benefits [3,4] and to alter the nitrogen (N) dynamics in soils [5]. Since the biochar and N cycling review of Clough and Condron [5] the interest in the potential of biochar has continued to escalate, such that, according to the Web of Science[®] data base, there have been 442 manuscripts published on various aspects of 'biochar' since the beginning of 2011, with 87 of these cross referenced to 'nitrogen'. The anthropogenically induced global N cascade is resulting in enhanced fluxes of nitrous oxide (N₂O), ammonia (NH₃), and nitrate (NO₃⁻) leaching as a consequence of the increasing intensification of agricultural systems [6,7]. In fact, humankind introduces more reactive N into the biosphere via the Haber-Bosch process and legume cultivation than all the natural processes taken together [7]. Thus mitigation options to reduce environmentally harmful N fluxes are keenly sought. Biochar has been shown to have promise in reducing inorganic-N leaching [8], N₂O emissions [9], and ammonia volatilisation [10], while also increasing biological nitrogen fixation [11]. This review focuses on the impacts of biochar on soil N dynamics, in particular the literature since 2010, and recommends future directions for research.

2. Mitigation of nitrogen leaching using biochar

Several mechanisms have been proposed to explain the apparent retention of N in biocharamended soils and the reduction of N leaching. These include adsorption of NH_3 or organic-N onto biochar, cation or anion exchange reactions, and enhanced immobilisation of N as a consequence of labile C addition in the biochar. Recent research on these mechanisms is considered below.

2.1 Nitrate adsorption and leaching

Recent research has clarified the potential role of biochars with respect to NO_3^- adsorption. Yao et al. [12] evaluated 13 biochar materials (sugarcane bagasse, peanut hull, Brazilian pepperwood, and bamboo that had been slowly pyrolysed at 300, 450 or 600°C, and a hydrochar) to determine their potential to remove NO₃⁻ from solution. It was found that four high temperature (600°C) biochars (bagasse, bamboo, peanut hull, and Brazilian pepperwood) were able to remove between 0.12 to 3.7% of NO_3^- (0.02-0.64 mg NO_3^- per g of biochar) from a solution (0.1 g: 50 mL of 34.4 mg L⁻¹ NO₃⁻) with variation in removal due to species of feedstock used. As Yao et al. [12] point out, the ability of the higher temperature biochars to remove NO_3^- is consistent with the earlier findings of Mizuta et al. [13] who found that bamboo biochar manufactured at 900°C had a high NO₃⁻ adsorption capacity. Kameyama et al. [14] performed a very informative study using sugarcane bagasse, where they determined NO₃⁻ adsorption properties of bagasse biochar manufactured at five pyrolysis temperatures (400-800°C). Significant NO₃⁻ adsorption occurred at pyrolysis temperatures \geq 700°C. At high pyrolysis temperatures the biochars had high pH (8.7-9.8) and Kameyama et al. [14] reasoned that the adsorption of NO₃⁻ was a result of base functional groups and not a result of physical adsorption since surface area and micropore volumes followed different trends when compared to observed NO₃⁻ adsorption. Similarly, Dempster et al. [15] also showed that a *Eucalyptus* sp. biochar (600°C) could adsorb NO₃⁻ when placed in an ammonium nitrate (NH₄NO₃) solution (10 g: 100 mL), with up to 80% adsorbed after 24 hours when the NO₃-N concentration was $2.5-5 \text{ mg NO}_3$ -N L⁻¹ (0.02-0.04 mg NO₃-N per g biochar), decreasing to 38% at 50 mg NO₃-N L⁻¹ although the adsorbtion rate had increased to 0.19 mg NO₃-N per g biochar.

Based on this increasing body of literature, for a biochar to have any NO_3^- adsorption potential, the pyrolysis process needs to occur at a temperature of at least 600°C. Clearly there is also a feedstock effect on NO_3^- adsorption potentials and more research is needed to better understand exactly how feedstock characteristics determine NO_3^- adsorption potentials given that high pyrolysis temperatures are deemed a prerequisite.

But what are the practical implications of adding such a NO_3^- retentive biochar to a soil when it comes to reducing NO_3^- leaching? Yao et al. [12] tested the significance of this NO_3^- retention mechanism, with respect to NO_3^- leaching, using two biochars with good NO_3^- retention properties (peanut hull and Brazilian pepperwood biochars made at 600°C). These were incorporated (2% by weight) into a sandy soil, in columns, and a nutrient solution was applied (34.4, 10.0, and 30.8 mg L⁻¹ of NO_3^- , NH_4^+ , and phosphate (PO_4^{3-}), respectively). After flushing the columns with 4 pore volumes of water over 4 days it was found that the biochar materials reduced NO_3^- leaching by 34% [12]. When the *Eucalyptus* sp. biochar, found by Dempster et al. [15] to adsorb NO_3^- , was placed in lysimeter pots (25 t ha⁻¹) holding a sandy soil, fertilised $((NH_4)_2SO_4; 40 \text{ kg N ha}^{-1})$ and irrigated over 21 days, the cumulative NO_3^{-1} leached was reduced by 25% when compared with a control treatment. Thus NO_3^{-1} adsorbing biochars can reduce NO_3^{-1} leaching.

However, other important issues with respect to the potential for biochar to reduce NO_3^{-1} leaching were also raised in the study of Kameyama et al. [14]. They questioned if adding biochar could significantly change a soil's physical characteristics with respect to hydraulic conductivity, and if so, could this affect the rate of NO_3^- leaching, negating or amplifying any effect of NO₃⁻ adsorption? And they also asked how permanent the adsorption of NO₃⁻ on to biochar was when incorporated in a soil? The answer to the first question will be influenced by the biochars physico-chemical characteristics such as pore size distribution, hydrophobicity and the rate of biochar addition. Kameyama et al. [14] found that when a bagasse biochar (800°C) was applied at a rate \geq 5% by weight to a calcaric dark red soil the saturated hydraulic conductivity increased, with the effect likely to also be a function of the meso- and micro-pore fractions in the soil and biochar. Thus, amending soil with biochar could potentially increase hydraulic conductivity, or preferential flow around larger particles, and thus lead to enhanced leaching of NO₃⁻. On the other hand, amendment of soil with biochar has been shown to increase water retention capacity [15-17] and this may decrease leaching of NO_3^- . Ideally, the water-holding properties of the soil should be known, and a biochar of suitable pore size distribution should be selected and applied at a rate not likely to enhance leaching. Kameyama et al. [14] also examined the permanence of adsorbed NO_3^- by measuring NO_3^- transport in soil columns amended with the same NO_3^- adsorbing bagasse biochar (0, 5, or 10% by weight). They found that when a 20 mg N L⁻¹ solution of KNO₃ was applied to the soil columns the maximum concentration of NO_3^- in the effluent was ~ 5% less than in unamended soil, however, cumulative discharge of NO_3^- was similar in all treatments [14]. The authors therefore concluded that NO₃⁻ was only weakly adsorbed onto biochar, that it could be desorbed by water infiltration, and that the net result may be an increased residence time for NO₃⁻ in the soil. This in turn may allow a greater opportunity for plant uptake of NO₃. Thus, in situ, the role of a biochar in reducing NO₃⁻ leaching will obviously depend on its NO₃⁻ adsorption capacity (initial pyrolysis temperature and feedstock), the biochar rate applied, the resulting rate of NO₃⁻ adsorption, the N loading of the given ecosystem, the resulting soil hydraulic characteristics, precipitation/irrigation events, soil type, plant and microbial N demand and potential biochar effects on these (e.g. changes in nitrification rates). Further in situ studies need to examine NO3⁻ leaching and biochar effects, e.g. in large lysimeter experiments over long durations, with both plants and standard fertilizer practices included. Since biochar effects on NO₃⁻ leaching may only develop over time. For example, Ventura et al. [18] observed a 75% reduction in the second year of a study in an apple orchard in Northern Italy. In addition, some consideration should be given to the potential impacts of biochar particle size on NO_3^- retention and loss from soil, and indeed on the overall effects of biochar amendment on soil N dynamics.

2.2 Immobilisation or NH₃ volatilisation as leaching retardation mechanisms.

Reduced leaching of N has also been observed in the absence of increased ion –retention by biochars. For example, Ippolito et al. [19] placed two switchgrass (*Panicum virgatum* L.)

biochars, manufactured at either 250 or 500°C in an Aridosol and determined cumulative NO3⁻ leaching 34, 62, 92, and 127 days after the experimental start. Ippolito et al. [19] found that less NO_3 leached when the lowest temperature biochar was present. This was explained by the presence of more easily degradable C compounds at the lowest temperature and greater N immobilisation, thus reducing NO₃⁻ leaching. A 2 M KCl extract of the incubated biochar-soil matrix also supported this reasoning with less NO3⁻ present in the low temperature biochartreated soils [19]. Schomberg et al. [20], incorporated five biochar materials into soil and after a 127 day incubation reduced N leaching was observed. The rationale supplied for this result was not greater N retention, but rather the promotion of NH₃ losses by the biochar as a consequence of the elevated soil pH resulting from biochar addition [20]. Pollution swapping (e.g. reduced NO₃⁻ leaching in exchange for greater NH₃ losses) does not solve the problem of N leakage from agricultural systems. It is important to examine the long-term net outcome of biochar in reducing leaching via N immobilisation, changes in nitrification, N sorption onto biochar or promotion of NH₃ volatilisation. Immobilisation of N may only occur for a short term following biochar application, and may lead to a delay in leaching of N. One relatively long-term result to date has shown reduced N leaching in an orchard system [18].

2.3 Ammonium adsorption and leaching

Biochar adsorption of NH_4^+ has been examined in several studies. Yao et al. [12] found that 9 of the 13 biochars tested in their sorption experiment could remove NH_4^+ from solution (0.1 g biochar in 50 mL of 10 mg NH_4^+ L⁻¹), with removal rates ranging from 1.8-15.7% (0.05 to 0.79 mg NH_4^+ per g biochar), varying widely with feedstock and pyrolysis temperature, but with no pyrolysis temperature trend. The *Eucalypt* sp. biochar (600°C) used by Dempster et al. [15] adsorbed 75% of the NH_4^+ in solution (10 g biochar in 100 mL) at 2.5 and 5 mg NH_4^+ -N L⁻¹ (0.02-0.04 mg NH_4^+ -N per g biochar) but this was reduced to 54% at 50 mg NH_4^+ -N L⁻¹, although the adsorbtion rate had increased to 0.25 mg NH_4^+ -N per g biochar.

Placing NH₄⁺ retentive biochars into soil has also been shown to affect the leaching of NH₄⁺. Ding et al. [21] found a bamboo charcoal (pyrolysed at 600°C and added at 0.5% by weight to 0-10 cm depth) affected soil solution NH₄⁺ concentrations at 20 cm depth when added at a rate of 400 kg N ha⁻¹ to a sandy silt soil, but no differences were observed at 40 cm depth after 70 days. Dempster et al. [15] observed that when a biochar with cation exchange capacity (CEC) of ~10 cmol_c kg⁻¹ was added to a sandy soil (CEC of ~2 cmol_c kg⁻¹) NH₄⁺ leaching was reduced (15.0 to 12.9 mg pot⁻¹) 21 days following fertilisation ((NH₄)₂SO₄; 40 kg N ha⁻¹).

The rationale generally given for the adsorption of NH_4^+ onto biochar and the observed reductions in NH_4^+ leaching is the CEC of the biochar. The NH_4^+ retention studies noted above were performed on fresh biochar materials which have relatively low CEC [17,22,23]. *In situ*, cation retention increases with biochar age and depends on climatic conditions, [24,25]. Thus, the practical long-term significance of freshly made biochar in reducing NH_4^+ leaching remains to be tested. However, the short-term practical impact of incorporating a new biochar material into soil on the total (soil+biochar) CEC can be inferred if both the soil's CEC, and the biochar's CEC and application rate are known. For example, Jones et al. [26] found no effects of biochar (50 t ha⁻¹) on NH_4^+ adsorption in a three year field trial where theoretical retention of NH_4^+ by

biochar and soil were 3 and 142 kg N ha⁻¹, respectively. In sandy soils this biochar input may be significant in terms of CEC, but may also be insignificant [27], while for many soils that already contain higher levels of organic matter and clay the impact of biochar may be inconsequential. Further studies need to report on the change in freshly incorporated biochar CEC values over time for biochars that have been placed *in situ*.

Ammonium contained onto biochar surfaces as a result of cation exchange should be readily removable with potassium chloride extraction. However, this was not the case when peanut hull biochar was exposed to NH_{4^+} solutions, with $\leq 0.39\%$ of the total sorbed NH_{4^+} released [28]. Although the exact mechanism for NH_{4^+} retention was not identified it was suggested that physical entrapment of NH_{4^+} in biochar pore structures may have been responsible [28]. Given that the NH_{4^+} ion has a diameter of 286 pm [29] and there is wide range of pore sizes in biochar materials [30] this is entirely possible. Prost et al. [31] also found surface areas of biochars decreased during composting due to compost derived materials clogging biochar pores with the biochar also absorbing leachate and nutrients. Thus it is also necessary to continue to advance our understanding of mechanisms responsible for the adsorption of N forms onto biochar surfaces and the effect of time on these processes.

2.4 Dissolved organic nitrogen retention and leaching

Relatively few studies have examined dissolved organic-N (DON) leaching from soil [32,33], and fewer still have looked at the role of biochar on this. Dempster et al. [15] found that biochar had no effect on levels of DON leached from a sandy soil, which initially contained 18.8 mg N kg⁻¹ in the 0-10 cm depth (actual values measured in treatments were not reported). However, DON mainly carries a net negative charge. Dempster et al. [15] therefore argued that this weakened the case for biochar reducing leaching via adsorption of NO₃⁻ (despite the fact that the biochar used was capable of NO₃⁻ adsorption). Thus the authors went on to propose that the observed reductions in NO₃⁻ leaching were the result of reduced rates of nitrification [34]. This rationale concurs with the results of Kameyama et al. [14], who found no differences in cumulative NO₃⁻ leaching from a sandy soil over a shorter experimental period when treating a soil with a biochar known to be NO₃⁻ retentive.

3. Plant nitrogen response to biochar amendment

Biochar addition to soils does not always result in consistent yield increases (e.g. [35]) and plant responses to biochar addition have been reported to vary considerably. Biochar effects on yield were reviewed by Spokas et al. [4] and occur as a result of changes in soil nutrition, water holding capacity and microbial activity, with results varying due to soil type. Positive yield increases were generally associated with hardwood biochars and chars possessing plant nutrients, such as high N content poultry manure biochars [4].

Prendergast-Miller et al. [36] also found biochar (charcoal fines from mixed deciduous hardwood, pH 9.3) produced elevated NO_3^- concentrations in the rhizosphere of wheat seedlings, increased wheat root length and decreased root N uptake but with no effect on plant biomass or plant N content. More recently, O'Toole et al. [37] reported on a pot experiment

growing ryegrass under 4 rates of N fertiliser with 2 rates of a wheat-straw biochar (500-600°C) and found foliar N concentrations were reduced, possibly due to adsorption or immobilisation of N being stimulated with biochar addition, but with unaffected yields. Kammann et al. [16] also observed reductions in foliar N concentrations in a pot trial with a relatively nutrient-rich peanut hull biochar, but in this case the reduction likely resulted from increased N use efficiency since the authors reported biomass increases of up to 60%.

Longer term in situ studies on agronomic effects of biochar, and N cycling in particular, are beginning to appear. Jones et al. [26] performed a three year field trial to examine the agronomic effect of biochar (various tree species at 450°C; 0, 25 and 50 t/ha) where maize was sown in the first year followed by a forage grass (Dactylis glomerata) in years 2 and 3. Maize yield and agronomic performance, along with nutrient content, were unaffected by biochar in the first year. However, subsequent grass crops in the second and third years resulted in greater foliar uptake of N as a result of biochar addition which was speculated to be due to the biochar interaction with crop rooting depths and soil water. In another 3-year study Unger and Killorn [38] found no differences in maize grain or biomass yields due to biochar addition and no interaction with urea fertiliser. Lentz et al. [39] found a hardwood biochar (500°C) had no effect on maize silage N content or yield after 1 year but decreased silage total-N, yield and cumulative uptake of total-N in year 2, which was reasoned to be due to lower soil mineralization in year 2. Application of a nutrient rich wheat-straw biochar (20 and 40 t ha⁻¹) to a calcareous loamy soil resulted in no changes in soil mineral N concentrations but nevertheless there was a significant maize yield increase, accompanied by increased total soil N content and agronomic N-use efficiency during a 4-month field trial [40]. Uzoma et al. [41] conducted a glasshouse experiment where a biochar manufactured from cow manure (500°C) was applied at increasing rates to a sandy soil, subsequently planted with maize. Both maize yield and N uptake increased with increasing biochar rate, indicating N release from the biochar. Thus, the latter study further supports the conclusion of Spokas et al. [4].

Only a few biochar studies have examined, in detail, the actual mechanism for enhanced N uptake by biomass, following biochar addition. One superlative study that does provide a mechanism to explain the contribution of high N content manure biochars to enhanced plant available N showed that low temperature biochar materials made from manures and biosolids contain hydrolysable organic N forms such as amino acids [42]. The hydrolysable N fractions in the biochars decreased as pyrolysis temperatures increased. These results were explained by either N becoming progressively embodied in the increased formation of aromatic and heterocyclic structures [43] or the degradation of labile N forms (e.g. proteins), as clearly shown in the derivative thermogravimetric profiles, as the pyrolysis temperatures increased. Given the fact that plants can assimilate organic N compounds [44], and that mineralization of organic N also provides inorganic-N, it can be inferred to be the cause of increased yields seen under manure derived biochars. Noguera et al. [45] examined the effect of a low temperature (350°C) *Eucalyptus sp.* biochar and earthworms on rice plant growth and associated plant physiological and gene regulation processes in the leaves, finding that biochar increased protein catabolism (proteolytic activity) as well as anabolism via enhanced gene expression of some (but not all) genes associated with the leaf protein turnover, respectively. Given these results and those of Wang et al. [42] it would be interesting to identify the available organic-N content of the biochar used by Noguera et al. [45] and to see if bioavailable organic-N forms contributed to the enhanced proteolytic activity. A ¹⁵N study by de la Rosa and Knicker [46] also examined biochar-¹⁵N bioavailability, and confirmed the release of biochar-¹⁵N to the soil. After 72 days incubation in an arable soil 10% of the biochar-15N, manufactured from Lolium perenne at 350°C, was found to be taken up by the new grass biomass, clearly showing that biochar N can be decomposed, with subsequent metabolites utilised by microbes and plants. Similarly, Schouten et al. [47] showed ¹⁵N labelled biochar-N (pyrolysed digested slurry) was recovered by plants. In the short-term, immobilisation and mineralization may be affected by biochar addition to soil (see below) while effects on soil water availability, root architecture, plant ecophysiology, nutrient supply, microbial form and function may also be affected. Where biochar materials induce a liming effect it may be advisable to also run a lime treatment to assist in differentiating biochar induced soil physical versus soil pH effects. However, knowledge of the buffering capacity of both the soil and biochar may also be required. Both short and long-term studies are still needed to further evaluate biochar-N and its effects on agronomic performance. In particular further use of the ¹⁵N stable isotope for this purpose is recommended.

5. Mitigation of nitrous oxide emissions using biochar

To date, several studies have shown that the addition of biochar to soils can mitigate N_2O emissions *in situ* from soybean and grass ecosystems [48], following ruminant urine deposition [49], in wheat plots [50] and during laboratory or greenhouse incubations under various conditions [8,9,47,51-61] while other studies have found no differences or even increases in cumulative N_2O emissions after biochar addition [61-63].

In many studies where biochar has been shown to reduce N_2O fluxes a number of mechanisms have been proposed based mainly on prior knowledge of the requirements of nitrifiers and denitrifiers. These include (i) enhanced soil aeration (reduced soil moisture) inhibiting denitrification due to more oxygen being present, (ii) labile C in the biochar promoting complete denitrification i.e. dinitrogen (N₂) formation, (iii) the elevated pH of the biochar creating an environment where N₂O reductase activity is enhanced thus promoting N₂ formation and higher N₂/N₂O ratios, and (iv) a reduction in the inorganic-N pool available for the nitrifiers and/or denitrifiers that produce N₂O, as a result of NH₄⁺ and/or NO₃⁻ adsorption, greater plant growth, NH₃ volatilisation loss, or immobilisation of N. Increases in N₂O fluxes have been attributed to (i) the release of biochar embodied-N or priming effects on SOM following biochar addition (ii) biochar providing inorganic-N and/or carbon substrate for microbes.

Many studies reporting biochar effects on N_2O emissions have lacked the rigorous experimental design needed to test the hypotheses proposed and interpret the results produced (e.g. N_2O source and fate) when investigating biochar effects on N_2O emissions. For example, Wang et al. [59] performed a 60 day aerobic incubation with paddy soils treated with rice husk biochar (350-500°C) and N fertilizers finding that the cumulative emission of N_2O from N fertilizer was reduced when the biochar was present. However, no detailed examination of the cause was pursued with the rationale for lower N_2O emissions being some of the previously

hypothesised theories as outlined above. More recently, Case et al. [53] hypothesised that observed reductions in N₂O emissions from a biochar-amended sandy soil (0-10% biochar by weight) occurred due to enhanced aeration. Case et al. [53] maintained the biochar-amended soils at field capacity while measuring N₂O flux and inorganic-N over 168 h. Reduced N₂O fluxes observed at > 2% biochar were hypothesised to occur as a result of greater immobilisation of NO₃⁻. However, no supporting measurements of the changes that may have resulted from biochar addition, in the pore size distribution or soil aeration were undertaken, to verify the hypothesis. Aquilar-Chavez et al. [51] investigated the effects of charcoal application on N₂O emissions, over 45 days, following the application of wastewater sludge to mesocosms cultivated with wheat. The net result was a decrease in N₂O emissions as charcoal rates increased but the experimental design and measurements provided inconclusive explanations and speculated on the rationales noted above.

However, other studies have included further measurement components in their experiments and have been more successful at describing mechanisms for reduced N₂O fluxes. Saarnio et al. [56] demonstrated a plant competition effect for N on N₂O fluxes, showing increased N₂O emissions when plants were absent with less effect from biochar when plants were present due to plant uptake competing with microbes for N. Kammann et al. [54] also performed detailed experiments that aimed to examine causes for the variation in N₂O fluxes in response to soil moisture. Kamman et al. [54] included wetting-drying cycles (assumed to stimulate microbial N₂O production), while measuring biomass yields and inorganic-N, observing that increased plant growth correlated with reduced N₂O emissions in the biochar treatments. A detailed examination of mechanisms for reduced N₂O emissions from biochar-amended soils was also carried out by Spokas et al. [64] who showed the origins and effects of ethylene on N₂O emissions and its role in reducing N₂O emissions. The effect that ethylene has on N₂O production from biochar amended soils needs to be investigated in more detail to further elucidate its role in biochar-N₂O dynamics.

While recent studies have provided an increased perspective on biochar induced decreases in N₂O fluxes further experiments are required to further elucidate the mechanism(s) responsible. The use of ¹⁵N stable isotopes can provide insight into the source(s) of the N₂O-N, the reduction of N₂O to N₂, and associated N dynamics of other organic and inorganic-N pools in both the biochar and soil [46,47,49,65,66]. The use of ¹⁵N enhances our understanding of biochar N dynamics, N₂O emissions and facilitates modelling [67]. Detailed studies examining the effects of biochar on soil physical conditions and the effect on N₂O emissions where both are measured simultaneously are overdue. We need to know how biochar rates and forms specifically affect gas diffusivities, soil moisture contents and water movement under given conditions. Studies are required that concurrently measure both the soil physical and chemical parameters, inorganic-N species and N₂O and N₂ fluxes following biochar incorporation into soil. Similarly, every opportunity should be taken to assess changes in microbial community form and function to elucidate biochar effects on N₂O emissions. For example, Yoo and Kang [61] attributed an increase in N₂O emissions, after biochar application, in a rice paddy field to abundant pre-existing denitrifiers.

Ultimately the magnitude and duration of any N₂O flux following biochar incorporation into soil is the net result of soil and biochar N availability, soil fertility and moisture, climate, and competition for available N between microbes and plants. While some of the studies measuring N₂O are relatively long-term in nature for incubation experiments (e.g. Kammann et al. [54]) they are relatively short when compared to the long term (e.g. 2-3 year) in situ experiments now beginning to emerge in the literature. Given that short-term initial effects on N pool dynamics, observed in short term field studies and lab incubations, were not persistent in these longer term trials [26] it might be argued that there will not be persistent changes in N₂O dynamics as a result of initial soil-biochar N dynamics. However, in rice paddies studies in Southern China (summarized by Liu et al. [68]) it was shown that N₂O reductions with biochar application rates of 20 and 40 t ha⁻¹ persisted beyond the first cropping season. Thus other mechanisms involving plant-microbe interactions, denitrifier gene expression or bacterial-to-fungal ratios may become more important over time in biochar-amended soils. Therefore, the effects of biochar on soil physical properties, microbial communities and microbial gene expression on N₂O emissions need to be evaluated in the long-term in situ. Sampling methods must be of sufficient quality and frequency that rigorous comparisons of seasonal N₂O emissions can be made. There is great scope here for employing automated chamber methodologies. Investigation of old charcoal-rich soils such as Terra preta or charcoal-kiln sites and comparisons with surrounding charcoal-free soils may offer insights into the long-term changes in N cycling, and N₂O emissions associated with biochar application to soils. The long-term measurements need to be made during periods of changing N inputs (e.g. regular fertiliser application) in particular to determine biochar's long-term in situ role in mitigating N₂O fluxes.

6. Impacts of biochar on nitrogen mineralization, immobilisation and nitrification

Mineralization and immobilisation rates in the soil are a function of the C and N pools available to microorganisms. Typically as C:N ratios increase immobilisation of N occurs. Adding biochar to the soil adds another dimension to both the C and N pools. Addition of biochar to soils has been shown to result in slower mineralisation of the biochar materials than the uncharred biomass [69], decrease net N mineralisation [34,50], cause increased net N mineralisation [50], have no effect on mineralization [20,70], and to have little effect on DON [15]. Furthermore, biochar addition has been shown to have no effect on soil-N immobilisation [71] or promote immobilisation [72].

The N embodied in plant derived biochar has previously been assumed to be of low availability due to it being in heterocyclic structures [73] but a ¹⁵N study by de la Rosa and Knicker [46] shows that biochar embodied N can be utilised by biomass. Wang et al. [42] showed that acid hydrolysable N (amino acids, amino sugars and ammonia) embodied in manure-derived biochars decreased as pyrolysis temperature increased (250-550°C) with a strong correlation between this acid hydrolysable N and CO₂ respiration, following biochar addition to soil, indicating that the total acid hydrolysable N represented the available N in the biochar.

Fresh low temperature biochars can contain significant amounts of labile C that can be readily utilised by soil microorganisms [74] which, when delivered to the soil may, in the short term, result in the microbially available soil N becoming immobilised. This was demonstrated by Bruun et al. [72] who produced biochar from wheat straw using slow or fast pyrolysis. Fast pyrolysis resulted in a biochar that still contained a labile, un-pyrolysed carbohydrate fraction. When the 'slow' and 'fast' biochars were placed in the soil the 'fast' biochar resulted in immobilisation of mineral N while the 'slow' biochar resulted in net N mineralization over a 65 day period. Because addition of biochar to soil involves multiple N pools, tracer studies are needed to elucidate the gross N immobilisation and mineralization rates. Nelissen et al. [67] used ¹⁵N labelling-tracing to examine and model gross N dynamics following biochar (ensilaged maize pyrolysed at 350° C or 550° C, C:N = 43 and 49, respectively) addition (10 g kg^{-1} soil) to a loamy sand (C:N = 9). The authors found that gross N mineralization was stimulated by biochar addition, with most of the N coming from a more recalcitrant fraction, whereas mineralization in the control was mainly from a labile N pool. This was reasoned to be the result of biochar having a priming effect, i.e. stimulating microorgansims to mineralize recalcitrant SOM [75,76]. This concurs with the results of Schomberg et al. [20] who also found differences in a recalcitrant N fraction when incubating several different biochars over 127 days. Increased turnover of SOM can result from the addition of biochar to soil as a result of priming effects, most likely induced by labile components of the biochar, and this may increase with increasing soil pH and decreasing pyrolysis temperature [77].

Thus, while biochar may contain bioavailable N forms, its mineralization and release will be dependent on how recalcitrant the biochar and soil N and C pools are, on the soil and biochar C:N ratio, the relative magnitude of the soil and biochar C and N pools, and the studied ecosystems. Further tracer studies with labelled biochar and/or SOM are required to fully understand the effects of various biochar forms on immobilisation and mineralization and to determine if the effects observed are of relatively short duration or more long-term. Long-term modelling of biochar and soil N pools, and processes, over the long-term will be required as previously demonstrated for soil C [78].

Biochar application may have no effect on gross or net nitrification rates in agricultural soils [50,71], but biochar application has been shown to promote net nitrification in natural ecosystems due to the liming effects of biochar or the removal of inhibiting substances such as polyphenols or tannins [50,79,80]. Volatile organic compounds associated with a biochar or ethylene production can decrease nitrification activity [62,64]. In agricultural ecosystems, the lack of positive effects from adding biochar on net nitrification rates may be because agricultural ecosystems are already characterised by high nitrification rates [80]. The first and apparently only study to date to record biochar stimulation of gross nitrification in an agricultural soil [67] showed that it was due to increased mineralization of NH₄⁺ from the recalcitrant soil N pool, where the flux was larger than the simultaneous incorporation of NH₄⁺ into the labile soil N pool. Thus, the authors reasoned that the increase in gross nitrification was mainly due to an increase in the NH₄⁺ substrate supply. Studies such as that of Nelissen et al. [67], using a stable isotope modelling approach, provide detailed information on not just N

pool sizes but also the gross N dynamics. These types of studies now need to be applied over longer terms.

7. Biochar and soil biota

The known effects of biochar addition to soils on soil biota were extensively covered by Lehmann et al. [81] who concluded that there was limited knowledge on the shifts in microbial consortia and that our knowledge of biochar effects in soil on soil biota is limited. This is even more so when confining the discussion to N. Since the review by Lehmann et al. [81] the study of Jones et al. [26] has measured higher growth rates of bacteria and fungi after incorporating biochar but this effect was not observed after storage of soil in the laboratory leading the authors to speculate that the effect was the result of an indirect rhizosphere effect. While Dempster et al. [34] found that the addition of a *Eucalypt* biochar at 25 t ha⁻¹ altered the ammonia oxidiser community structure when it was present with inorganic-N, with lower nitrification rates ensuing. The latter was thought to be due to a negative priming effect on the soil organic matter resulting in lower NH4⁺ concentrations, since the potential for NO3⁻ adsorption to remove NO3⁻ was minimal when biochar was mixed with soil. Anderson et al. [75] examined biochar induced soil microbial community changes from soil where biochar had been incorporated during pasture renewal, and found that compared to control soils the abundance of the bacterial families Bradyrhizobiaceae and Hyphomicrobiaceae increased. During anaerobic phases members of these families can utilise NO₃, N₂ and NH₃ and they are capable of N₂ fixation and denitrification. Anderson et al. [75] concluded that adding biochar to the soil potentially increased microbial N cycling, especially the abundance of those organisms that may decrease N₂O fluxes and NH₄⁺ concentrations. Conversely, Yoo and Kang [61] suggested the higher N₂O fluxes observed in the presence of swine manure-derived biochar in paddy soils was partially a consequence of higher denitrifier abundance.

Noguera et al. [82] hypothesised that earthworms and biochar would have a synergistic effect on nutrient availability and plant growth. However, while differences in mineral N were observed with treatments these were soil type dependant and they found few interactions between earthworms and biochar, and no interaction with respect to mineral N, possibly as a result of the short term nature of the mesocosm study. The study by Augustenborg et al. [52] found biochar reduced earthworm-induced N₂O fluxes although the mechanisms for this were not clear. Thus, the systematic and rigorous experimentation, called for by Lehmann et al. [81], to assess biochar induced effects on soil biota with regard to soil N cycling is still needed.

8. Biochar as a carrier for nitrogen fertiliser

Besides the release of N intrinsically embodied in the biochar ([42,46,47] there have been attempts to further enhance the delivery of N using biochar by adding nutrients to the biochar prior to soil incorporation. For example, Sarkhot et al. [83] mixed biochar with filtered liquid dairy manure, by shaking the mixture for 24 h and then oven drying the biochar, which increased the biochar's N content by 8.3%. When the unamended and N enriched biochars were added to the soil in an 8 week incubation experiment, reductions in net nitrification were 68 and 75%, respectively, while net ammonification rates were reduced by 221 and 229%,

respectively. However, enriching biochar with N did not alter the N_2O flux which averaged a 26% reduction in the biochar treatments [83]. These results were interpreted as being due to adsorption processes rather than enhanced immobilisation, since CO_2 fluxes were also lower under biochar treatments. Sarkhot et al. [83]thus concluded that N enriched biochar could be used as a slow release N fertilizer.

Adsorption of NH₃ onto black carbon (defined as thermal-chemical by-products, encompassing graphite's to biochars [84] has been previously recognized [85]. Mechanisms responsible for NH₃ adsorption have been reviewed [86,87] and discussed by Spokas et al. [84]. As a result of NH₃ adsorption amides and amines are formed on the black carbon surface [88]. Adsorption of NH₃ by black carbon has been shown to be correlated to the quantity of surface acidic groups on the black carbon (Spokas et al. [84] and references therein). The potential for biochar to be used as an N fertilizer, by increasing its N content via NH₃ adsorption, was demonstrated in a study by Taghizadeh-Toosi et al. [65] who exposed biochar materials to ¹⁵Nlabelled NH₃ which became enriched in ¹⁵N. The increase in biochar-N and its ¹⁵N enrichment was higher in the more acidic biochars. Twenty five days after the application of these ¹⁵Nenriched biochar materials to soil, plant biomass had increased up to 3-fold (non-labeled biochar treatments showed no differences from the controls in terms of biomass yield) and there was transfer of the ${}^{15}N$ from the biochar to the soil (2.5 – 10.6% ${}^{15}N$ recovery) and subsequent plant N uptake (10.9 – 26.1% ¹⁵N recovery). Taghizadeh-Toosi et al. [66] then showed that NH₃ generated in the soil from ¹⁵N-labelled ruminant urine patches, that are dominated by urea, could also be captured by biochar present in the soil, which effectively acted as a N 'sponge' subsequently delivering the ¹⁵N to the soil and plant biomass when the biochar was transferred to non-urine treated soil.

The ability for biochar to act as a sink for NH₃ was further demonstrated by Doydora et al. [89] who acidified pine chip and peanut hull biochars (400°C) with HCl and then applied the acidified biochars to soil and either surface applied or incorporated poultry litter, hypothesising that NH₃ volatilisation would be reduced. After 21 days incubation they found that NH₃ volatilisation from poultry litter decreased by 58-63% and 56-60% for surface applied and incorporated poultry litters, respectively. Notably the amount of leached inorganic-N was higher due to the retention of non-volatilised N in the soil where acidified biochar had been used. However, plants were not present in this incubation and it can be expected that in a field trial situation poultry litter applications (with acidified biochar) timed to enhance plant N uptake would offset synthetic fertiliser inputs. The role of NH₃ uptake, i.e. reduced NH₃ volatilization and loss, has also been postulated or observed in composting experiments [10,90,91]. Clearly there is role for biochar in capturing NH₃ and for this captured N to be released upon plant demand. However, much remains to be examined. Is all the NH₃ adsorbed on the biochar surface released, or plant-available? What is the repeatability of the NH₃ adsorption effect? If NH₃ adsorbed onto biochar as an amide or amine, for example, is released, can the resulting biochar surface immediately take up further NH₃ or is a period of surface restoration required? How will soil moisture conditions affect NH₃ uptake? Results from long term field trials are needed to improve our understanding of biochar as a slow-release N fertilizer after N loading or N capture, subsequent N dynamics, and its effect on N_2O emissions and N_2O -emission-to-yield ratios.

9. Conclusions

The increasing body of biochar literature provides further evidence that biochar affects N cycling in soils and that it offers potential options for tightening the N cycle in agricultural ecosystems. Much of the biochar-soil research to date, with respect to N cycling, is fragmented with results as diverse as the types of biochar used and the biochar-soil combinations tested. However, trends do emerge. It is apparent biochar can take up N via ion exchange, remove NH₃ via adsorption, and stimulate immobilisation with flow on consequences for NO_3^- leaching. Biochar can also stimulate mineralization, supply N embodied in the biochar to biomass, and reduce N₂O emissions. Future research efforts need to continue the assessment of biochars role in soil N cycling, but in a systematic way, making use of N isotopes where possible, so that mechanisms responsible for variations in N cycling and the potential mitigation tools are more fully understood and identified. In particular there is a dearth of soil microbial studies and in situ studies examining the role of biochar and N cycling over the longer term. Furthermore, biochar N studies should also aim to elucidate the effects and potential risks, if any, that biochar may have in the future by investigating long-term analogs such as charcoal-rich soils, or aged versus fresh biochars. As an environmentally beneficial agricultural management tool, the most promising prospects for biochar, to date, are: (i) the reduction of NH₃ volatilisation via adsorption processes (urine patches, animal housing filters, composting), (ii) the development of slow release N fertilisers, and (iii) the reduction of N₂O emissions using fresh biochar additions to soils. However, even these areas require further research since the use of biochar as a mitigation tool demands a deeper mechanistic understanding and at the same time an increase in our ability to predict net effects over time.

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