



OPEN

Biochar and urease inhibitor mitigate NH₃ and N₂O emissions and improve wheat yield in a urea fertilized alkaline soil

Khadim Dawar¹, Shah Fahad^{2,3}, M. M. R. Jahangir⁴, Iqbal Munir⁵, Syed Sartaj Alam⁶, Shah Alam Khan⁷, Ishaq Ahmad Mian¹, Rahul Datta⁸, Shah Saud^{9,10}, Jan Banout¹⁰, Muhammad Adnan¹¹, Muhammad Nauman Ahmad¹², Aamir Khan¹, Raf Dewil¹³, Muhammad Habib-ur-Rahman^{14,15}, Mohammad Javed Ansari¹⁶ & Subhan Danish^{8,17}

In this study, we explored the role of biochar (BC) and/or urease inhibitor (UI) in mitigating ammonia (NH₃) and nitrous oxide (N₂O) discharge from urea fertilized wheat cultivated fields in Pakistan (34.01°N, 71.71°E). The experiment included five treatments [control, urea (150 kg N ha⁻¹), BC (10 Mg ha⁻¹), urea + BC and urea + BC + UI (1 L ton⁻¹)], which were all repeated four times and were carried out in a randomized complete block design. Urea supplementation along with BC and BC + UI reduced soil NH₃ emissions by 27% and 69%, respectively, compared to sole urea application. Nitrous oxide emissions from urea fertilized plots were also reduced by 24% and 53% applying BC and BC + UI, respectively, compared to urea alone. Application of BC with urea improved the grain yield, shoot biomass, and total N uptake of wheat by 13%, 24%, and 12%, respectively, compared to urea alone. Moreover, UI further promoted biomass and grain yield, and N assimilation in wheat by 38%, 22% and 27%, respectively, over sole urea application. In conclusion, application of BC and/or UI can mitigate NH₃ and N₂O emissions from urea fertilized soil, improve N use efficiency (NUE) and overall crop productivity.

In terrestrial ecosystems, nitrogen (N) deficiency is the main growth limiting factor, thereby adversely affecting crop productivity^{1–4}. Therefore, an exogenous supply of N along with phosphorus as fertilizers is key farming practice for improving crop yield³. Conferring to⁵, a huge quantity of chemically synthesized N compounds will be required to fulfill the food requirements of 9 billion people by 2050. However, the higher land application of N compounds as chemical fertilizers has a negative influence on the environment through the release of N

¹Department of Soil and Environmental Science (SES), The University of Agriculture, Peshawar, KPK, Pakistan. ²Department of Agronomy, The University of Haripur, Haripur, Khyber Pakhtunkhwa 22620, Pakistan. ³Hainan Key Laboratory for Sustainable Utilization of Tropical Bioresource, College of Tropical Crops, Hainan University, Haikou 570228, Hainan, China. ⁴Department of Soil Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh. ⁵Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar, Pakistan. ⁶Department of Plant Pathology, The University of Agriculture, Peshawar, Pakistan. ⁷Department of Plant Protection, The University of Agriculture, Peshawar, Pakistan. ⁸Department of Geology and Pedology, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemedelska1, 61300 Brno, Czech Republic. ⁹Department of Horticulture, Northeast Agricultural University, Harbin, China. ¹⁰Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Prague, Czech Republic. ¹¹Department of Agriculture, University of Swabi, Swabi, Khyber Pakhtunkhwa, Pakistan. ¹²Department of Agricultural Chemistry, The University of Agriculture, Peshawar, Pakistan. ¹³Process and Environmental Technology Lab, Department of Chemical Engineering, KU Leuven (University of Leuven), Leuven, Belgium. ¹⁴Crop Science, Institute of Crop Science and Resources Conservation (INRES), University of Bonn, Bonn, Germany. ¹⁵Department of Agronomy, MNS University of Agriculture Multan, Multan, Pakistan. ¹⁶Department of Botany, Hindu College Moradabad (Mahatma Jyotiba Phule Rohilkhand University Bareilly), Moradabad 244001, India. ¹⁷Department of Soil Science, Faculty of Agricultural Sciences & Technology, Bahauddin Zakariya University, Multan, Punjab 60800, Pakistan. ✉email: khadimdawar@yahoo.com; shah_fahad80@yahoo.com; rahulmedcure@gmail.com; sd96850@gmail.com

containing gasses such as NH_3 and N_2O , and its losses to the ground and surface water by nitrate leaching and runoff^{6–8}. At current, the N use efficiency of synthetic N fertilizers is too low, only amounting approximately 50%^{9,10}, which adds to the adverse environmental, agronomic and economic impact^{11,12}.

Ammonia volatilization is a major source for N losses and is considered to be the main cause of low N use efficiency^{9,13}. Ammonia is a major alkaline atmospheric pollutant that plays an important role in the formation of aerosols^{14,15}, which badly affect human health¹⁶, reduce visibility^{17,18}, alter Earth's radiative balance, and contribute to a global redistribution of N through atmospheric deposition^{19,20}. Although ammonia is not considered a potential greenhouse gas (GHS), its emission and re-deposition can adversely affect the environment²¹, and it may act as a secondary cause of N_2O emission in soil^{22,23}.

Nitrous oxide is a potential (150 years lifetime) GHS, which is 298 times more efficient than CO_2 for its heat-trapping capacity with a 7% contribution to the total GHS emission and 0.26% annual growth rate²⁴. Agricultural soils annually emit 4.1 Tg N to the global atmospheric N_2O emission of 14 Tg N^{24,25}. In agricultural soils, N_2O is emitted mainly by biological and chemical processes such as nitrification and denitrification^{26–28}. Additionally, N_2O emissions may also happen via nitrification–denitrification through autotrophic NH_3 -oxidizing bacteria, where ammonia is oxidized to NO_2^- , followed by its reduction into NO, N_2O and N_2 ^{26,29,30}. It can also be produced during a hybrid reaction by co-denitrification where two N atoms are released one each from organic N by mineralization and NO_2^- by denitrification³¹. In soil containing high organic matter, it can be released by heterotrophic reduction of nitrite by the oxidation of organic N^{32,33}.

Various studies have been aiming to develop sustainable and eco-friendly management practices for reducing N losses, improving NUE and crop yield^{34,35}. Such practices include application of nitrification inhibitors, urease inhibitor (UI), elemental S and polymers³⁶, removal of crop residues from the land in various ratios^{37,38} and application of biochar^{39,40}. Biochar, a carbon-rich pyrolytic product of organic waste, has received ample attention for its environmental benefits⁴¹. Utilization of biochar as a soil amendment/conditioner not only provides a way to recycle environmental waste but also optimizes soil health, crop yield and stimulates soil C sequestration^{42–50}. Studies have demonstrated that biochar may be used as a compound fertilizer in conjunction with mineral sources^{51,52}, as a slow-release fertilizer^{53–55}, which could enhance fertilizer⁵⁶ and may increase N use efficiency under different soil conditions^{51,52}. Biochar may have the capacity to sorb NH_4^+ or NH_3 gas released during composting, thereby lowering NH_3 emission. Biochar may also adsorb organic N compounds, thus decreasing their mineralisation⁵⁷ and consequently NH_3 emission. This gradual availability of N may be partially explained by lower atmospheric N_2O emissions from N fertilizer applied in the presence of biochar^{58,59}.

The application of UI [N-(n-butyl)] and thiophosphoric triamide (nBTPT or NBPT for short) have been reported to reduce urease activity in soil and slow down NH_4^+ release from urea, thus reducing NH_3 emission^{34,35,60}. Urease inhibitor significantly decreases urea hydrolysis, reduced NH_4^+ concentrations and, thereby limited NO_3^- supply and nitrification rate thus reducing N_2O emission and improves crop yield^{61–64}.

So far, many experiments have focused the effect of biochar and urease inhibitor sole application on gaseous N emissions, N-transformation processes and crop yield in variable agro-climatic circumstances. However, limited work is documented regarding the influence of urea treated with urease inhibitor and biochar or alone on N_2O and NH_3 emission from arable agricultural system under hot climate of Pakistan. Therefore, main objectives of current study were to explore the effect of urea together with urease inhibitor and biochar or alone on crop productivity, N_2O and NH_3 emission and N efficiency.

Results

Soil mineral N dynamics. The NH_4^+ -N concentration observed in urea treated plots on day 1 was significantly ($p < 0.05$) higher (5–25 mg N kg^{-1} soil) compared to the control (Fig. 1). In urea treatment, the NH_4^+ -N concentration increased because of the applied urea's fast hydrolysis during the first 3 days after first fertilization. Thereafter, a sharp decrease was observed (Fig. 1). The plots treated with biochar + urea showed maximum soil NH_4^+ -N concentration 28 days after fertilization, which was significantly higher than the plots treated with sole urea (Fig. 1). Furthermore, the application of UI considerably decreased the average soil NO_3^- -N content. After the first fertilization, soil NO_3^- -N content slightly increased, and most mineral N was observed as NH_4^+ -N. Soil NO_3^- -N concentration peaked just after the second urea application in the urea treatment and then displayed a rapidly decreasing trend within 14 days in after fertilizer application (Fig. 1). Additionally, biochar supplementation significantly decreased NO_3^- -N concentration in the soil.

Ammonia volatilization. The results concerning NH_3 emission are presented in Fig. 2 and Table 1. A maximum NH_3 discharge was detected between days 1 and 3 after every application. The application of BC and UI with urea significantly affected daily and total cumulative NH_3 discharge during the initial 10 to 12 days. Total NH_3 losses as kg N ha^{-1} amounted 14.4 with BC and 6.2 with BC + UI compared to sole urea (19.7), representing 27% and 69% reduction by BC and BC + UI, respectively, relative to sole urea application.

Nitrous oxide emissions. The application of urea, BC, and UI significantly affected the daily and cumulative N_2O emissions (Fig. 3 and Table 2). The highest and lowest cumulative emissions of N_2O were measured for urea and urea + BC + UI treatments, respectively (1.7 and 0.8 kg N_2O -N ha^{-1}) (Table 2). Biochar + urea significantly reduced N_2O emissions by 24% compared to urea alone. Urea applied in combination with BC and UI reduced N_2O emission by 53% over urea alone treatment.

N-use efficiency, yield and N uptake by wheat. Wheat N uptake, yield and NUE were significantly improved due to biochar and/or UI application (Table 3). Wheat biomass (24%) and grain yields (13%) were improved by BC over sole urea. In contrast, urea's combined application with BC and UI increased wheat bio-

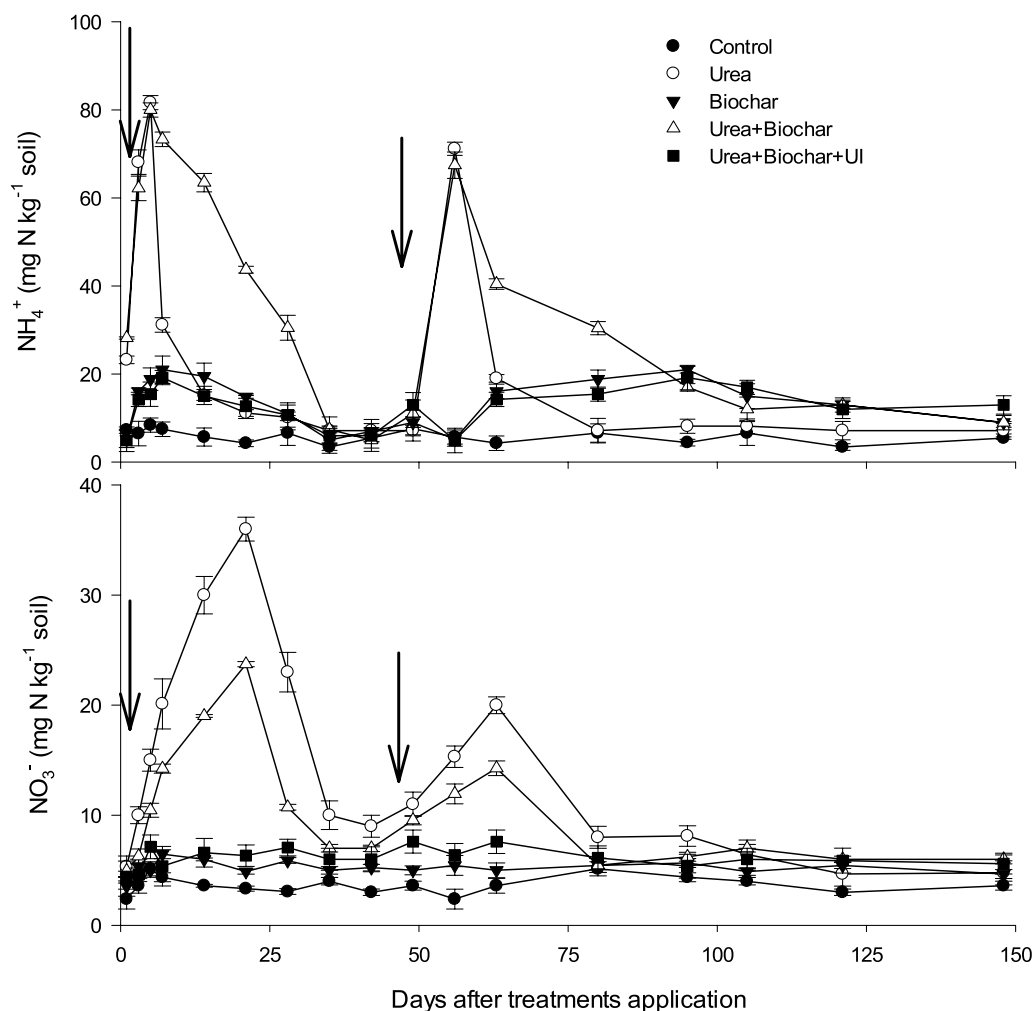


Figure 1. Soil NH_4^+ and NO_3^- contents as affected by nitrogen application with and without biochar and/or urease inhibitor. Bars on means represent SE for $n=4$. The arrows show time of N application.

mass and grain yields by 38% and 22% over the sole urea treatment. The highest total N uptake, above ground wheat biomass, contained 101 and 114 kg N ha^{-1} from urea + BC and urea + BC + UI treated plots, respectively (Table 3). The BC and UI application increased total N uptake by 12% and 27%, respectively, over sole urea (Table 3). Similarly, the highest N uptake and NUE were observed in plots treated with urea + BC + UI, followed by urea + BC (Table 3). The NUE observed for sole urea, urea + BC and urea + BC + UI were 27%, 35%, and 43%, respectively (Table 3).

Discussion

Ammonia volatilization as affected by biochar and urease inhibitor. A 27% decrease was observed in NH_3 volatilization with BC compared to sole urea (Table 1), which can be attributed to its highly porous structure, surface area and high sorption capacities^{60,61}. Furthermore, BC may absorb NH_4^+ -N⁶² due to the presence of acidic functional groups⁶³, hence decreasing the NH_3 volatilization⁶⁴. Biochar application with N fertilizers could avoid soil alkalization^{65,66} and decrease organic N mineralization through the adsorption of organic N compounds^{32,57}.

On day 1 after application, a rapid increase in NH_4^+ -N content was observed with sole application of urea (Fig. 1), which could be ascribed to the fast hydrolysis of urea. As a result, more NH_4^+ -N and OH^- ions are produced and allowing significant NH_3 losses (Fig. 1). Urease inhibitor reduce the effect of pH increasing by delaying urea hydrolysis, as demonstrated by lower NH_4^+ -N (Fig. 1) which could significantly reduce NH_3 emissions. Slower urea hydrolysis due to UI application could also be associated with increasing pH around urea particles, which prevents hydrolytic action on urea via the enzyme urease^{10,35,67}. It has also been reported that UI slows down the microbial decomposition of ammonia⁶⁸⁻⁷¹. Additionally, slower urea hydrolysis due to application of UI could also provide more time for rainfall or irrigation to move the applied urea from the soil surface to the sub-soil layers vertically as well as and laterally consequently protects the applied N from volatilization^{72,73}.

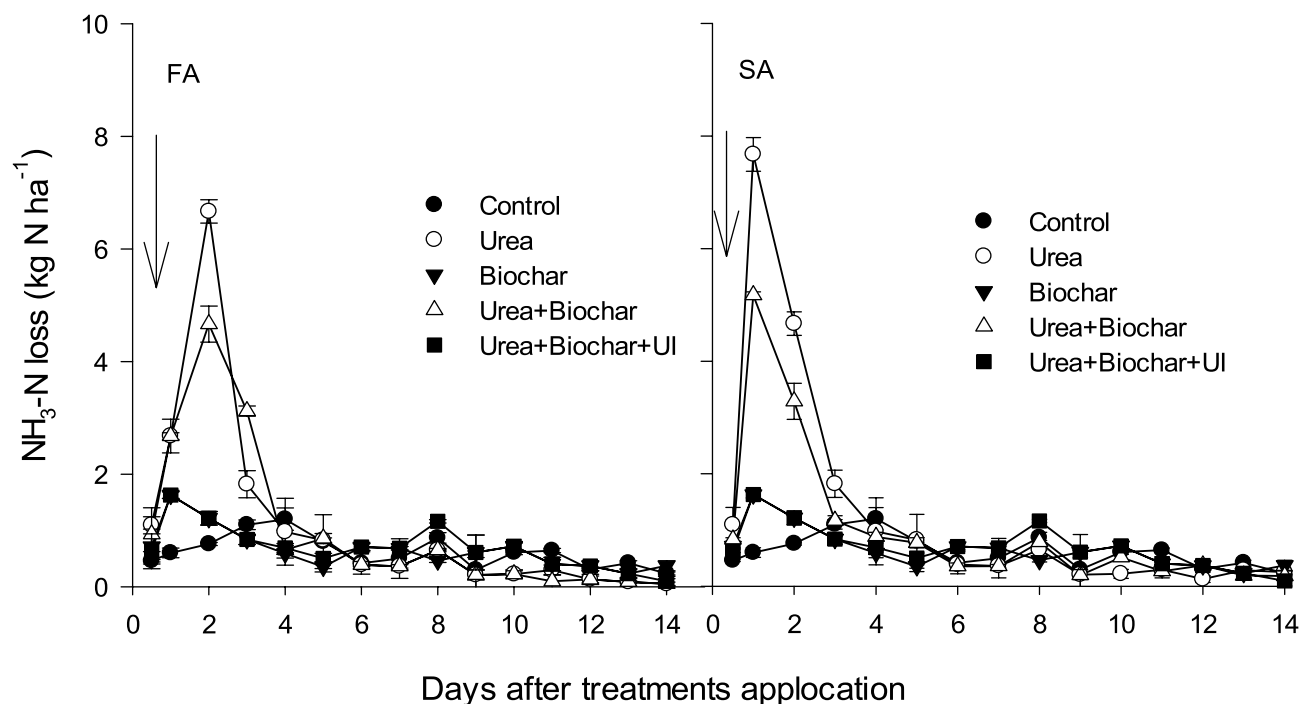


Figure 2. Soil NH_3 Fluxes as influenced by nitrogen application with and without biochar and/or urease inhibitor. Bars on means represent SE for $n=4$. The arrows show time of N application. Where, FA (First dose application) and SA (Second dose application).

Treatments	$\text{NH}_3\text{-N}$ losses (kg ha^{-1})	N lost as NH_3 (% of the applied N)	% Changes in NH_3 relative to urea
Control	0.7 ± 0.12^a	–	–
Urea	19.7 ± 0.12^e	12.7	–
Biochar	4.5 ± 0.12^b	2.5	–
Urea + biochar	14.4 ± 0.12^d	9.1	– 27
Urea + biochar + UI	6.2 ± 0.12^c	3.7	– 69

Table 1. Nitrogen losses as ammonia volatilization as affected by nitrogen application with and without biochar and/or urease inhibitor. Means followed by same letter(s) within columns are statistically non-significant ($p < 0.05$).

N_2O emission as affected by biochar and urease inhibitor. Nitrous oxide emissions are directly related to the amount of mineral N available in the soil. A two-way ANOVA indicated that seasonal N_2O emissions during the wheat growing season were significantly affected by biochar application (Fig. 2), in agreement with previous results Zhang et al.³³ and Schirrmann et al.⁷⁴. Our results confirm that the application of biochar reduced N_2O emissions, because biochar resulted in lower NO_3^- contents and higher NH_4^+ concentrations after N addition (Fig. 2) which affect N availability in the soil, either physically by sorption to surfaces or microbiologically⁷⁵. These results are in agreement with the findings of Lehmann et al.⁷⁶ and Kammann et al.⁷⁷ who reported that after application of N fertilizer, the adsorption of soil NH_4^+ by biochar on its surface, especially by biochar with a maximum C/N ratio reduce N_2O emissions, subsequently resulting a decrease in the processes of ammonification and nitrification.

Nitrous oxide is generated by both nitrification and denitrification processes; therefore, it may be closely related to soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations⁷⁸. Under high nitrification activity, when a lot of NO_3^- is produced in urea-treated soils during the first week of treatment application (Fig. 1) the observed N_2O may actually be related to nitrification which produces N_2O as a byproduct¹⁰. Apart from high water content also a high microbial activity can cause O_2 depletion and therefore anoxic conditions that might promote denitrification. Due to the strong nitrification usually taking place during aerobic conditions, NO_3^- predominates in soils. As a result, denitrification would be prompted by the large amounts of NO_3^- accumulated in soil, especially under high soil moisture conditions. For a given soil, whether nitrification or denitrification contributed more to N_2O emission may be closely related to soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations when the initial physicochemical factors of the tested soil are almost identical⁷⁹.

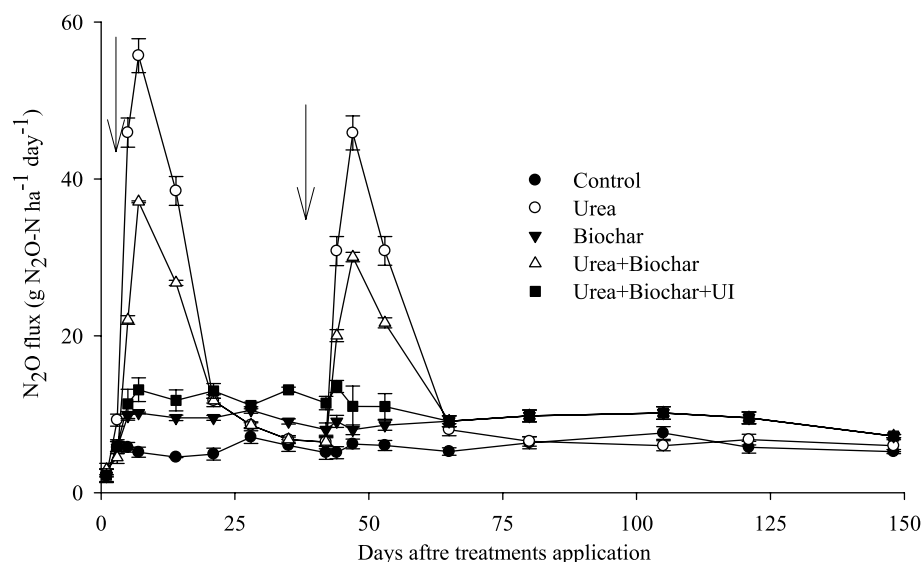


Figure 3. Soil N_2O flux in response to nitrogen application with and without biochar and/or urease inhibitor. Bars on means represent SE for $n=4$. The arrows show time of N application.

Treatments	Total N_2O losses ($kg\ ha^{-1}$)	N lost as N_2O (% of the applied N)	% Changes in N_2O relative to urea
Control	0.4 ± 0.12^a	–	–
Urea	1.7 ± 0.34^c	1.4	–
Biochar	0.6 ± 0.15^b		–
Urea + biochar	1.3 ± 0.22^d	1.0	– 24
Urea + biochar + UI	0.8 ± 0.17^c	0.40	– 53

Table 2. Nitrogen losses as nitrous oxide in response to urea supplementation with and without biochar and/or urease inhibitor. Means followed by same letter(s) within columns are statistically non-significant ($p < 0.05$).

Treatments	Biomass yield ($kg\ ha^{-1}$)	% Increase over urea	Grain yield ($kg\ ha^{-1}$)	% Increase over urea	Total N uptake ($kg\ ha^{-1}$)	% Increase over urea	NUE (%)
Control	5604 ± 62^a		2683 ± 32^a		49 ± 11^a		
Urea	7930 ± 68^c		3827 ± 47^c		90 ± 15^c		27
Biochar	6731 ± 52^b		3328 ± 38^b		62 ± 0.09^b		9
Urea + biochar	9855 ± 62^d	24	4327 ± 46^d	13	101 ± 17^d	12	35
Urea + biochar + UI	$10,978 \pm 58^e$	38	4651 ± 54^e	22	114 ± 21^e	27	43

Table 3. Wheat biomass and grain yields ($kg\ ha^{-1}$), N uptake ($kg\ ha^{-1}$) and NUE (%) as affected by urea fertilization with and without biochar and/or urease inhibitor. Means followed by same letter(s) within columns are statistically non-significant ($p < 0.05$).

The results showed lower NH_4^+ -N concentrations in plots treated with urea + BC + UI than sole urea treated plots, while NO_3^- -N concentrations were higher in urea treated plots than in urea + BC + UI (Fig. 1 and Fig. 3). It is likely that the combination of biochar and UI significantly decreased NO_3^- supply and thus reduced the activity of denitrifiers. Here, it is evident that UI application reduced the activity of ammonia oxidase and nitrification process and played a significant role in converting NH_4^+ -N to NO_3^- -N, which is significantly related to N_2O discharge from soil^{10,36,67}. These findings imply that applying either BC or BC + UI plays a significant role in reducing NH_3 and N_2O emissions from urea fertilized soils. Additional investigations are recommended to elucidate the role of BC and BC + UI in reducing NH_3 and N_2O emissions at varying soil and climatic conditions.

Wheat yield and N uptake as affected by biochar and urease inhibitor. Both BC and UI significantly improved total N uptake and yield of wheat (Table 3). Application of BC and/or UI with N fertilizers can reduce N losses as GHS and may prevent NO_3^- leaching, thus enhancing the bioavailability of N^{36,48,60}. Dawar et al.¹³ reported that the retention of mineral N in the form of NH_4^+ , rather than NO_3^- , for several days after

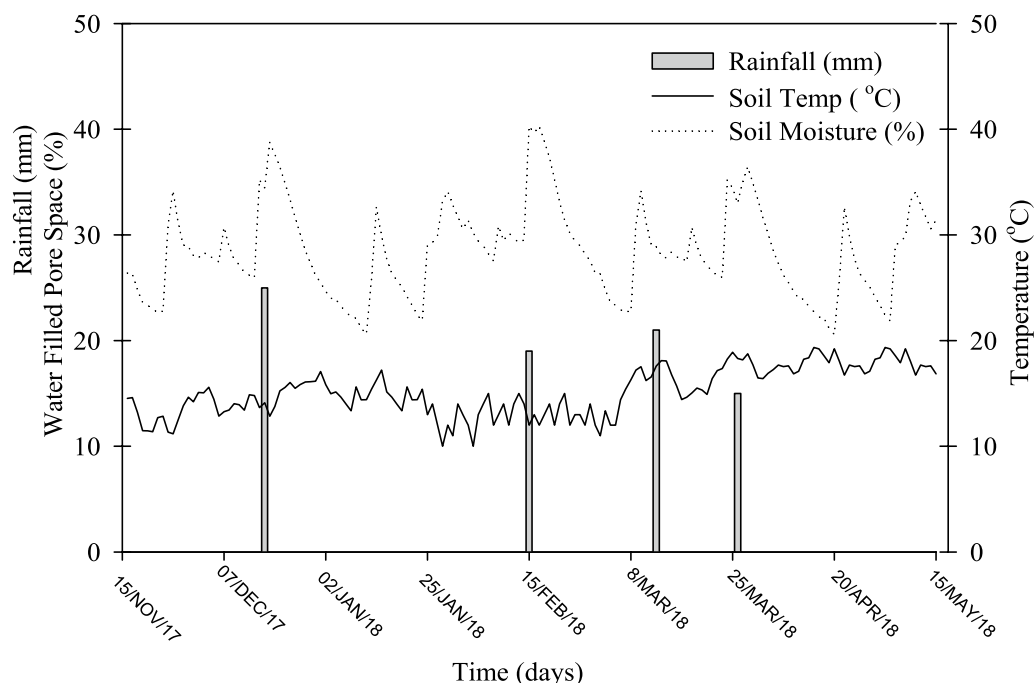


Figure 4. Soil (0–10 cm) temperature, moisture and precipitation throughout the growing season.

urea application increasing N uptake and thus improved crop yield. The results also show that BC improved soil WHC, allowing the wheat to retain a proper moisture level between irrigation periods, with a subsequent positive impact on final grain yields⁸⁰. Furthermore, BC + UI could significantly improve soil conditions such as soil organic content, pH, and total N content⁸¹, improving N availability by reducing NH_3 and N_2O from the soil. Urease inhibitor and BC retain NH_4^+ in the soil for a longer time and improve its subsequent uptake by wheat, thus may improve crop yield. This not only offers environmental benefits by preventing and NO_3^- leaching and sinking NH_3 and N_2O release^{13,36}, but also includes economic benefits using an increase in NUE. The plant uses relatively less energy to absorb NH_4^+ than NO_3^- as the transformation of NO_3^- into NH_4^+ via amides, amines, and amino acids is energy-consuming. Thus, higher availability of NH_4^+ facilitates improved crop yield and nutrition¹⁰.

Conclusions

It was observed that BC and UI have the highest potential to reduced NH_3 and N_2O emissions in urea fertilized soils. Furthermore, applying BC and/or UI with urea significantly improved wheat biomass and grain yield compared to sole urea. Therefore, the application of BC and/or UI with urea plays a significant role in mitigating NH_3 and N_2O emissions from cultivable land and improving crop yield under the hot and semi-arid agro-climatic conditions of Pakistan.

Materials and methods

Location. This experiment was executed at the National Institute of Food and Agriculture (NIFA), Peshawar (34.01°N, 71.71°E), Pakistan. The study area is semi-arid to arid and humid in the north to dry in the southern parts with a mean yearly precipitation of 384 mm and temperature of 22.7 °C (Fig. 4). The field had been conventionally cultivated with maize-wheat cropping system for approximately 10 years. The main soil (0–15 cm) properties of the composite sample are provided in Table 4.

Experiments. The field experiment including five treatments [control; urea (150 kg N ha⁻¹); BC (10 t ha⁻¹); urea + BC and urea + BC + UI] was designed following a randomized complete block design (RCBD) with 4 replications. The ‘Pirsabak-2013’ variety of wheat was sown in 5 m × 3 m plots containing 10 rows with row-to-row distance was kept 30 cm apart at a seed ratio of 120 kg ha⁻¹ on 15th Nov, 2017. The conventional tillage was implemented using a mouldboard plow (40–50 cm deep) followed by disking (20–25 cm deep) and land leveller operations for seed bed preparation. Before seeding, 90 kg P ha⁻¹ and 60 kg K₂O ha⁻¹ as single super phosphate and sulphate of potassium, respectively, were applied as a basal dose. The biochar used was produced from eucalyptus wastes (branches) pyrolyzed at 350 °C and sieved through a 5 mm sieve. Nitrogen was supplemented in the form of urea as a top dressing in two splits, half each a sowing and tillering. Urease inhibitor was supplemented as 0.1% solution. Granular urea with UI containing 25% NBPT was added at 150 kg N ha⁻¹ as a surface application followed by surface incorporation and irrigation (10 mm) to ensure its proper distribution in the root zone.

Property	Quantity
Texture	Silt loam
Electrical conductivity (EC)	0.26 d Sm ⁻¹
pH	7.80
Soil Bulk density (BD)	1.27 g cm ⁻³
Organic matter (OM)	0.88%
AB-DTPA extractable P	2.42 mg kg ⁻¹
Total nitrogen	0.089%

Table 4. Characterization of experimental field.

N₂O measurement. The N₂O discharge from soil was measured from November, 2017 to May, 2018 using opaque manual spherical static chambers, following the procedure described in Sanz-Cobena et al.³⁴. For sample collection, the cylindrical (radius 12.5 and height 20 cm) chambers were inserted in the soil at 15 cm depth 24 h before sampling. The chambers were properly sealed, and samples were taken after 0, 30 and 60 min once a week in the morning between 7:00 and 10:00 by a butyl septum installed on the upper part of the chamber. The samples were injected in 20 ml glass vials (Agilent Technologies, USA) via syringes (50 ml) equipped with inner three-way stopcocks (0.7-mm). The air and soil temperatures inside the chambers were recorded during gas sampling via a portable digital thermometer. Gas samples were analyzed on gas chromatographs (Varian Aerograph Series 2800 in NZ; Perkin Elmer Auto system XL B5902) equipped with 63Ni electron capture detectors (Pye Unicam) and two manual switching valves (Valco Instruments Co., Inc.)

The mean variation in gas concentration was calculated through linear regression while the ideal gas law was applied for quantification of gas-fluxes as follows:

$$F = \rho \times (P/760) \times (V/A) \times (\Delta C/\Delta t) \times [273/(273 + T)] \quad (1)$$

where F, P, V, A, $\Delta C/\Delta t$ and T represent N₂O flux ($\mu\text{g m}^{-2} \text{h}^{-1}$), density (mg m^{-3}), chamber size (m^3), base size (m^2) of the chamber, mean rate of variation in gas concentration per unit time ($\text{mg kg}^{-1} \text{h}^{-1}$) and chamber inner temperature ($^{\circ}\text{C}$), respectively. The acceptable range of R² for N₂O fluxes was taken as 0.80 for the static chamber, except where the maximum change in concentration was lower than the gas-specific GC detection limit (< 10 ppb for N₂O), where no filtering criterion was adopted (Järveoja et al.⁸²). Using these criteria, 10% of fluxes (N₂O) were subtracted from succeeding data analysis.

The following formula was used for the determination of cumulative N₂O emissions (Ec, kg N₂O-N ha⁻¹):

$$\sum_{i=1}^n (F_i + F_{i+1})/2 \times (t_{i+1} - t_i) \times 24$$

where F_i and F_{i+1} is the *i*th and (*i* + 1)th measured value, respectively, of N₂O flux ($\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$); t_i and t_{i+1} is the day when the *i*th and (*i* + 1)th measurement of N₂O flux is taken, respectively (d); and n is the total number of the measurements.

Ammonia measurement and analysis. Ammonia volatilization was quantified by a 5 L (plastic bottle) semi-static open chamber, according to Araujo et al.⁸³. In each plot, a single chamber was installed 5 mm above the ground surface and was randomized daily as advised by Jantalia et al.⁸⁴. A foam strip pre-soaked in 1 molar H₂SO₄ and 4% (V/V) glycerol was kept moist throughout sampling duration by immersing one end into a polypropylene jar containing 15 mL 1 molar H₂SO₄ solution suspended inside the chamber. In each plastic bottle, the solution and foam strip were initially replaced by fresh solution after 12 h and then every 24 h and 14 days and processed for NH₄⁺-N concentration via the steam distillation method⁸⁵.

The following equation was applied for the quantification of NH₃ fluxes (kg N ha⁻¹ d⁻¹):

$$F = \frac{2 \times C \times V \times 14 \times 10^{-2}}{\pi \times r^2} \times \frac{24}{t}$$

where; C, V, t and r represent the molar concentration of H₂SO₄, amount of H₂SO₄ consumed during titration (ml), sampling time (hours) and chamber radius (m). The cumulative NH₃ flux was determined by adding the NH₃ fluxes for all sampling days for their respective treatment plots.

This method showed 57% NH₃ recovery by calibration with ¹⁵N isotope equilibrium technique (Araújo et al.⁸³). Therefore, for the accurate estimation of cumulative NH₃ emissions and flows, a correction factor of 1.74 was applied (Araújo et al.⁸⁶). According to Jantalia et al.⁸⁴, this method for quantification of NH₃ emissions is more suitable than the wind tunnel procedure³⁵ for comparison of different treatments.

Soil and plant analysis. From each plot, five soil samples (0–10 cm) were randomly collected, well mixed, sieved (2 mm) and analyzed for key soil properties (Table 4). To determine mineral N (NO₃⁻ and NH₄⁺), extraction was done with 1:5, 2 M KCl for 1 h on a rotary shaker¹⁰, filtered and analyzed via ultraviolet spectrophoto-

tometry (Jenway, 6305 UV/Vis, UK). Soil moisture was measured by oven-drying and was transformed to water-filled pore space (WFPS) using the formula of Li et al.⁸⁵.

Total N in soil and plant samples was determined by the Kjeldhal method of Keeney and Nelson⁸⁶. In this method, 0.2 g of finely ground samples of dry materials were digested with 3 ml of concentrated H₂SO₄ in the presence of 1.1 g digestion mixture containing CuSO₄, K₂SO₄ and Se on a heating mantle for about 1 h. The digest was transferred quantitatively to the distillation flask and distilled in the presence of 10 ml of 10 M NaOH solutions. The distillate was collected in 5 ml boric acid mixed indicator solution and then titrated against 0.01 M HCl solution by adding 5 ml boric acid mix Indicator. Using the follow formula total N was calculated.

$$\text{Total Nitrogen \%} = \frac{(\text{Sample} - \text{Blank}) \times 0.005 \times 0.014 \times 100}{\text{Weight of soil} \times \text{volume made}}$$

For obtaining the data on grain and biological yield, the central four rows were harvested from each plot at physiological maturity and data were recorded on various agronomical traits (biomass, grain yield and straw yield) and total N uptake in crop. Biomass yield was separated into grain and above ground plant tissue (i.e. shoot and leaves) and record their fresh bulk weight immediately. Five randomly chosen plant tissue sub-samples (ca. 1000 g fresh weight) from each sub-plot; were transferred to sealable plastic bags, and transferred to lab in container with ice to ensure no water losses occur from collected plant tissue. After transporting the plant tissue samples to the lab, fresh weight was immediately recorded. After recording the fresh weight, harvested material was placed in pre-weighed paper bags and dried at 65 °C for 7 days. Dry weights of the plant tissue after 7 days were recorded in order to calculate its moisture content or fraction. The grain yield was adjusted for moisture fraction, prior to obtaining its dry weight, using a moisture tester. For N uptake by above ground plant tissues (i.e. shoot and leaves) and by the grain, the two tissues separately samples were ground to a fine powder (for determination of the total N).

Grains yield was recorded after threshing of wheat plants taken from central four rows of each treatment and then converted into kg ha⁻¹ by using the following formula.

$$\text{Grain yield (kg ha}^{-1}\text{)} = \frac{\text{Grain yield in four central rows}}{\text{Row} - \text{row distance} \times \text{Row length} \times \text{No. of rows}} \times 10000$$

Biological yield was recorded by harvesting 4 central rows in each plot, dried and weighed and then weight was converted into kg ha⁻¹ using the following formula;

$$\text{Biological yield (kg ha}^{-1}\text{)} = \frac{\text{Biological yield in four central rows}}{\text{Row} - \text{row distance} \times \text{Row length} \times \text{No. of rows}} \times 10000$$

Nitrogen uptake and NUE were then calculated as follows:

$$\text{Total N uptake} = \frac{\%N \text{ in grains} \times \text{grain yield (kg ha}^{-1}\text{)}}{100}$$

$$\text{NUE (\%)} = \frac{\text{Total N. uptake (kg ha}^{-1}\text{) in fertilized plot} - \text{Total N. uptake (kg ha}^{-1}\text{) in control plot}}{\text{N.applied (kg ha}^{-1}\text{)}} \times 100$$

Statistical analysis. Standard error (SE) of mean (n=4) was quantified via descriptive statistics. The replicated data were processed using analysis of variance (ANOVA) followed by least significant difference (LSD) test (P < 0.05) using general linear model (GLM)⁸⁷.

Plant material collection and use permission. No permission is required for plant material as it was purchased from certified dealer of local area.

Ethics approval and consent to participate. We all declare that manuscripts reporting studies do not involve any human participants, human data, or human tissue. So, it is not applicable.

Complies with international, national and/or institutional guidelines. Experimental research and field studies on plants (either cultivated or wild), comply with relevant institutional, national, and international guidelines and legislation.

Received: 29 April 2021; Accepted: 17 August 2021

Published online: 31 August 2021

References

1. Battaglia, M. L., Lee, C. & Thomason, W. Corn yield components and yield responses to defoliation at different row widths. *Agron. J.* **110**, 210–225 (2018).
2. Liu, X.Y. et al. Nitrate is an important nitrogen source for Arctic tundra plants. *Proc. Natl. Acad. Sci.* **115**, 3398–3403 (2018).
3. Kumar, P. et al. Impacts of nitrogen fertilization rate and landscape position on select soil properties in switchgrass field at four sites in the USA. *CATENA* **180**, 183–193 (2019).

4. Diatta, A. A. *et al.* Assessment of nitrogen fixation by mungbean genotypes in different soil textures using ^{15}N natural abundance method. *J. Soil Sci. Plant Nutr.* <https://doi.org/10.1007/s42729-020-00290-2> (2020).
5. Müller, C., Laughlin, R. J., Spott, O. & Rütting, T. Quantification of N_2O emission pathways via a ^{15}N tracing model. *Soil Biol. Biochem.* **72**, 44–54 (2014).
6. Yi, Q. *et al.* Effects of nitrogen application rate, nitrogen synergist and biochar on nitrous oxide emissions from vegetable field in south China. *PLoS ONE* **12**, e0175325 (2017).
7. Adnan, M. *et al.* Coupling phosphate-solubilizing bacteria with phosphorus supplements improve maize phosphorus acquisition and growth under lime induced salinity stress. *Plants* **9**, 900 (2020).
8. Dawar, K. *et al.* Effects of the nitrification inhibitor nitrapyrin and the plant growth regulator gibberellic acid on yield-scale nitrous oxide emission in maize fields under hot climatic conditions. *Pedosphere* **31**, 323–331 (2021).
9. Li, H., Liang, X., Chen, Y., Tian, G. & Zhang, Z. Ammonia volatilization from urea in rice fields with zero-drainage water management. *Agric. Water Manag.* **95**, 887–894 (2008).
10. Dawar, K., Zaman, M., Rowarth, J. S., Blennerhassett, J. & Turnbull, M. H. Urease inhibitor reduces N losses and improves plant-bioavailability of urea applied in fine particle and granular forms under field conditions. *Agric. Ecosyst. Environ.* **144**, 41–50 (2011).
11. Galloway, J. N. *et al.* Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* **320**, 889–892 (2008).
12. Schlesinger, W. H. On the fate of anthropogenic nitrogen. *Proc. Natl. Acad. Sci. USA*. **106**, 203–208 (2009).
13. Dawar, K. *et al.* The effect of biochar and nitrogen inhibitor on ammonia and nitrous oxide emissions and wheat productivity. *J. Plant Growth Regul.* <https://doi.org/10.1007/s00344-020-10283-1> (2021).
14. Finlayson-Pitts, B. J. & Pitts, J. N. Jr. *Chemistry of the Upper and Lower Atmosphere: Theory, Experiments, and Applications* (Academic Press Inc. Elsevier, 1999) <https://doi.org/10.1016/B978-0-12-257060-5.X5000-X>.
15. Saylor, R., Myles, L., Sibble, D., Caldwell, J. & Xing, J. Recent trends in gas-phase ammonia and $\text{PM}_{2.5}$ ammonium in the Southeast United States. *J. Air Waste Manag. Assoc.* **65**, 347–357 (2015).
16. Chow, J. C. *et al.* Health effects of fine particulate air pollution: Lines that connect. *J. Air Waste Manag. Assoc.* **56**, 1368–1380 (2006).
17. Batty, W. H. *et al.* Evaluating ammonia (NH_3) predictions in the NOAA National Air Quality Forecast Capability (NAQFC) using in situ aircraft, ground-level, and satellite measurements from the DISCOVER-AQ Colorado campaign. *Atmos. Environ.* **140**, 342–351 (2016).
18. Bray, C. D. *et al.* Evaluating ammonia (NH_3) predictions in the NOAA National Air Quality Forecast Capability (NAQFC) using in-situ aircraft and satellite measurements from the CalNex2010 campaign. *Atmos. Environ.* **163**, 65–76 (2017).
19. Galloway, J. N., Leach, A. M., Bleeker, A. & Erismann, J. W. A chronology of human understanding of the nitrogen cycle. *Philos. Trans. R. Soc. B Biol. Sci.* **368**, 20130120 (2013).
20. Pinder, G. F. & Gray, W. G. *Essentials of Multiphase Flow and Transport in Porous Media* (Wiley, 2008).
21. Megaritis, A. G., Fountoukis, C., Charalampidis, P. E., Pilinis, C. & Pandis, S. N. Response of fine particulate matter concentrations to changes of emissions and temperature in Europe. *Atmos. Chem. Phys.* **13**, 3423–3443 (2013).
22. Beusen, A. H. W., Bouwman, A. F., Heuberger, P. S. C., Van Der Drecht, G. & Van Der Hoek, K. W. Bottom-up uncertainty estimates of global ammonia emissions from global agricultural production systems. *Atmos. Environ.* **42**, 6067–6077 (2008).
23. Del Grosso, S. J., Wirth, T., Ogle, S. M. & Parton, W. J. Estimating agricultural nitrous oxide emissions. *EOS Trans. Am. Geophys. Union* **89**, 529 (2008).
24. IPCC (Intergovernmental Panel on Climate Change). Climate change 2013: The physical science basis. In *Working Group I contribution to the IPCC Fifth Assessment Report*. (Cambridge Univ. Press, 2014) <https://doi.org/10.1017/cbo9781107415324.023>.
25. Fowler, D. *et al.* Atmospheric composition change: Ecosystems–atmosphere interactions. *Atmos. Environ.* **43**, 5193–5267 (2009).
26. Wrage, N., Velthof, G. L., Van Beusichem, M. L. & Oenema, O. Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biol. Biochem.* **33**, 1723–1732 (2001).
27. Saggat, S. *et al.* Denitrification and $\text{N}_2\text{O}:\text{N}_2$ production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. *Sci. Total Environ.* **465**, 173–195 (2013).
28. Zhu, T. *et al.* Nitrogen mineralization, immobilization turnover, heterotrophic nitrification, and microbial groups in acid forest soils of subtropical China. *Biol. Fertil. Soils* **49**, 323–331 (2013).
29. Mueller, N. D. *et al.* Closing yield gaps through nutrient and water management. *Nature* **490**, 254–257 (2012).
30. Wrage-Mönnig, N. *et al.* The role of nitrifier denitrification in the production of nitrous oxide revisited. *Soil Biol. Biochem.* **123**, A3–A16 (2018).
31. Hayatsu, M., Tago, K. & Saito, M. Various players in the nitrogen cycle: Diversity and functions of the microorganisms involved in nitrification and denitrification. *Soil Sci. Plant Nutr.* **54**, 33–45 (2008).
32. Chen, L., Zheng, H. & Wang, Z. Y. The formation of toxic compounds during biochar production. *Appl. Mech. Mater.* **361**, 867–870 (2013).
33. Zhang, M. *et al.* A 2-yr field assessment of the effects of chemical and biological nitrification inhibitors on nitrous oxide emissions and nitrogen use efficiency in an intensively managed vegetable cropping system. *Agric. Ecosyst. Environ.* **201**, 43–50 (2015).
34. Sanz-Cobena, A., Sánchez-Martín, L. & García-Torres, V. A. Gaseous emissions of N_2O and NO and NO_3^- leaching from urea applied with urease and nitrification inhibitors to a maize (*Zea mays*) crop. *Agric. Ecosyst. Environ.* **149**, 64–73 (2012).
35. Sanz-Cobena, A. *et al.* Yield-scaled mitigation of ammonia emission from N fertilization: The Spanish case. *Environ. Res. Lett.* **9**, 125005 (2014).
36. He, T. *et al.* Effects of application of inhibitors and biochar to fertilizer on gaseous nitrogen emissions from an intensively managed wheat field. *Sci. Total Environ.* **628–629**, 121–130 (2018).
37. Battaglia, M., Groover, G. & Thomason, W. *Harvesting and nutrient replacement costs associated with corn stover removal in Virginia. Virginia Cooperative Extension Publication CSES-229NP* https://pubs.ext.vt.edu/content/dam/pubs_ext_vt_edu/CSES/cses-229/CSES-229.pdf (2018).
38. Battaglia, M. *et al.* The broad impacts of corn stover and wheat straw removal for biofuel production on crop productivity, soil health and greenhouse gas emissions: A review. *GCB Bioenergy*. **13**(1), 45–57 (2021).
39. Saarnio, S., Heimonen, K. & Kettunen, R. Biochar addition indirectly affects N_2O emissions via soil moisture and plant N uptake. *Soil Biol. Biochem.* **58**, 99–106 (2013).
40. Malińska, K., Zabochnicka-Świątek, M. & Dach, J. Effects of biochar amendment on ammonia emission during composting of sewage sludge. *Ecol. Eng.* **71**, 474–478 (2014).
41. Lehmann, J., Gaunt, J. & Rondon, M. Bio-char sequestration in terrestrial ecosystems—A review. *Mitig. Adapt. Strateg. Glob. Chang.* **11**, 395–419 (2006).
42. Scheer, C., Grace, P. R., Rowlings, D. W., Kimber, S. & Van Zwieten, L. Effect of biochar amendment on the soil-atmosphere exchange of greenhouse gases from an intensive subtropical pasture in northern New South Wales, Australia. *Plant Soil* **345**, 47–58 (2011).
43. Taghizadeh-Toosi, A., Clough, T. J., Sherlock, R. R. & Condron, L. M. Biochar adsorbed ammonia is bioavailable. *Plant Soil* **350**, 57–69 (2012).
44. Ameloot, N. *et al.* Short-term CO_2 and N_2O emissions and microbial properties of biochar amended sandy loam soils. *Soil Biol. Biochem.* **57**, 401–410 (2013).

45. Nelissen, V., Saha, B. K., Ruysschaert, G. & Boeckx, P. Effect of different biochar and fertilizer types on N₂O and NO emissions. *Soil Biol. Biochem.* **70**, 244–255 (2014).
46. Sánchez-García, M., Roig, A., Sánchez-Monedero, M. A. & Cayuela, M. L. Biochar increases soil N₂O emissions produced by nitrification-mediated pathways. *Front. Environ. Sci.* **2**, 25 (2014).
47. Sun, H., Zhang, H., Powlson, D., Min, J. & Shi, W. Rice production, nitrous oxide emission and ammonia volatilization as impacted by the nitrification inhibitor 2-chloro-6-(trichloromethyl)-pyridine. *Food Crop. Res.* **173**, 1–7 (2015).
48. Sun, H., Lu, H., Chu, L., Shao, H. & Shi, W. Biochar applied with appropriate rates can reduce N leaching, keep N retention and not increase NH₃ volatilization in a coastal saline soil. *Sci. Total Environ.* **575**, 820–825 (2017).
49. Idrees, M. *et al.* Animal manure-derived biochars produced via fast pyrolysis for the removal of divalent copper from aqueous media. *J. Environ. Manag.* **213**, 109–118 (2018).
50. Diatta, A. A., Fike, J. H., Battaglia, M. L., Galbraith, J. M. & Baig, M. B. Effects of biochar on soil fertility and crop productivity in arid regions: A review. *Arab. J. Geosci.* **13**, 595 (2020).
51. Joseph, S. *et al.* Shifting paradigms: Development of high-efficiency biochar fertilizers based on nano-structures and soluble components. *Carbon Manag.* **4**, 323–343 (2013).
52. Zheng, J. *et al.* Biochar compound fertilizer increases nitrogen productivity and economic benefits but decreases carbon emission of maize production. *Agric. Ecosyst. Environ.* **241**, 70–78 (2017).
53. Zhou, Z. *et al.* Biodegradation of a biochar-modified waterborne polyacrylate membrane coating for controlled-release fertilizer and its effects on soil bacterial community profiles. *Environ. Sci. Pollut. Res.* **22**, 8672–8682 (2015).
54. Wen, P. *et al.* Microwave-assisted synthesis of a novel biochar-based slow-release nitrogen fertilizer with enhanced water-retention capacity. *ACS Sustain. Chem. Eng.* **5**, 7374–7382 (2017).
55. El Sharkawi, H. M., Tojo, S., Chosa, T., Malhat, F. M. & Youssef, A. M. Biochar-ammonium phosphate as an uncoated-slow release fertilizer in sandy soil. *Biomass Bioenergy* **117**, 154–160 (2018).
56. Chunxue, Y. A. O. *et al.* Developing more effective enhanced biochar fertilisers for improvement of pepper yield and quality. *Pedosphere* **25**, 703–712 (2015).
57. Cole, D. P., Smith, E. A. & Lee, Y. J. High-resolution mass spectrometric characterization of molecules on biochar from pyrolysis and gasification of switchgrass. *Energy Fuels* **26**, 3803–3809 (2012).
58. Clough, T., Condon, L., Kammann, C. & Müller, C. A review of biochar and soil nitrogen dynamics. *Agronomy* **3**, 275–293 (2013).
59. Nelissen, V., Rütting, T., Huygens, D., Ruysschaert, G. & Boeckx, P. Temporal evolution of biochar's impact on soil nitrogen processes—a 15N tracing study. *Gcb Bioenergy* **7**, 635–645 (2015).
60. Mandal, S. *et al.* Biochar-induced concomitant decrease in ammonia volatilization and increase in nitrogen use efficiency by wheat. *Chemosphere* **142**, 120–127 (2016).
61. Chen, W. *et al.* Effects of different types of biochar on methane and ammonia mitigation during layer manure composting. *Waste Manag.* **61**, 506–515 (2017).
62. Thies, J. & Rillig, M. C. *Characteristics of biochar: Biological properties*. In *Biochar for Environmental Management: Science and Technology* (2009).
63. Thangarajan, R. *et al.* The potential value of biochar in the mitigation of gaseous emission of nitrogen. *Sci. Total Environ.* **612**, 257–268 (2018).
64. Agyarko-Mintah, E. *et al.* Biochar lowers ammonia emission and improves nitrogen retention in poultry litter composting. *Waste Manag.* **61**, 129–137 (2017).
65. Feng, Y. *et al.* Biochar applied at an appropriate rate can avoid increasing NH₃ volatilization dramatically in rice paddy soil. *Chemosphere* **168**, 1277–1284 (2017).
66. Wang, S. *et al.* Different effects of biochar and a nitrification inhibitor application on paddy soil denitrification: A field experiment over two consecutive rice-growing seasons. *Sci. Total Environ.* **593–594**, 347–356 (2017).
67. Abalos, D., Sanz-Cobena, A., Misselbrook, T. & Vallejo, A. Effectiveness of urease inhibition on the abatement of ammonia, nitrous oxide and nitric oxide emissions in a non-irrigated Mediterranean barley field. *Chemosphere* **89**, 310–318 (2012).
68. Cantarella, H., Otto, R., Soares, J. R. & de Brito Silva, A. G. Agronomic efficiency of NBPT as a urease inhibitor: A review. *J. Adv. Res.* **13**, 19–27 (2018).
69. Mira, A. B. *et al.* Optimizing urease inhibitor usage to reduce ammonia emission following urea application over crop residues. *Agric. Ecosyst. Environ.* **248**, 105–112 (2017).
70. Li, Q. *et al.* Effect of a new urease inhibitor on ammonia volatilization and nitrogen utilization in wheat in north and northwest China. *Food Crop. Res.* **175**, 96–105 (2015).
71. Marcinkowski, T. & Kierończyk, M. Effectiveness of urease inhibitor NBPT in mitigation ammonia emission from urea and urea ammonium nitrate solutions (UAN) applied in mineral fertilization of plants. *J. Civil. Eng. Environ. Architect* **62**, 271–279 (2015).
72. Engel, R. E., Williams, E., Wallander, R. & Hilmer, J. Apparent persistence of N-(n-butyl) thiophosphoric triamide is greater in alkaline soils. *Soil Sci. Soc. Am. J.* **77**, 1424–1429 (2013).
73. Dawar, K., Zaman, M., Rowarth, J. S., Blennerhassett, J. & Turnbull, M. H. Urea hydrolysis and lateral and vertical movement in the soil: Effects of urease inhibitor and irrigation. *Biol. Fertil. Soils* **47**, 139–146 (2011).
74. Schirrmann, M. *et al.* Biochar reduces N₂O emissions from soils: A meta-analysis. in *EGU General Assembly Conference Abstracts* 8265 (2017).
75. Case, S. D. C., McNamara, N. P., Reay, D. S. & Whitaker, J. Can biochar reduce soil greenhouse gas emissions from a Miscanthus bioenergy crop?. *GCB Bioenergy* **6**, 76–89 (2014).
76. Sharma, S. P. Biochar for carbon sequestration. In *Omic Technologies and Bio-Engineering* 365–385 <https://doi.org/10.1016/b978-0-12-815870-8.00020-6> (2018).
77. Kammann, C., Ratering, S., Eckhard, C. & Müller, C. Biochar and hydrochar effects on greenhouse gas (carbon dioxide, nitrous oxide, and methane) fluxes from soils. *J. Environ. Qual.* **41**, 1052 (2012).
78. Müller, C. & Sherlock, R. R. Nitrous oxide emissions from temperate grassland ecosystems in the Northern and Southern Hemispheres. *Glob. Biogeochem. Cycles* **18**, GB1045 (2004).
79. Liu, S. *et al.* A meta-analysis of fertilizer-induced soil NO and combined NO+ N₂O emissions. *Glob. Chang. Biol.* **23**, 2520–2532 (2017).
80. Niu, Y. *et al.* Yield-scaled N₂O emissions were effectively reduced by biochar amendment of sandy loam soil under maize-wheat rotation in the North China Plain. *Atmos. Environ.* **170**, 58–70 (2017).
81. Zhang, W. *et al.* Effect of biochar on root morphological and physiological characteristics and yield in rice. *Acta Agron. Sin.* **39**, 1445–1451 (2013).
82. Järveoja, J. *et al.* Impact of water table level on annual carbon and greenhouse gas balances of a restored peat extraction area. *Biogeosciences* **13**, 2637–2651 (2016).
83. Araújo, E. D. S. *et al.* Calibration of a semi-opened static chamber for the quantification of volatilized ammonia from soil. *Pesqui. Agropecuária Bras.* **44**, 769–776 (2009).
84. Jantalia, C. P. *et al.* Nitrogen source effects on ammonia volatilization as measured with semi-static chambers. *Agron. J.* **104**, 1595–1603 (2012).

85. Li, B., Fan, C. H., Xiong, Z. Q., Li, Q. L. & Zhang, M. The combined effects of nitrification inhibitor and biochar incorporation on yield-scaled N₂O emissions from an intensively managed vegetable field in southeastern China. *Biogeosciences* **12**, 2003–2017 (2015).
86. Keeney, D. R. & Nelson, D. W. Nitrogen-inorganic forms. In *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties*, 9.2.2 (ed Page, A. L.) Vol. 9 643–698 (American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, 1983).
87. Steel, R. G., Torrie, J. H. & Dickey, D. A. *Principles and Procedures of Statistics: A Biometrical Approach* (McGraw Hill Book International Co., 1997).

Author contributions

Conceptualization: S.F., K.D.; Data curation: I.M., S.S.A; Formal analysis: I.A.M., S.D., R.D.S.S.; Investigation: S.F., K.D.; Methodology: M.M.R.J.; A.K.; M.A.; resources: S.A.K., M.N.A.; Writing—original draft: K.D.; Writing—review and editing: S.F., R.D., S.D., R.D., J.B., M.H., M.J.A.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to K.D., S.F., R.D. or S.D.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2021