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Urease Activity, pH Buffer Capacity, & Ammonia Volatilization in a Biochar amended Rice Growing Entisol under Two Water Regimes

Jayarathna, Nelum Wasana[1]*; Dharmakeerthi, Randombage Saman[1] & Igalavithana, Avanthi Deshani[2]

[1]*Department of Soil Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka*
[2]*Future Industries Institute, University of South Australia, Mawson Lakes Campus, Mawson Lakes, 5095, SA, Australia*

*Correspondence: nelumjayarathne94@gmail.com

ABSTRACT

Ammonia volatilization (AV) is a primary mechanism for nitrogen (N) loss from fertilized paddy soils amended with biochar, yet limited information is available on the underlying mechanisms. We investigated the impact of rice husk biochar (RHBC) on urease activity (UA), pH buffer capacity (pHBC) and N dynamics in a paddy growing Entisol applied with urea under two water regimes; saturated and flooded conditions. A closed chamber dynamic incubation experiment was conducted under laboratory conditions by amending the soil with 0, 0.5 and 2 % (w/w) of RHBC with or without urea (115 mg Urea-N/kg). Temporal variation (14 days) of pore water pH, NO_3^- and NH_4^+ concentrations, AV, and changes in UA and pHBC by the end of 14 days were measured. While urease activity decreased (17-22%), pHBC increased (6-8%) in soil amended with 2% RHBC depending on the water regime. Cumulative AV loss was very low (<3% of added N) under the conditions tested in this study, probably due to low urea application rate, and near neutral pH conditions (7.1 - 7.2) in pore water. However, application of 2% RHBC significantly decreased pore water NH_4^+ concentration (19%) and hence AV loss in flooded soil when compared to the 0% RHBC with urea treatment. It appears that RHBC has the potential to reduce AV losses from paddy growing acidic Entisols when applied at high rates. Extending the study to field conditions is required to validate the potential of RHBC to enhance N use efficiency of urea in paddy soils.

Keywords: **Ammonia volatilization, biochar, Entisols, rice, urea**

INTRODUCTION

Urea is a widely used nitrogen (N) fertilizer in rice cultivation. One of the primary processes that leads to the loss of added urea-N from rice paddies is ammonia volatilization (AV). Up to about 60% of applied N can be lost via AV (Wang and Huang, 2021), thus contributing to low use efficiency of added urea in rice (Buresh *et al.*, 2008). Volatilization of ammonia from paddy fields is affected by meteorological conditions, soil and crop properties, fertilization and irrigation strategies, and soil amendments (Hayashi *et al.*, 2006). Broadcasting of urea into puddled saturate soil or flood water leads to the accumulation of NH_4^+ ions in the surface water and encourages AV (Sommer *et al.*, 2004). The extent of AV in flooded soils depends on flood water pH, flood water NH_4^+-N concentration, depth of floodwater, temperature and wind speed (Sha *et al.*, 2019; Macnack *et al.*, 2013; Xie *et al.,* 2020). Therefore, better understanding of factors that govern AV in paddy growing soils is essential when attempting to increase efficiency of applied N in paddy fields. Urea undergoes hydrolysis after its soil application and releases ammonia into the soil solution [$NH_{3(aq)}$] which then will be in an equilibrium with $NH_4^+{}_{(aq)}$. Urease activity in soil governs the urea hydrolysis process (Cartes *et al.,* 2009). $NH_4^+{}_{(aq)}$ in the soil solution can diffuse into standing floodwater in paddy fields (Ethan, 2015) and the pH of the flood water or pore water determines the loss of $NH_4^+{}_{(aq)}$ as $NH_{3(g)}$. Changes in the pore water pH during urea hydrolysis can be influenced by pH buffer capacity (pHBC) of the soil (Curtin *et al.*, 2020). The AV loss from flooded soils is proportionate to changes in soil pH (Zhenghu and Honglang, 2000). Even though the pH of continuously submerged soil is neutral (6.7-7.2), the pH of the standing water can be above 8 and can even be reached to values as high as 9 due to high temperature and and is also influenced by the pH of the irrigation water (Ding *et al*., 2019; Ventura and Yoshida, 1977). AV is not commercially significant for fertilized acidic soils unless the pH of the soil surface increases over 7.5 (Dari and Rogers, 2021; Ferguson *et al.*, 1984). Moreover, a critical factor influencing soil pH changes is the soil's pHBC (Nelson and Su, 2010). Soil amendments can influence urease activity, soil pH, pHBC and hence AV losses. Owing to its multiple benefits, application of biochar is becoming a popular soil fertility management strategy (Scmidt *et al.,* 2021).

Biochar has been used as a soil amendment to reduce N losses form soil. The magnitude of the effects of soil application of biochar is dependent on the type of feedstock, application rate, and pyrolysis temperature (Awad *et al.*, 2018). Application of biochar can alter key soil factors that affect AV. For instance, the initial pH of very acidic soils is greatly changed compared to the neutral soil and predicts an overall increase of 11.5%-11.9% after application of biochar to rice soil (Awad *et al.*, 2018). Biochar can increase the soil pHBC by increasing the cation exchange capacity (CEC) in acid soils as a result of both high charge density on biochar surfaces and high surface area of biochar particles (Xu *et al.*, 2012; Liang *et al.*, 2006) and the main mechanism is identified as protonation and deprotonation of functional groups with oxygen on biochar surfaces (Shi *et al.*, 2017). At low pyrolysis temperatures the are quantitatively increased and facilitates ion adsorption and at high temperatures most organic substances are volatilized (Kwak *et al.*, 2019). Biochar affects soil enzyme driven pathways such as urea hydrolysis, ammonification, denitrification and nitrification (Oladele, 2019; Rajput *et al.*, 2019). The urease activity (UA) of soil can be either increased by biochar (Jiang *et al.,* 2021, Zhao *et al.,* 2022) as increased organic C, microbial biomass C and N which provides substrates for soil enzymes (Oladele, 2019); however, a decrease in activity can also occur due to inhibition effects on the enzyme activity can occur due to the adsorption of enzymes on the surface of biochar by the charged particles or blocks the reaction sites of enzymes (Day and Mavi, 2021; Hu *et al.*, 2020).

Although the application of rice husk biochar (RHBC) is an environmentally friendly soil management practice in paddy growing soils (Novair *et al.,* 2023), there is limited studies and information available on the behaviour and mechanisms of ammonia volatilization in Sri Lankan paddy soils amended with locally produced RHBC and how RHBC influences urease activity, pHBC, specially under different water regimes. We hypothesized that the application of RHBC could change the AV losses significantly in lowland rice fields, but the magnitude and the direction are dependent on the net effect on soil properties such as pH, urease activity, pH buffer capacity. This study was aimed to address this research gap and conducted a closed chamber experiment to quantify the effect of RHBC on AV losses and studied the pH changes, ammonium and nitrate ion concentrations in the pore water and the effect of RHBC application on soil urease activity and pHBC in a rice growing Entisol in Sri Lanka.

MATERIALS AND METHODS

Soil sampling and characterization

A bulk soil sample (Typic Endoaquents) of about 10 kg was collected (0-15 cm depth) from a rice cultivating farmer field in Kurunegala area (7°35' N 80°23' E). Soil was air dried and sieved (2-mm). Soil was initially characterized for pH and electrical conductivity (EC) with 1:5 soil water suspension (Brown, 1943), organic carbon Walkley and Black (1934) method, total N (Bremner and Mulvaney, 1983), cation exchange capacity (Sumner and Miller, 1996), pH buffer capacity (Liyanage *et al.,* 2012), urease activity (Sahrawat, 1980), available nutrients (P and K) after extracting with Melich III extraction (Mehlich, 1984) and soil texture (Sheldrick and Wang, 1993).

Biochar production and characterization

Biochar was prepared using rice husk pyrolyzed at 500 °C using a Down Draft Double Chamber (DDDC) pyrolyzer (Alahakoon *et al.,* 2018) and they were initially characterized for basic chemical parameters; pH, EC (IBI, international biochar initiative standards), CEC (Enders *et al.,* 2012), total carbon and total N using CN analyzer (SKALAR Primacs SNC100, the Netherlands), available nutrients (P and K) after extracting with Melich III extraction (Mehlich, 1984).

Closed chamber experiment

The closed chamber was conducted based on the method described by Jayarathna *et al.* (2024). Glass bottles (Diameter 7cm x height 12cm) were used as closed chambers. In the lid of the glass bottle there were one inlet and two outlets. The inlet was connected to an air pump (BOUY 9900) and a flow rate of ≈ 0.1 L min^{-1} was maintained. One outlet was connected to a fixed Rhizonflex® sampler and the other to a conical flask with 25 ml of 4% boric acid. The chambers were filled with 200 g of soil and biochar (at three rates; 0%, 0.5%, 2% w/w) mixture and well puddled with excess water. The three rates of biochar were selected based on previous literature and the production capacity of biochar by farmers. Rhizonflex® soil solution samplers (pore diameter 0.12-0.18 µm) were installed horizontally at 0.05 m below the soil surface in each pot. Then pre-incubate for two weeks under saturated conditions without standing water and similar set of pots were pre-incubated under flooded conditions with 1cm of sanding water. At the end of the pre-incubation, urea was applied at two rates to soils in the two sets (saturated and flooded) of bottles; 0 and 23 mg of N as urea (U). All treatments were arranged according to a complete randomized design with three replicates and kept under laboratory conditions. The trapped NH_3 in boric acid was collected in 1, 2, 3, 4, 5, 7, 9, 11 and 14 days after fertilizer application (DAF). Then samples were titrated with 0.01M HCl acid and reacted ammonia amount was calculated. Pore water was collected through RhizonFlex® samplers at the same time and analyzed for NH_4^+-N concentration (Markus *et al.,* 1985), NO_3^- -N concentration (Bremner and Mulvaney, 1983), and pH (Hanna Instruments HI 2020, USA) as described in Jayarathna *et al.,* 2024. At the beginning (0 DAF) and after two weeks (14 DAF) soil pHBC (Liyanage *et al.,* 2012) and UA (Sahrawat, 1980) were measured in soils of each pot.

Statistical analysis

Univariate ANOVA was conducted for UA and pHBC across treatment. Significantly different treatment means ($P < 0.05$) were separated using LSD method. Repeated measures analysis was conducted on AV, pH, NH_4^+-N, and NO_3^- -N concentrations using SPSS software (IBM Corp., 2012). Statistical analyses were done separately for saturated and flooded soils. Briefly, between subject effects were N treatments and within subject effects were time (DAF) and where the sphericity was detected from Mauchly's test, the significance was tested using Greenhouse-Geisser correction. If the interaction effects were significant (time × treatment) Bonferroni correction was adopted for the mean separation.

RESULTS AND DISCUSSION

The selected soil had an acidic pH and sandy loam texture. total N and organic carbon content (OC) were low. The soil is non-saline and poor in available P and K contents. This soil has shown a high response to N fertilizer application in previous studies (Kulasinghe *et al.*, 2020).

Prepared RHBC had high pH (>8.0). The EC value of RHBC was comparatively high (334 dS cm^{-1}), and it had a very high CEC (32.70 cmols$_+$ kg^{-1}), indicating a high cationic retention capacity (**Table 1**).

Table 1: Characterization of soil and biochar ±SD (n=3)

Property	Soil	Rice Husk Biochar
pH†	5.82 ±0.03	8.22 ±0.02
EC (dS cm^{-1})†	33.70 ±1.31 (1:5)	334.30 ±2.81 (1:20)
CEC (cmols$_+$ kg^{-1})	4.60 ±0.57	32.70±3.66
OC (g kg^{-1})	13.38 ±0.22	N/A*
TC (g kg^{-1})	N/A*	500.00 ±3.42
Total N (g kg^{-1})	0.40 ±0.12	0.46 ±0.25
Available P (mg kg^{-1})	1.24 ±0.19	24.44 ±2.42
Available K (mg kg^{-1})	55.63 ±2.55	324.20 ±6.38
Texture (USDA)	Sandy loam (Sand 81.6%, Clay17.0%)	N/A*

Note: † pH and EC was measured in 1:5 and 1:20 water suspensions in soil and biochar samples, respectively; *Not analyzed

Effects of Biochar Application and water regime on Soil pH Buffer Capacity

The pH buffer capacity varied from 11.2 to 12.2 mmols kg^{-1}pH^{-1} depending on the moisture regime and biochar rate. In the unamended soils pHBC was 11.22± 0.1 and increased significantly (*P*<0.05) by about 6-8% in 2% biochar added treatment, irrespective of the moisture regime (**Figure 1**).

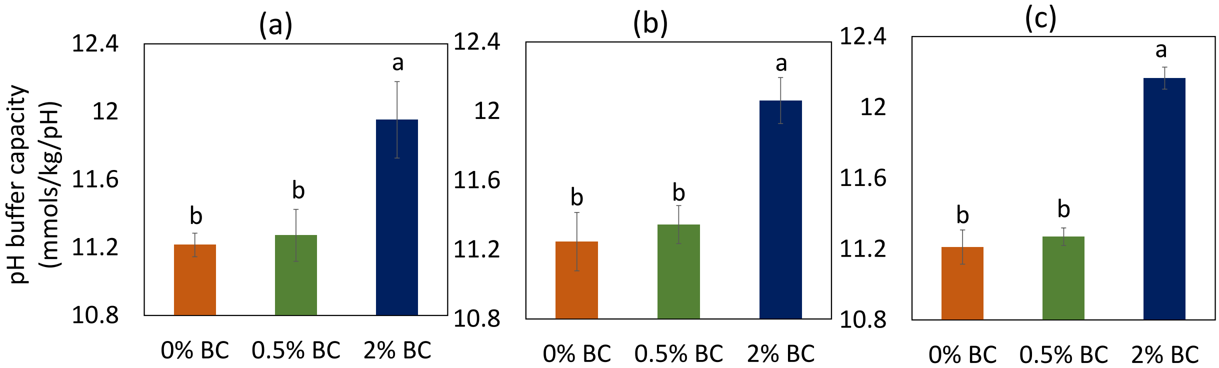

Figure 1: Variation in pHBC in the initial dry soil (a), saturated soil (b) and flooded soil (c) after incubated for 4 weeks with three biochar levels. The same lowercase letters for a given soil are not significantly different at p<0.05. Error bars on each bar indicate the SE (n=6).

The pHBC of this soil is generally low compared to the values reported elsewhere for lowland rice growing soils (Lu *et al.*, 2022) probably since our soil contains a lower amount of clay and organic matter. Biochar improves the soil pH buffering systems by increasing the soil CEC. The increase in soil's specific surface area while adding surface functional groups and the presence of residual volatile matter in the biochar structure contributes to the increase in CEC (Liang *et al.*, 2006; Purakayastha *et al.*, 2019). The main processes causing the rise in soil pHBC at pH 4.0−7.0 in biochar applied soil are protonation and deprotonation of functional groups on biochar surfaces that contain oxygen and dissolution of carbonates. Reaction of the protons with rice husk biochar derived soluble silica resulting $H_3SiO_4^-$ precipitation could also account for increased pHBC (Shi *et al.*, 2017). In addition to the moisture regime, the pHBC of a soil could also be influenced by the soil suspension valence, ionic strength, reaction time and biological processes (Wang *et al.*, 2015).

Effects of Biochar Application and water regime on Soil Urease Activity

The UA in unamended soils ranged from 5.6 to 8.4 NH_4^+-N µg $g^{-1}hr^{-1}$ depending on the moisture regime. Unlike pHBC, the UA was lower in biochar added treatments and the reduction was about 17-22% (**Figure 2**). However, the reductions were not statistically significant (*P*>0.05) under saturated conditions. Even though we could not statistically compare the differences in moisture regime, UA under the two moisture regimes is more or less similar (**Figure 2**).

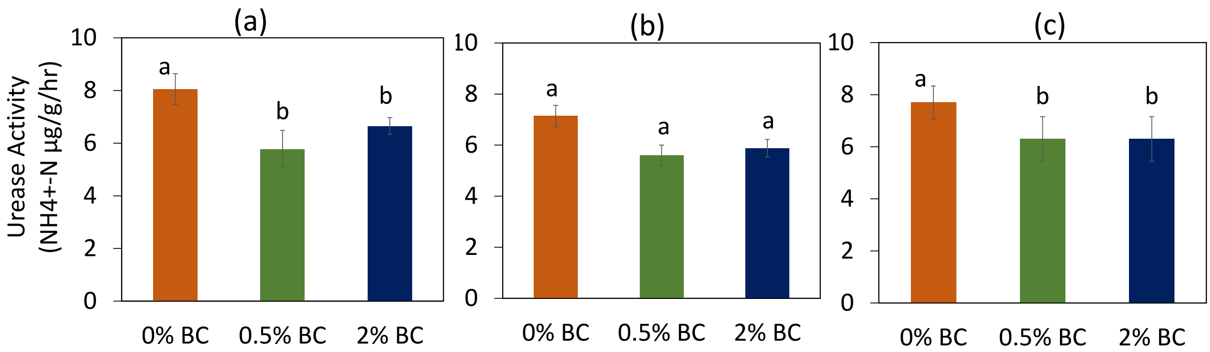

Figure 2: Variation in urease activity in the initial dry soil (a), saturated soil (b) and flooded soil (c) after incubation for 4 weeks with three biochar levels. The same lowercase letters for a given soil are not significantly different at p<0.05. Error bars on each bar indicate the SE (n=6).

Huang *et al*. (2017) has observed that the soil urease activity has significantly reduced at biochar application rates of 20 and 40 Mg ha^{-1} and there was not any significant difference between the rates. In rice soil UA is positively correlated with soil total N and OC content (Zantua *et al.*, 1977; Sahrawat, 1983). Although biochar application increases organic C contents in soil, suggesting an increase in UA, high specific surface area and the porosity of biochar can adsorb substrates (urea) on to the functional groups of the surfaces and/ or blocks the reaction sites of soil enzymes (Bailey *et al.*, 2011) resulting a decrease in enzyme activities. Enzymes can be adsorbed by charged particles on the surface of biochar resulting in inhibition of soil enzyme activity (Hu *et al.*, 2020). There have been conflicting reports regarding how soil moisture content affects UA. Delaune and Patrick, (1970) has observed that UA is not significantly changed with moisture content (field capacity Vs flooded). But the effect of redox potential is significant in urease activity where the rate is greater under oxidized conditions than the reduced conditions (Lindau *et al.*, 1989). This two-week incubation study with small quantity of soils could not have decreased the redox potential in flooded or saturated soils significantly during this period, hence no marked difference was observed in our study.

Changes in pore water NH_4^+ concentrations after urea and biochar application

Soil solution NH_4^+-N concentration has significantly (*P*<0.001) varied throughout the studied period. Under saturated conditions, a gradual decrease in NH_4^+-N concentration shows up to 5-7 DAFs (from 31 to 7 mg L^{-1}) and slight increase at 9 DAFs and then decreased again until 14 DAF (**Figure 3a**). Under flooding conditions, it decreased slightly until 7 DAF and then increased before decreasing after 11 DAF. It fluctuated between 18-35 mg L^{-1} (**Figure 3b**). Application of biochar did not significantly change the average NH_4^+-N concentrations in soil solution under saturated condition. But under flooded conditions, it was significantly (*P*<0.001) lower (22.1 mg L^{-1}) in soils amended with 2% biochar than that of unamended (27.3 mg L^{-1}) or 0.5% amended (25.7 mg L^{-1}) soils. Urea application has significantly increased the pore water average NH_4^+-N concentration in both saturated (*P*<0.05) and flooded (*P*<0.001) soils. Under saturated conditions average pore water NH_4^+-N concentrations in urea added and not added soils were 18.2 mg L^{-1} and 14.2 mg L^{-1}, respectively. The corresponding values under flooded conditions were 26.8 mg L^{-1} and 23.3 mg L^{-1}.

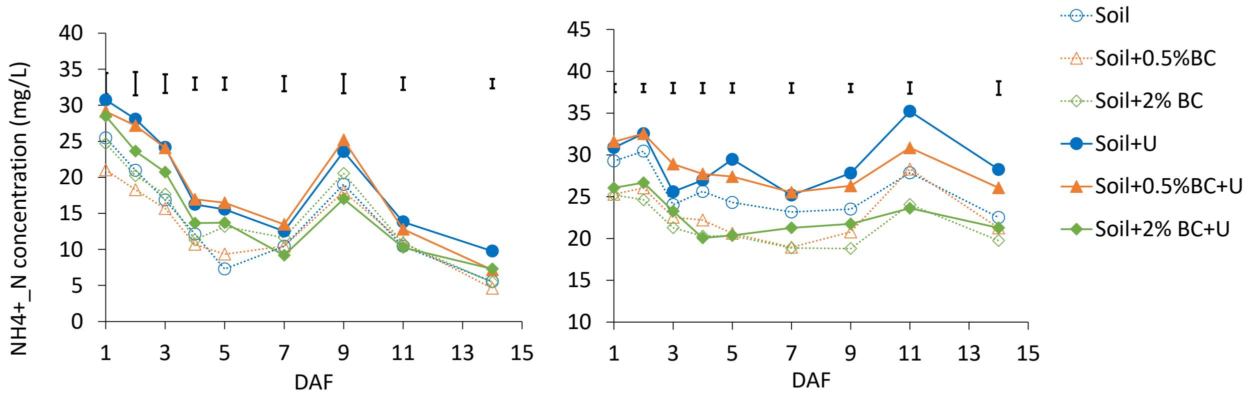

Figure 3: Changes in NH_4^+-N concentrations of pore water under (a) saturated and (b) flooded conditions with days after fertilizer application (DAF). Vertical bars at each DAF are ±SE (n=3). BC-biochar, U- urea.

He *et al.* (2019) has reported that ammonia oxidizer abundance rises following biochar amendment and stimulates nitrification in acidic paddy soil decreasing NH_4^+ ion concentration. High NH_4^+ adsorption capacity of biochar on the other hand limits the NH_4^+ ion availability and slower the nitrification (Lv *et al.*, 2021) reducing the impact of biochar application on NH_4^+ concentration in the pore water.

Changes in pore water NO_3^- concentrations after urea and biochar application

There were no significant interaction effects among DAFs, biochar rate and urea application on NO_3^- -N concentrations in the pore water under both moisture regimes. In saturated soils, NO_3^- -N concentrations increased significantly (P<0.005) from 7.8 mg L^{-1} to 21.1 mg L^{-1} during the initial 4 DAFs and then decreased to levels similar to initial levels after 9 DAF (Figure 4a). Under flooded conditions, the NO_3^- -N concentrations decreased continuously from 14.0 mg L^{-1} to 4.3 mg L^{-1} till 9 DAFs and remained constant thereafter (Figure 4b). Urea application has significantly increased (P<0.01) the average the NO_3^- ion concentration in pore water under saturated conditions, but not under flooded conditions. Biochar application increased the average NO_3^- -N concentration from 7.4 mg L^{-1} at 0% biochar to 8.6 mg L^{-1} at 2% biochar (P=0.06) under flooded conditions but not changed under saturated conditions.

Increase in NO_3^- concentration in the pore water with time could be a result of nitrification and this argument is supported from the decrease in NH_4^+ concentrations during such periods. After urea application to soil, urea hydrolyses to NH_4^+ which will then be subjected to nitrification increasing NO_3^- concentration in the pore water. Under flooded conditions denitrification losses of NO_3^- -N could be greater than that in saturated soil. Under the conditions tested in this experiment, NO_3^- -N can be lost mostly due to denitrification, could also be reduced due to microbial assimilation or ammonification by Archaea (Cabello *et al.*, 2004). Qi *et al.* (2022) observed that a reduction of AOA and AOB abundance initially with biochar application, possibly due to toxic pyrolysis residues and high localized NH_4^+ toxicity. But over time, nitrification activity increased, as NH_4^+ was consumed and NO_3^- content rose, enhancing AOA and AOB abundance later in the growth cycle.

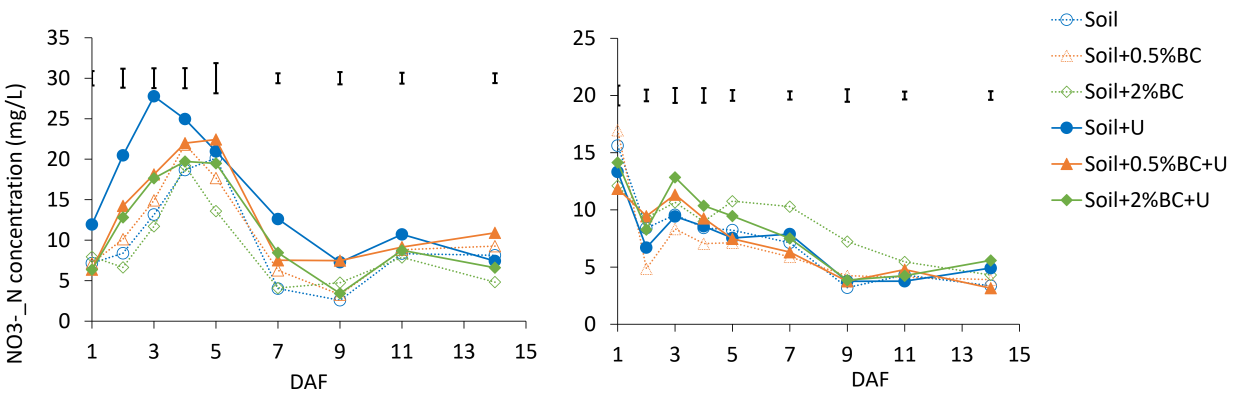

Figure 4: Changes in NO_3^--N concentrations of pore water under (a) saturated and (b) flooded conditions with days after fertilizer application (DAF). Vertical bars at each DAF are ±SE (n=3). BC-biochar, U- urea.

Biochar has the ability to reduce denitrification loss (Cayuela *et al.*, 2013) and therefore the impact on NO_3^- concentration should be more prominent in flooded soils where denitrification losses are expected to be higher than in saturated soils. Denitrifier activity can be suppressed in the anaerobic zone by the application of biochar due to limited NO_3^- availability (Borchard *et al.*, 2019). Nitrification can occur in the surface aerobic zones with diffused O_2 and convert remaining NH_4^+ into NO_3^- (Buresh *et al.*, 2008). Moreover, the NH_4^+ and NO_3^- ion adsorption capacity of biochar could effectively govern the ion concentration in the pore water (Thao *et al.*, 2021) and reduce their availability for different processes.

Effects of urea and biochar application on Pore Water pH

The pH of the soil used was 5.8 and after the pre incubation period with either saturated or flooded conditions, pH of the pore water has been increased up to near neutral pH (7.0 – 7.2) in all treatments. During the incubation period, the pH of the pore water stayed near neutral values. Under saturated conditions, pore water pH has not been significantly (P<0.05) changed by the addition of either biochar or urea throughout the incubation period (Figure 5a). Under flooded conditions the urea × biochar interaction effect was significant (P<0.008) (Figure 5b). As an average across the incubation period, the pH was reduced only by 0.01 unit after biochar application and the pH was increased by 0.01 units after urea application. Overall, the pH of the pore water under flooded conditions is less than that of saturated conditions and the changes with time and treatments are less than 1%.

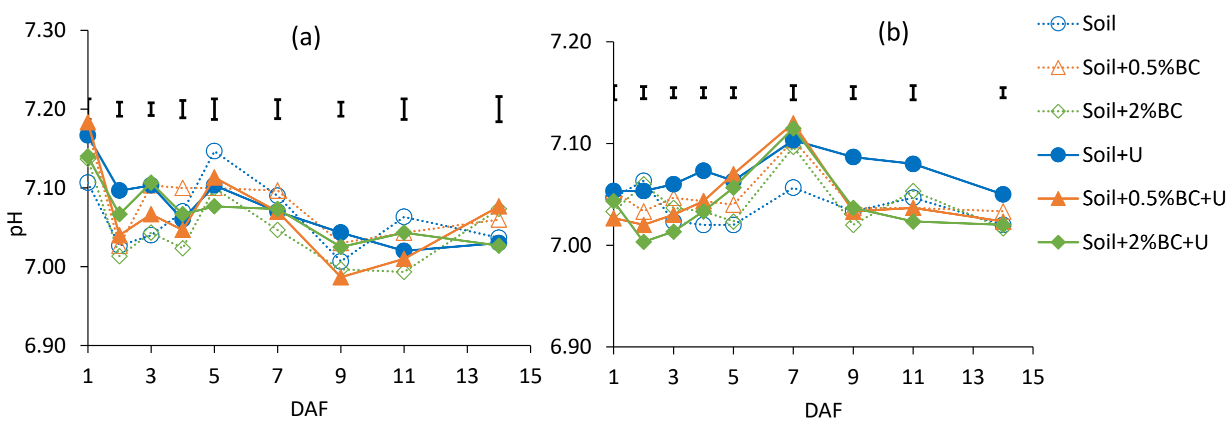

Figure 5: Changes in pH of pore water in different treatments with days after fertilization under (a) saturated and (b) flooded moisture regimes. The vertical bars at each data point are ±SE (n=3). BC-biochar, U- urea.

The slight reduction of pH can be due to the behavior of the NH_4^+ ions in aqueous solutions which did not dissociate completely and act as a conjugated acid in aqueous solutions (Silberberg, 2007). When acidic soils become reduced, it increases pH mainly by the H^+ consumption during the reduction reactions (Ponnamperuma, 1972). Furthermore, NH_4^+ and OH^- ions are created during urea hydrolysis, and rapid urea hydrolysis could accelerate their accumulation, raising the pH of the soil (Gao *et al.*, 2022). Urease activity was quite low in the investigated soil, and during the incubation period, there were no noticeable fast pH changes. Further, nitrification releases H^+ and NO_3^- over time, which may progressively lower soil pH (Curtin *et al.*, 2020).

Effects of urea and biochar application on ammonia volatilization

A detectable level of AV was observed only in urea applied treatments and the N losses from urea added soils as AV were very low, <3% of added N, under both moisture regimes in this acidic soil (P<0.001). While application of RHBC did not significantly affect AV losses in saturated soils (Figure 6a), 2% biochar added flooded soils did not release a detectable level of NH_3-N (Figure 6b). The highest AV was detected at 1 DAF under both moisture regimes and no detectable level of NH_3 was released after 3 DAF.

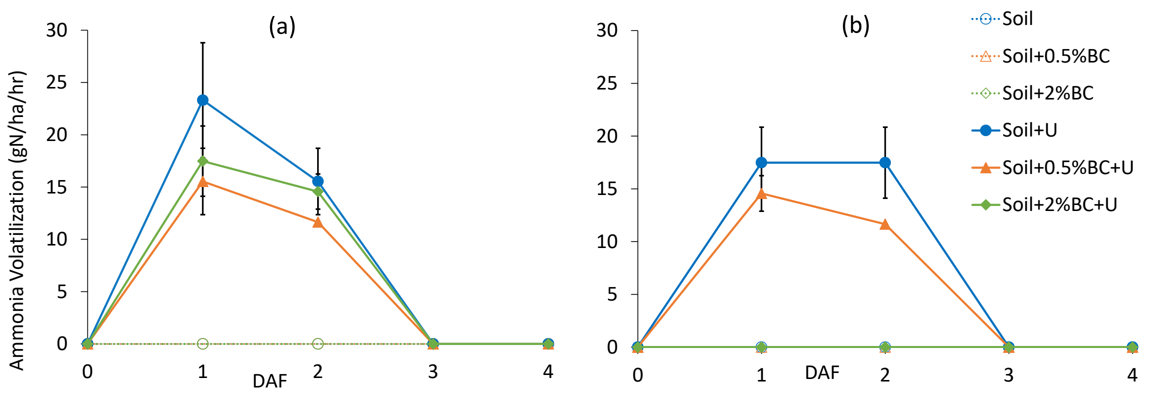

Figure 6: Changes in ammonia volatilization (mg N/kg of soil/day) with days after fertilization under (a) saturated and (b) flooded moisture regimes. The vertical bars at each data point are ±SE (n=3). BC-biochar, U- urea.

Qi *et al*. (2022) reported a similar observation under field conditions that AV mainly occurs shortly (1–3 days) after urea fertilization, driven by rapid NH_4^+ production and pH increase. In their study, biochar application (40 t ha^{-1}) reduced ammonia volatilization (up to 12.27%) compared to non-biochar treatment. Hayashi *et al.* (2006) observed that AV reached its highest peak at 2 DAFs (30 g N $ha^{-1}hr^{-1}$) in acidic rice soil (pH 5.7) with low urea rates (30 kg N ha^{-1}) under field conditions in acidic rice soil (pH 5.7). Selvarajh *et al.* (2020) has conducted a similar experiment under flooded conditions, where RHBC at a rate of 5-10 t ha^{-1} along with urea (175kg ha^{-1}) reduced the ammonia volatilization by about 30%. Application of urea increases NH_4^+ availability, which in turn increases the rate at which urea hydrolyzes to initiate the AV. (Sigurdarson *et al.*, 2018). As the soil urease activity has reduced by 17-22% in the respective soil after addition of biochar, the urea hydrolysis retards and can reduce the NH_4^+ availability. Although the 0 and 0.5% biochar application showed an AV, the 2% biochar rate is higher enough to suppress the AV under flooded conditions. This observation can be affected by various factors that are cumulative. AV is affected by soil solution pH (Jayaweera and Mikkelsen, 1991; Sha *et al.*, 2019). Compared to alkaline soils, acidic to neutral soils (pH 5.0–7.0) are less vulnerable to AV (Overrein and Moe, 1967). The pH of the pore water has stayed close to neutral according to the observations as increase in pHBC after biochar amendment at the same time. The pH between 7.0 - 8.5 has been recorded as optimal for nitrification in flooded soil which further reduces NH_4^+ ions in pore water (Katyal *et al.*, 1988). Adsorption of NH_4^+ to the biochar particles limits the availability by chemisorption and physisorption of ions into surface functional groups and to micro pore structure of the biochar particles (Yu *et al.*, 2016; Fidel *et al.*, 2018). Sun *et al.* (2019) reported that rice straw biochar (22.5t ha^{-1}) significantly reduced cumulative NH_3 volatilization by about 20% compared to straw, while also increasing rice yields, plant nitrogen uptake, and ammonia-oxidizing gene abundance. They observed a lower NH_4^+-N concentrations in surface water and improved ammonia oxidation contributed to reduced NH_3 losses under biochar treatments due to enhanced soil pH and cation exchange capacity (CEC), promoting nitrogen retention by biochar.

Under submerged irrigation systems, oxidized N species diffuse downward from the oxidized layers to the reduced layers, while incorporated ammonium diffuses upward from the anaerobic layer to the aerobic layer (Hanif, 1987). The flood water layer serves as a barrier where the ammonium ions dissolve and prevents volatilization. However, it is more oxidized in saturated conditions 316 than in flooded soil. Since there is no flood water layer, volatilization plays a direct role in removing ammonium ions (Ventura and Yoshida, 1977). Therefore, the N use efficiency is around 65% under continuous flooded conditions while it is around 25% in alternate flooded conditions (Hanif *et al.*,1987).

The comparatively low urea application rate utilized in this investigation, which limited the NH_4^+ ion concentration in the pore water, and the pore water's nearly neutral pH, which delayed the NH_4^+ dissociation in water, may be the cause of the very low AV values compared to other studies. Moreover, the near neutral soil pH favored the adsorption capacity of biochar over its alkalizing effect, aligning with Qi *et al.* (2022). Furthermore, in this system, there may exist other N transformation processes besides AV that are more significant including nitrification on the surface soil layer, denitrification, N fixation and the NH_4^+ ions that underwent denitrification in the reduced layer after being subjected to nitrification in the surface oxidized layer (Ponnamperuma, 1972; Reddy *et al.*, 1989). To better understand the underlying reasons for the low AV seen in this study, future research investigations under field conditions on rate of nitrification and denitrification are required to quantify the actual AV losses and formulate eco-friendly technologies to increase the use efficiency of urea-N in paddy cultivations in Sri Lanka.

CONCLUSION

The study revealed that biochar application up to 2% rate has significantly increased the soil pHBC by 6- 8% and reduced the UA by 17-22% and did not significantly affect the AV. The near neutral pH and high pHBC with UA reduction after application of biochar could have suppressed the AV under the experimental conditions. Nitrogen application has significantly increased the AV by increasing the NH_4^+ concentration. In comparison, the pore water NH_4^+ content was much lower in the flooded treatments with biochar added than it was in the treatments with urea added under saturated conditions. The application of rice husk biochar and/or keeping a flood water layer in place for a few days after N application could further reduce the AV loss (<3%) that occurred after N application. However, this study was conducted under controlled laboratory conditions, which limits the generalizability of the results to field conditions. Field-scale studies across different eco-physiological conditions are necessary to validate and refine these findings. Additionally, the long-term effects of continuous RHBC application on soil health, nutrient cycling, and greenhouse gas emissions and economic feasibility and farmer adoption potential of RHBC need to be studied.

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