https://doi.org/10.17221/112/2022-PSE

Urease inhibitor and biochar independently affected N_2O emissions from *Camellia oleifera* soils

Bangliang Deng[1,2], Fangfang Shen[1], Xiaomin Guo[2], Evan Siemann[3], Ling Zhang[2]*

[1]*Jiangxi Provincial Key Laboratory for Restoration of Degraded Ecosystems and Watershed Ecohydrology, College of Water Conservancy and Ecological Engineering, Nanchang Institute of Technology, Nanchang, P.R. China*

[2]*Key Laboratory of Silviculture, College of Forestry, Jiangxi Agricultural University, Nanchang, P.R. China*

[3]*Department of Biosciences, Rice University, Houston, USA*

**Corresponding author: gxmjxau@163.com*

Citation: Deng B.L., Shen F.F., Guo X.M., Siemann E., Zhang L. (2022): Urease inhibitor and biochar independently affected N_2O emissions from *Camellia oleifera* soils. Plant Soil Environ., 68: 424–430.

Abstract: Nitrous oxide (N_2O) is a long-lived greenhouse gas that impacts climate change. Agricultural soils with intensive nitrogen (N) application are the main source of N_2O emissions. Reducing N_2O emissions from N-fertilised soils is, therefore, important for climate change mitigation. The application of urease inhibitor and/or biochar provides the potential for mitigating N_2O emissions. However, the interactive effect of urease inhibitor and biochar on N_2O emissions remains limited. In this study, an incubation experiment was performed to investigate the gradients of urease inhibitor *N*-(*n*-butyl) thiophosphoric triamide (NBPT) (0, 0.08, 0.16, and 0.24%) and biochar additions (0, 2.5, and 5%) on N_2O emissions from urea-fertilised *Camellia oleifera* soils. Results showed that urease inhibitor decreased, but biochar increased cumulative N_2O emissions. No significant interactive effects were observed between urease inhibitor and biochar on the cumulative N_2O emissions, but cumulative N_2O emissions were decreased by NBPT under a 2.5% biochar addition rate. Soil N_2O emission rates were negatively correlated with net ammonification and N mineralisation rates and positively correlated with net nitrification rates. This study indicates that NBPT, with the characteristic of delaying urea hydrolysis, can be better than biochar in mitigating N_2O emissions from urea-fertilised soils of *C. oleifera* plantations.

Keywords: biowaste management; soil ameliorant; global warming; nitrogen transformation; tea-oil tree

Nitrous oxide (N_2O) is a major greenhouse gas with 265-fold trapping heat that of carbon dioxide (CO_2) on a 100-year frame (IPCC 2014). Moreover, soils contribute to approximately 70% of N_2O emissions to the atmosphere and overuse of inorganic and organic nitrogen (N) fertilisers increased the risk of soil N_2O emissions (Fowler et al. 2013, Zaw Oo et al. 2018). Urea is the most commonly used N fertiliser because of its low cost and high N content (Fan et al. 2018). Nevertheless, compared to other inorganic N fertilisers such as ammonium nitrate and ammonium chloride, urea application generally results in higher soil N_2O emissions (Nelissen et al. 2014, Deng et al. 2019a). Therefore, urea-fertilised soils have great potential in mitigating N_2O emissions.

Both urease inhibitor and biochar applications are potential strategies for mitigating soil N_2O emissions (Recio et al. 2020, Aamer et al. 2021). *N*-(*n*-butyl) thiophosphoric triamide (NBPT) is one major urease inhibitor that has been widely used in agriculture

Supported by the National Natural Science Foundation of China, Grants No. 41967017 and 31760223, and by the Science Foundation of Jiangxi Education Department, Grant No. GJJ211934.

https://doi.org/10.17221/112/2022-PSE

for delaying urea hydrolysis and improving the use efficiency of urea. For example, the efficiency of NBPT in reducing soil N_2O emissions was up to 79% (Recio et al. 2020). On the other hand, biochar produces from biomass pyrolysis at high-temperature ranges (250–700 °C) and oxygen-limited conditions. Although biochar effects on greenhouse gas emissions were influenced by various fertiliser management and pyrolysis process (Sosulski et al. 2020a, Sri Shalini et al. 2021), biochar has been widely applied as soil ameliorants for mitigating N_2O emissions and biochar application can reduce up to 33% of N_2O emissions from N-fertilised soils (He et al. 2017).

Tea-oil tree (*Camellia oleifera* Abel.) is an important woody edible oil plant widely cultivated in subtropical China. A field study found that N_2O emitted from N-fertilised soils was three times greater than that emitted from unfertilised soils in *C. oleifera* plantations (Deng et al. 2019b). In addition, the fruit shell of *C. oleifera*, as a biowaste, has not been utilised effectively and has generally been discarded, although it is a suitable feedstock with high carbon content for producing biochar. Previous studies reported that *C. oleifera* fruit shell-derived biochar was an excellent soil ameliorant for mitigating N_2O emissions (Deng et al. 2019b, Gao et al. 2022). For example, biochar and modified biochar can reduce 34.3% and 25.8% N_2O emissions from urea-fertilised soils, respectively (Gao et al. 2022).

Based on the possible interaction of urease inhibitor and biochar, this study hypothesised that urease inhibitor and biochar could synergistically mitigate N_2O emissions from urea-fertilised soils. To test this hypothesis, an incubation experiment was conducted to examine the interactive effect of urease inhibitor and biochar with different rates on N_2O emissions and N transformations from urea-fertilised soils of *C. oleifera* plantation.

MATERIAL AND METHODS

Soil sampling and biochar production. Red soil (Nitisols) samples of the upper layer (0–20 cm) were collected from *C. oleifera* plantation (29.16°N, 115.77°E) in Jiangxi, China, and were sieved to 2 mm. On the other hand, after producing the tested biochar from fruit shells of *C. oleifera* at 450 °C and low oxygen-limited conditions for 1 h, it was sieved to 2 mm. Physicochemical parameters such as NH_4^+-N, nitrate N (NO_3^--N), dissolved organic carbon (DOC) and dissolved organic N, pH, total organic carbon (TOC) and total N (TN) of biochar and soil were analysed following the methods described in Deng et al. (2020).

Experimental design. A two-factor incubation experiment was conducted in three replications with four urease inhibitor rates and three biochar rates to examine the interactive effect of urease inhibitor and biochar on soil N_2O emissions and N transformations. Fresh soils (25 g oven-dry soil) were added in 250 mL conical flasks. Biochar was added at the rate of 0, 2.5, and 5%, corresponding to 0, 25, and 50 g biochar/kg soil, respectively. Afterwards, urea (200 mg N/kg soil) was applied to all soil samples using urea solutions. NBPT was added to the soil using NBPT solutions at the rate of 0, 0.08, 0.16, and 0.24%, corresponding to 0, 0.8, 1.6, and 2.4 g NBPT/kg urea-N, respectively. Subsequently, all treated soil samples were incubated in the dark and aerobic conditions at a water holding capacity of 60% and a temperature of 25 °C (Constant temperature incubator, Zhongyi Guoke Technology, Beijing, China). Soil N_2O emission rates, soil NH_4^+-N, and soil NO_3^--N concentrations at each rate were monitored on 2, 4, 7, 12, 15, 19, 28, 39, 52, 69, 86, and 93 days by analysing a total of 432 independent soil samples (4 urease inhibitor rates × 3 biochar rates × 3 replicates × 12 times). Generally, changes in the emission (amplitude) of greenhouse gases (e.g. N_2O, CO_2) were widely fluctuating in the early stage of the incubation experiment (Deng et al. 2020); thus, gas sampling was not carried out in constant periods (Sosulski et al. 2019, 2020b).

Gas samples were collected following the method described in Deng et al. (2019a). The N_2O concentration was analysed using a gas chromatographic method (Gas chromatograph, Agilent 7890B, Santa Clara, USA). The equation used in the calculation of the cumulative soil N_2O emission was described by Deng et al. (2019b). Net ammonification, net nitrification, and net N mineralisation rates were calculated following the equations described in Deng et al. (2020).

Statistical analyses. A two-way ANOVA test was performed to determine whether the difference in the cumulative soil N_2O emission between urease inhibitor, biochar, and their interactions was significant. Tukey's honestly significant difference (Tukey *HSD* (honestly significant difference)) test was used to compare means of N_2O emissions between rates. The cumulative soil N_2O emission data was normalised (Shapiro-Wilk test) using inverse square root transformation ($y = 1/\sqrt{x}$, x = original data values). Moreover, Pearson correlation analysis was used to examine the relationships between soil N_2O emis-

https://doi.org/10.17221/112/2022-PSE

Table 1. Pairwise correlation coefficients among soil nitrous oxide (N_2O) emission rates and nitrogen transformation rates

Parameter	N_2O emission rate (ng/g/h)
Net ammonification rate (mg NH_4^+-N/kg/day)	–0.239***
Net nitrification rate (mg NO_3^--N/kg/day)	0.458**
Net N mineralisation rate (mg N/kg/day)	–0.221***

**P < 0.01; ***P < 0.001. NH_4^+-N – ammonium nitrogen; NO_3^--N – nitrate nitrogen

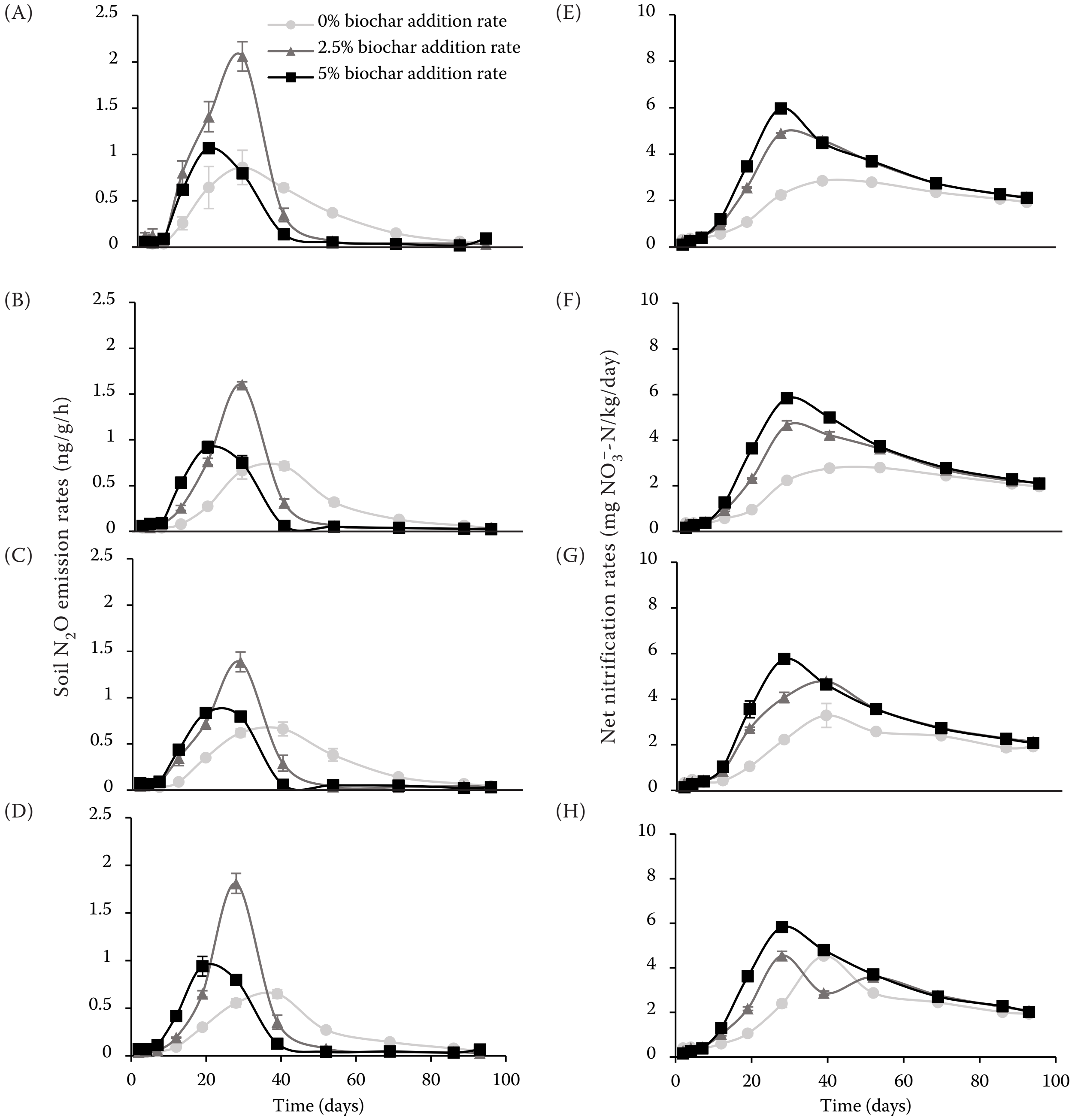

Figure 1. Dynamics of soil nitrous oxide (N_2O) emission rates (A, B, C and D) and net nitrification rates (E, F, G and H) under (A, E) 0%, (B, F) 0.08%, (C, G) 0.16%, or (D, H) 0.24% urease inhibitor addition rate. Means ± standard error. NO_3^--N – nitrate nitrogen

https://doi.org/10.17221/112/2022-PSE

Table 2. Dependence of cumulative soil nitrous oxide (N_2O) emissions on urease inhibitor and biochar

Factor	Cumulative soil N_2O emission		
	df	*F*	*P*
Urease inhibitor	3	14.002	< 0.001
Biochar	2	56.465	< 0.001
Urease inhibitor × biochar	6	1.931	0.117

sion and soil N transformation rates. The SPSS 20.0 software (IBM, Armonk, USA) was used to perform statistical tests. All significant results were set at α = 0.05.

RESULTS AND DISCUSSION

Effects of urease inhibitor on soil N_2O emissions. A positive relationship was observed between soil N_2O emission rates and net nitrification rates (Table 1, $P < 0.01$), indicating that nitrification may be the main source of N_2O emissions. Indeed, N_2O emissions and net nitrification rates showed similar dynamics and peaks (Figure 1). Urease can hydrolyse urea to NH_4^+, but the amount of NH_4^+ produced may provide sufficient N substrates for the nitrification process, thus, resulting in N_2O emissions (Kuypers et al. 2018). Numerous studies have revealed that the application of urease inhibitor can effectively reduce N_2O emissions from urea-fertilised soils (Krol et al. 2020, Recio et al. 2020), which is consistent with our results (Table 2, Figure 2A, $P < 0.001$). In fact, although urease is an enzyme with two nickel (Ni) atoms, only one Ni atom can specifically bind to urea and catalyse urea hydrolysis. Thus, NBPT inhibits the urease activity by binding to both Ni atoms, acting as a tridentate ligand. On the other hand, the transformation of NH_4^+ to NO_3^- *via* the nitrification process is accompanied by the release of two protons, resulting in a decrease in soil pH. As a key factor, soil pH regulates N_2O emissions and decreased soil pH (3.3–8.7) increased N_2O emissions (Wang et al. 2017). However, increasing soil pH with lime amelioration not consistently increased N_2O emission, and pH at 4.3–4.4 and > 6.6 was predicted as optimum pH in N_2O emissions (Sosulski et al. 2016a). Thus, the effects of soil pH on N_2O emissions were complicated which may influence by multiple factors. The decrease in soil pH increased the N_2O emission in acidic soils, which may be related to the reduced N_2O-reductase activity (Aamer et al. 2021). Therefore, suppressed effects of NBPT on mitigation of soil N_2O emissions may be attributed to NBPT first delayed urea hydrolysis and second retarded nitrification and acidification.

Effects of biochar on soil N_2O emissions. Positive effects of biochar on N_2O emissions (Figure 2B and Figure 3) may be induced by the water or acid soluble fraction of the biochar, especially the N (Wang et al. 2020). Indeed, compared to the soil N contents, fruit shells-derived biochar was rich in TN and available N (Table 3). However, higher biochar addition rates showed lower N_2O emissions (Figure 2B, $P < 0.001$), which may relative with biochar addition increasing

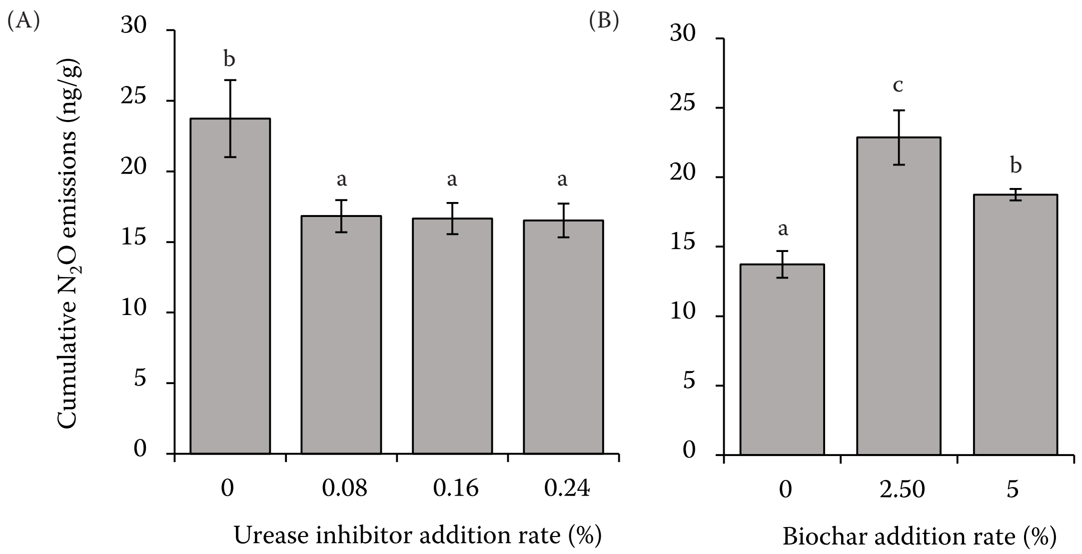

Figure 2. Effects of (A) urease inhibitor or (B) biochar on cumulative soil nitrous oxide (N_2O) emissions. Means ± standard error

https://doi.org/10.17221/112/2022-PSE

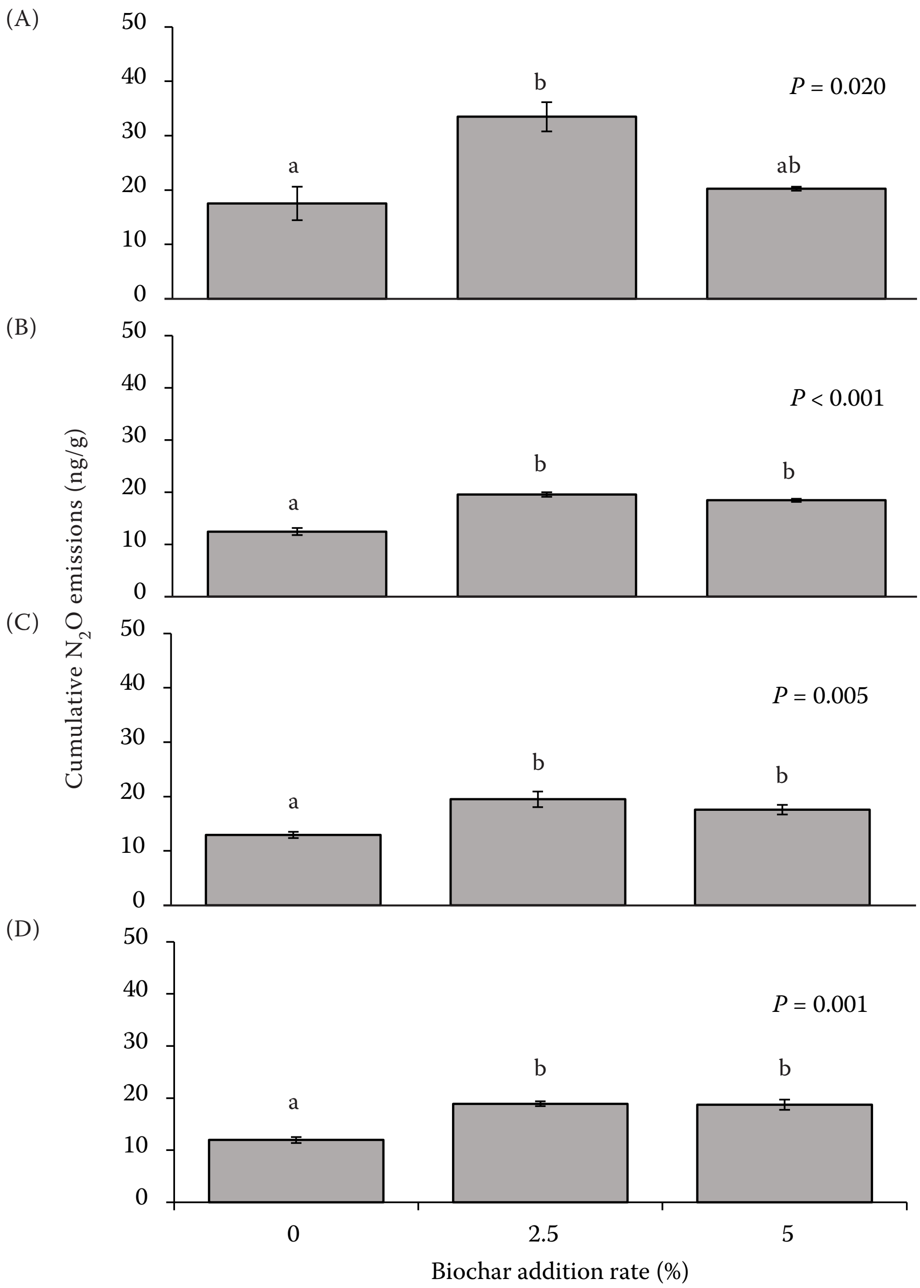

Figure 3. Effects of biochar on cumulative soil nitrous oxide (N_2O) emissions under (A) 0%, (B) 0.08%, (C) 0.16% or (D) 0.24% urease inhibitor addition rate. Means ± standard error

soil pH (Li et al. 2021). Indeed, a higher biochar addition rate can suppress N_2O emissions, which may be relative to the increased copy number of the N_2O reductase gene (*nosZ*) and soil pH (Aamer et al. 2021). In addition, biochar containing polycyclic aromatic hydrocarbons and metal oxides may negatively af-

Table 3. Physicochemical properties of incubated soil and fruit shells-derived biochar

Variable	pH	NH_4^+-N	NO_3^--N	TOC	TN	DOC	DON
		(mg/kg)		(g/kg)			(mg/kg)
Soil	4.45 ± 0.00	8.06 ± 0.07	1.09 ± 0.07	15.96 ± 0.14	0.98 ± 0.04	0.30 ± 0.03	26.67 ± 0.27
Biochar	9.80 ± 0.00	36.54 ± 1.15	4.79 ± 0.13	719.91 ± 19.67	5.54 ± 0.03	1.43 ± 0.08	17.25 ± 0.50

NH_4^+-N – ammonium nitrogen; NO_3^--N – nitrate nitrogen; TOC – total organic carbon; TN – total nitrogen; DOC – dissolved organic carbon; DON – dissolved organic nitrogen. Means ± standard error

https://doi.org/10.17221/112/2022-PSE

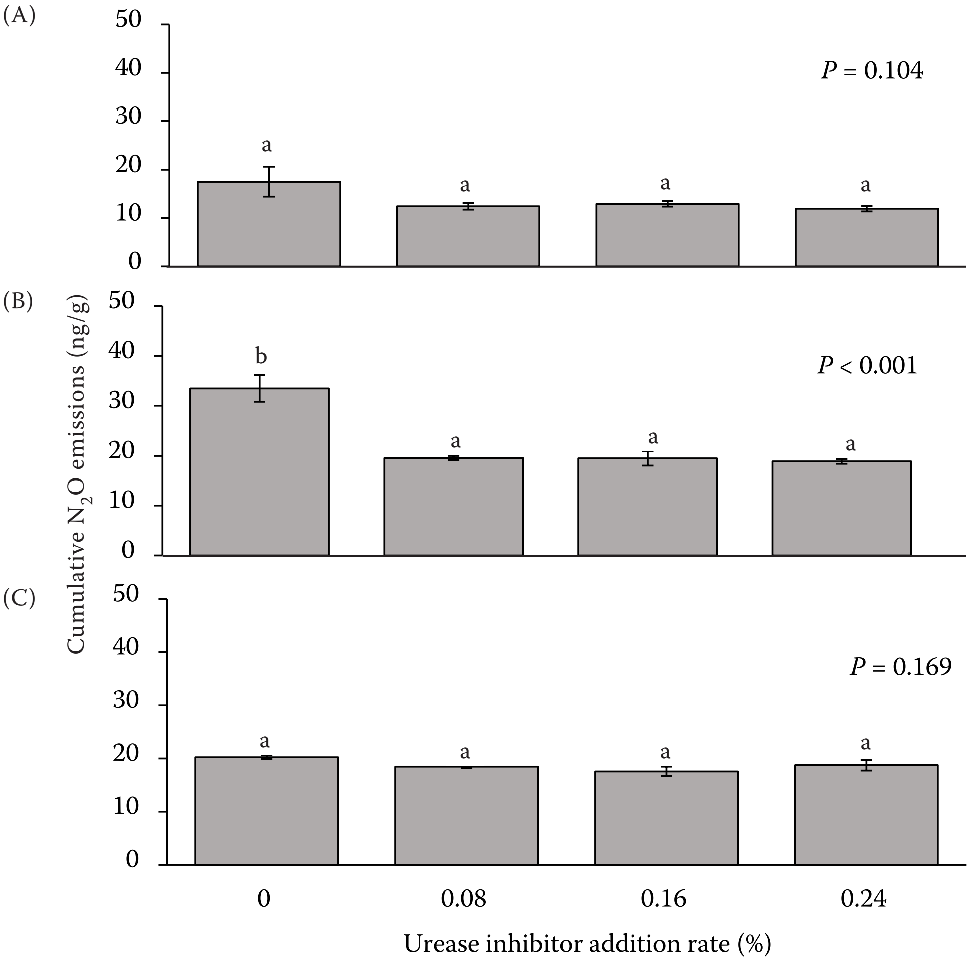

Figure 4. Effects of urease inhibitor on cumulative soil nitrous oxide (N_2O) emissions under (A) 0%, (B) 2.5% or (C) 5% biochar addition rate. Means ± standard error

fect urease activity (Liu et al. 2018), thus reducing the NH_4^+ substrate for N_2O-producing bacteria. On the other hand, soil N_2O emissions were various in different soil types, fertiliser management and water conditions. Although the conditions were similar, biochar effects on soil N_2O emissions were different between our study and a previous study (Gao et al. 2022), which may be relative to the different conditions of water holding capacity (60% *vs*. 70%) and different contents of soil TOC (15.96 *vs*. 6.99 g/kg) and TN (0.98 *vs*. 0.57 g/kg). Additionally, the laboratory incubation method might not effectively reflect natural field conditions, such as various soil temperatures and moisture (Sosulski et al. 2016b, Deng et al. 2019b); thus *in situ* experiment is recommended in the future study.

Interactive effects of urease inhibitor and biochar on soil N_2O emissions. No significant differences in N_2O emissions were observed between NBPT rates under no biochar treatment (Figure 4A, $P = 0.104$). Indeed, a similar result was reported in a previous study (Volpi et al. 2017). However, NBPT reduced the N_2O emission under a 2.5% biochar treatment rate (Figure 4B, $P < 0.001$), while no significant effects of NBPT treatment rates were observed on N_2O emissions under a 5% biochar treatment rate (Figure 4C, $P = 0.169$). This indicates that NBPT can mitigate N_2O emissions caused by biochar application, while higher biochar rates offset these suppressed effects. A meta-analysis showed that NBPT application was more effective in mitigating N_2O emissions in alkaline soil than in acid soil (Fan et al. 2018). Therefore, pH may play a key role in the mitigation of soil N_2O emissions by NBPT. In the future, NBPT should be preferentially considered rather than biochar to mitigate N_2O emissions from urea-fertilised soils in *C. oleifera* plantations.

REFERENCES

Aamer M., Chattha M.B., Mahmood A., Naqve M., Hassan M.U., Shaaban M., Rasul F., Battol M., Rasheed A., Tang H.Y., Chuan Z., Shao J.H., Huang G.Q. (2021): Rice residue-based biochar mitigates N_2O emission from acid red soil. Agronomy, 11: 2462.

https://doi.org/10.17221/112/2022-PSE

Deng B.L., Wang S.L., Xu X.T., Wang H., Hu D.N., Guo X.M., Shi Q.H., Siemann E., Zhang L. (2019a): Effects of biochar and dicyandiamide combination on nitrous oxide emissions from *Camellia oleifera* field soil. Environmental Science and Pollution Research, 26: 4070–4077.

Deng B.L., Fang H.F., Jiang N.F., Feng W.X., Luo L.C., Wang J.W., Wang H., Hu D.N., Guo X.M., Zhang L. (2019b): Biochar is comparable to dicyandiamide in the mitigation of nitrous oxide emissions from *Camellia oleifera* Abel. fields. Forests, 10: 1076.

Deng B.L., Shi Y.Z., Zhang L., Fang H.F., Gao Y., Luo L.C., Feng W.X., Hu X.F., Wan S.Z., Huang W., Guo X.M., Siemann E. (2020): Effects of spent mushroom substrate-derived biochar on soil CO_2 and N_2O emissions depend on pyrolysis temperature. Chemosphere, 246: 125608.

Fan X.P., Yin C., Yan G.C., Cui P.Y., Shen Q., Wang Q., Chen H., Zhang N., Ye M.J., Zhao Y.H., Li T.Q., Liang Y.C. (2018): The contrasting effects of *N*-(*n*-butyl) thiophosphoric triamide (NBPT) on N_2O emissions in arable soils differing in pH are underlain by complex microbial mechanisms. Science of The Total Environment, 642: 155–167.

Fowler D., Coyle M., Skiba U., Sutton M.A., Cape J.N., Reis S., Sheppard L.J., Jenkins A., Grizzetti B., Galloway J.N., Vitousek P., Leach A., Bouwman A.F., Butterbach-Bahl K., Dentener F., Stevenson D., Amann M., Voss M. (2013): The global nitrogen cycle in the twenty-first century. Philosophical Transactions of the Royal Society B: Biological Sciences, 368: 20130164.

Gao Y., Wang B.H., Luo L.C., Deng B.L., Shad N., Hu D.N., Aly H.M., Zhang L. (2022): Effects of hydroxyapatite and modified biochar derived from *Camellia oleifera* fruit shell on soil Cd contamination and N_2O emissions. Industrial Crops and Products, 177: 114476.

He Y.H., Zhou X.H., Jiang L.L., Li M., Du Z.G., Zhou G.Y., Shao J.J., Wang X.H., Xu Z.H., Bai S.H., Wallace H., Xu C.Y. (2017): Effects of biochar application on soil greenhouse gas fluxes: a meta-analysis. GCB – Bioenergy, 9: 743–755.

IPCC (2014): Synthesis Report, Climate Change 2014. Geneva, Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC), 1–164.

Krol D.J., Forrestal P.J., Wall D., Lanigan G.J., Sanz-Gomez J., Richards K.G. (2020): Nitrogen fertilisers with urease inhibitors reduce nitrous oxide and ammonia losses, while retaining yield in temperate grassland. Science of The Total Environment, 725: 138329.

Kuypers M.M.M., Marchant H.K., Kartal B. (2018): The microbial nitrogen-cycling network. Nature Reviews Microbiology, 16: 263–276.

Li S.P., Li Z.L., Feng X., Zhou F.W., Wang J.P., Li Y. (2021): Effects of biochar additions on the soil chemical properties, bacterial community structure and rape growth in an acid purple soil. Plant, Soil and Environment, 67: 121–129.

Liu Y., Dai Q.Y., Jin X.Q., Dong X.D., Peng J., Wu M., Liang N., Pan B., Xing B.S. (2018): Negative impacts of biochars on urease activity: high pH, heavy metals, polycyclic aromatic hydrocarbons, or free radicals? Environmental Science and Technology, 52: 12740–12747.

Nelissen V., Saha B.K., Ruysschaert G., Boeckx P. (2014): Effect of different biochar and fertilizer types on N_2O and NO emissions. Soil Biology and Biochemistry, 70: 244–255.

Recio J., Montoya M., Ginés C., Sanz-Cobena A., Vallejo A., Alvarez J.M. (2020): Joint mitigation of NH_3 and N_2O emissions by using two synthetic inhibitors in an irrigated cropping soil. Geoderma, 373: 114423.

Sosulski T., Szara E., Stepien W., Szymanska M. (2016a): Impact of liming management on N_2O emissions from arable soils in three long-term fertilization experiments in Central Poland. Fresenius Environmental Bulletin, 25: 6111–6119.

Sosulski T., Szara E., Stępień W., Rutkowska B. (2016b): The influence of mineral fertilization and legumes cultivation on the N_2O soil emissions. Plant, Soil and Environment, 61: 529–536.

Sosulski T., Szara E., Szymańska M., Stępień W., Rutkowska B., Szulc W. (2019): Soil N_2O emissions under conventional tillage conditions and from forest soil. Soil and Tillage Research, 190: 86–91.

Sosulski T., Stępień W., Wąs A., Szymańska M. (2020a): N_2O and CO_2 emissions from bare soil: effect of fertilizer management. Agriculture, 10: 602.

Sosulski T., Szymańska M., Szara E. (2020b): CO_2 emissions from soil under fodder maize cultivation. Agronomy, 10: 1087.

Sri Shalini S., Palanivelu K., Ramachandran A., Vijaya R. (2021): Biochar from biomass waste as a renewable carbon material for climate change mitigation in reducing greenhouse gas emissions – a review. Biomass Conversion and Biorefinery, 11: 2247–2267.

Volpi I., Laville P., Bonari E., Nasso N.N.O.D., Bosco S. (2017): Improving the management of mineral fertilizers for nitrous oxide mitigation: the effect of nitrogen fertilizer type, urease and nitrification inhibitors in two different textured soils. Geoderma, 307: 181–188.

Wang Y.J., Guo J.H., Vogt R.D., Mulder J., Wang J.G., Zhang X.S. (2017): Soil pH as the chief modifier for regional nitrous oxide emissions: new evidence and implications for global estimates and mitigation. Global Change Biology, 24: e617–e626.

Wang L., Yang K., Gao C.C., Zhu L.Z. (2020): Effect and mechanism of biochar on CO_2 and N_2O emissions under different nitrogen fertilization gradient from an acidic soil. Science of The Total Environment, 747: 141265.

Zaw Oo A., Gonai T., Sudo S., Thuzar Win K., Shibata A. (2018): Surface application of fertilizers and residue biochar on N_2O emission from Japanese pear orchard soil. Plant, Soil and Environment, 64: 597–604.

Received: May 31, 2022
Accepted: August 29, 2022
Published online: September 13, 2022