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DOES PLANTAIN REDUCE N_2O EMISSIONS FROM A PASTURE SOIL PREVIOUSLY EXPOSED TO ELEVATED ATMOSPHERIC CO_2?

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Abstract

We tested the legacy effect of elevated atmospheric carbon dioxide (eCO_2) on nitrous oxide (N_2O) emissions from a pasture soil previously exposed to long-term CO_2 enrichment and assessed the efficacy of plantain (*Plantago lanceolata* L.) to reduce N_2O emissions from the pasture soil. Soils were sampled from the New Zealand grassland Free Air CO_2 Enrichment (NZ-FACE) site ten months after the cessation of CO_2 enrichment. The soils were transferred into mesocosms/columns and monocultures of plantain and perennial ryegrass (*Lolium perenne* L.) were established in a glasshouse. Periodic measurements of soil N_2O emissions were carried out after urine application, and comparisons were made between plant species under eCO_2 and ambient CO_2 (aCO_2) soils. We found greater ($P < 0.001$) N_2O production from the eCO_2 soil. Plantain reduced N_2O emissions by 39% ($P < 0.001$) across all the treatments. Our study shows that the effect of eCO_2 on soil microbiome (and hence N_2O production) is long-lasting. It also highlights the efficacy of plantain as an N_2O mitigation strategy on pasture soils under future atmospheric CO_2 levels.

Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas (GHG) that has also been linked to stratospheric ozone depletion (Ravishankara *et al.*, 2009; IPCC, 2014). It is largely produced on grazed pasture soils because urinary nitrogen (N) loading rate often exceeds pasture N requirement (Selbie et al., 2015). In fact, grazed pasture soils have been identified as the single largest source (54%) of agricultural N_2O emissions (Dangal *et al.*, 2019). Therefore, understanding what influences N_2O emissions from pasture soils under future climate change scenarios is important as this would aid in the adoption of effective farm management strategies to limit GHG emissions.

Elevated atmospheric carbon dioxide (eCO_2) increases N_2O emissions from agricultural soils by modifying the interactions between plants, soils and microbes (Du *et al.*, 2022). Because CO_2 is a raw material for photosynthesis, eCO_2 directly stimulates photosynthesis which in turn often leads to greater rhizodeposition (Kim *et al.*, 2001; Allard *et al.*, 2005). The greater rhizodeposition under eCO_2 increases microbial activity which supports N_2O production processes such as nitrification and denitrification, especially under the high soil N levels prevalent on grazed pasture soils (Zhong *et al.*, 2015; Zhong *et al.*, 2018). Moreover, changes is soil moisture content via greater water use efficiency under eCO_2 can also promote N_2O emissions (Butterbach-Bahl and Dannenmann, 2011).

Plantain (*Plantago lanceolata* L.) has been identified as an effective strategy to limit N losses from pasture soils (Dietz *et al.*, 2013; Luo *et al.*, 2018; Simon *et al.*, 2019; Gardiner *et al.*, 2020). Reductions in N_2O emissions by plantain is linked to several mechanisms, including decreases in urine N output, biological nitrification inhibition (BNI), increased N immobilization, modification of soil microclimate and plant N uptake (de Klein *et al.*, 2020). There is limited information on the efficacy of plantain to reduce N_2O emissions from pasture soils exposed to eCO_2. The current study was designed to help fill this knowledge gap. Specifically, the study tested the legacy effect of eCO_2 on N_2O emissions from urine on a pasture soil and the efficacy of plantain to reduce N_2O emissions in this scenario. It is worth noting that though the concentration of atmospheric CO_2 is predicted to continue increasing even under the most promising climate change mitigation scenario (IPCC, 2021), eCO_2 legacy studies allow for ongoing ecosystem responses to eCO_2 to be identified and unambiguously linked to CO_2 enrichment (Stiling *et al.*, 2013).

Methods

The New Zealand grassland Free Air CO_2 Enrichment (NZ-FACE) site is located on the west coast of the North Island of New Zealand (40°14′S, 175°16′E). It was established on a long-term pasture composed of a diverse mix of C_3 and C_4 grasses, legumes, and forbs (Edwards et al., 2001). The experiment comprises of three 12 m diameter rings enriched with CO_2 and three additional 12 m diameter rings kept at ambient CO_2 (aCO_2). Additional information, including technical details, soil, grazing and fertility regimes can be found in Newton *et al.* (2001), Ross *et al.* (2013) and Newton *et al.* (2014).

The eCO_2 rings had been enriched with CO_2 (aCO_2 plus 83-111 ppm) for ~23 years. Ten months after the cessation of CO_2 enrichment, soils were sampled (0-10 cm soil depth) from all around each NZ-FACE ring after mowing the site. The collected soils were passed through a 4 mm sieve and transferred into 12 mesocosms/columns per ring (column size: 19 cm internal diameter by 16 cm height). Each column contained about 3.3 kg of soil. Using a split-split plot design consisting of 'CO_2 soil' as the main factor, and plant species and urine as the other factors, a semi-controlled experiment was established in the glasshouse. Each factor had two levels, namely eCO_2 and aCO_2 for the CO_2 soil, plantain (*Plantago lanceolata* L.) and perennial ryegrass (*Lolium perenne* L.) for plant species, and +urine and –urine for the urine treatment/factor. Each treatment combination had nine replicates (i.e. 3 replicates per ring × 3 CO_2 rings). Monocultures of plantain and ryegrass were established on the appropriate columns and all columns were maintained at a uniform soil moisture content. After four months of plant growth, synthetic urine (600 kg N ha^{-1}) was applied to the appropriate columns and N_2O emissions were measured periodically. Supporting soil and plant parameters were also measured to aid in result interpretation.

A repeated measurements model under mixed effects model framework was used to statistically analyse the generated data on R (Version 4.20, The R Foundation). In this model, CO_2 soil, plant species, urine, date and their interactions were the fixed effects, while the random effect was column nested within ring nested within block (from the field experiment).

Key results and implications

The daily N_2O flux was greater on the eCO_2 soil relative to the aCO_2 soil (Figure 1a). We also found a significant 'CO_2 × Urine' effect on the total N_2O emissions, whereby the soils (eCO_2

and aCO_2) differed in N_2O production only when urine was applied (Figure 1b). We found non-significant ($P = 0.640$) eCO_2 legacy effect on herbage production (data not shown). Therefore, the observed legacy effect of eCO_2 on N_2O emissions is likely associated with the long-term effect of eCO_2 on soil microbial processes that support N_2O production. Our observations are consistent with a previous NZ-FACE study (Zhong *et al.*, 2015) which showed that changes in the abundance and activity of soil microbes persisted for eighteen months after CO_2 enrichment was discontinued to conduct technical maintenance of the facility. Zhong *et al.* (2015) also noted that the changes in soil microbiome under eCO_2 were more prominent when livestock excreta were returned to the soil.

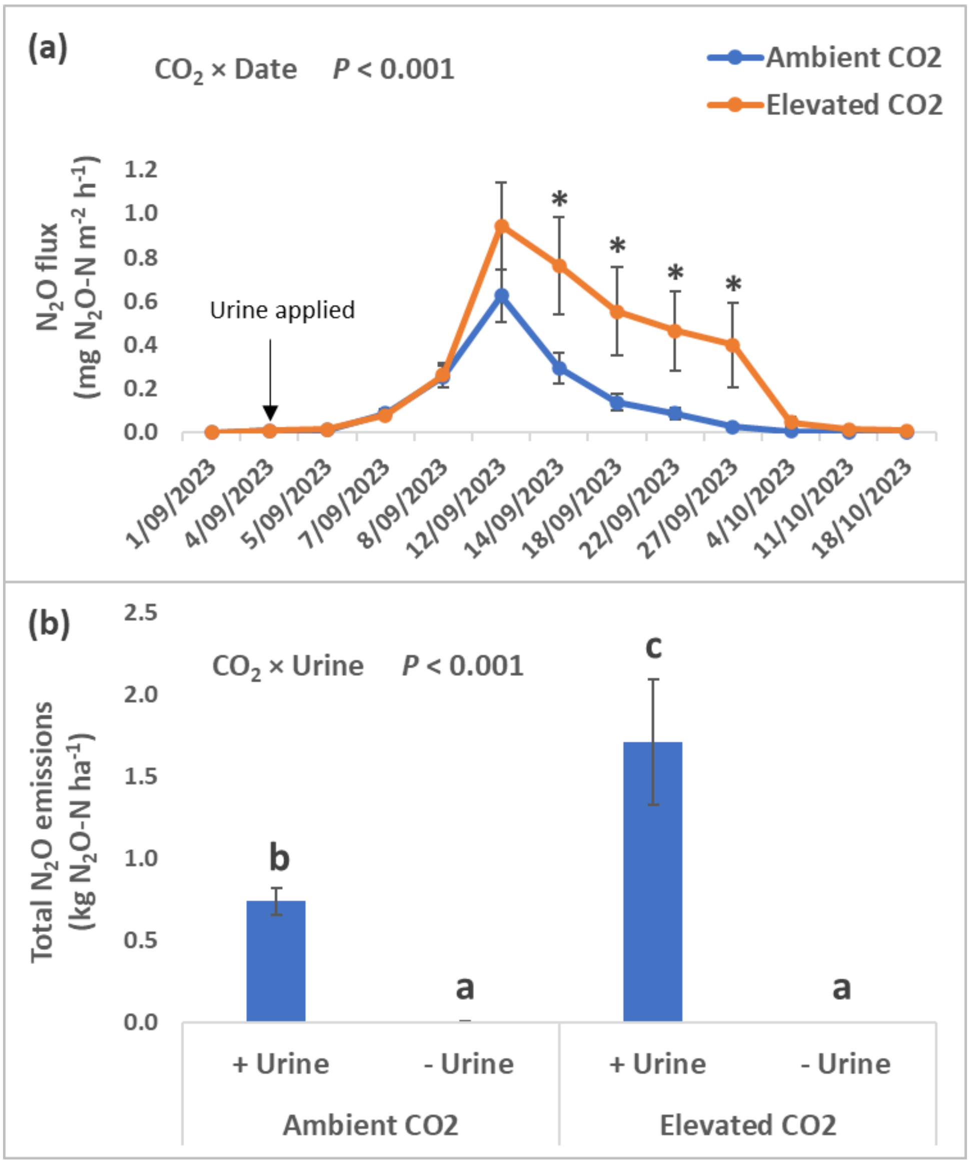

Figure 1: (a) Daily N_2O flux and (b) total N_2O emissions from the treatments. Asterisk indicates significant '$CO_2 \times$ Date' effect. Different letters indicate significant difference across treatment combinations. Error bars are s.e.m. (n = 36 [a] and 18 [b]).

Our results show plantain reduced N_2O emissions by 39% (Figure 2). We found non-significant '$CO_2 \times$ Plant species' ($P = 0.637$) and 'Urine × Plant species' ($P = 0.687$) effects. This indicates the efficacy of plantain to mitigate N_2O emissions was consistent across all the treatments. Our finding agrees with previous research that reported lower N_2O emissions from plantain swards on pasture soils (Luo *et al.*, 2018; Bowatte *et al.*, 2018; Simon *et al.*, 2019). The lower N_2O emissions from plantain was likely due to reduced nitrification as plantain had a negative effect ($P < 0.001$) on the daily and total soil nitrate production (data not shown). The soil pH of the plantain treatment was also less acidic ($P < 0.001$) than the ryegrass treatment (data not shown), which suggests slower soil acidification due to reduced nitrification in the plantain treatment (Gardiner *et al.*, 2020). Therefore, our data suggests BNI was likely the mechanism by which plantain reduced N_2O emissions from the examined soils. The plantain treatment also had greater ($P = 0.03$) herbage dry matter (data not shown). Thus, greater shoot N uptake by plantain could have also contributed to the observed lower N_2O production. We maintained a uniform soil moisture across treatments so it is unlikely that the modification of soil microclimate (moisture content) by plantain (Luo *et al.*, 2018) contributed to the observed lower N_2O emissions. Overall, our study suggests that under future atmospheric CO_2 levels, plantain will remain an effective strategy to limit N_2O emissions from pasture soils. Further research is needed to test this finding under actual eCO_2 conditions.

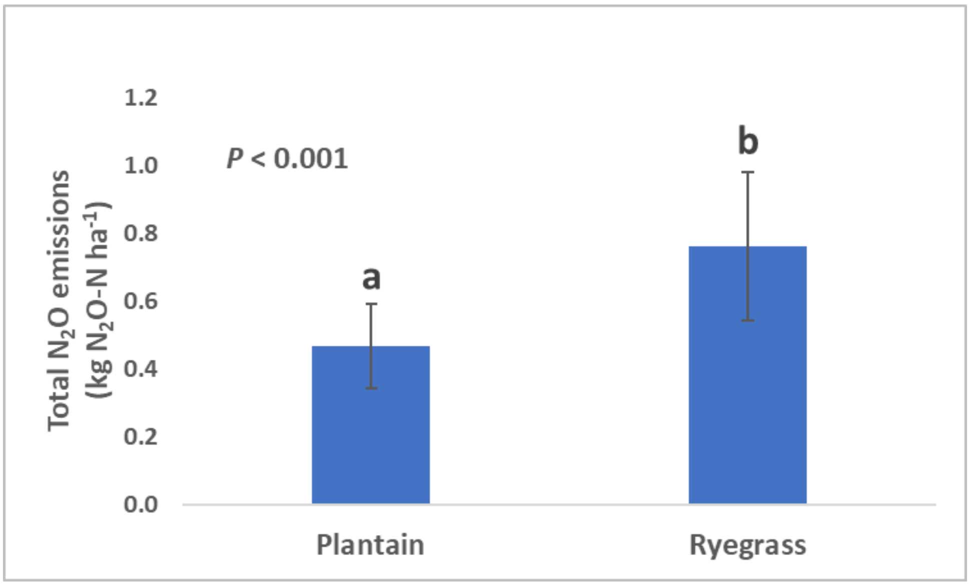

Figure 2: Total N_2O emissions as influenced by plant species. Different letters indicate significant difference between plant species. Error bars are s.e.m. (n = 36).

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References

Allard, V., Newton, P. C. D., Lieffering, M., Soussana, J. F., Carran, R. A. & Matthew, C. (2005). Increased quantity and quality of coarse soil organic matter fraction at elevated CO_2 in a grazed grassland are a consequence of enhanced root growth rate and turnover. *Plant and Soil* 276(1): 49-60.

Bowatte, S., Hoogendoorn, C. J., Newton, P. C. D., Liu, Y., Brock, S. C. & Theobald, P. W. (2018). Grassland plant species and cultivar effects on nitrous oxide emissions after urine application. *Geoderma* 323: 74-82.

Butterbach-Bahl, K. & Dannenmann, M. (2011). Denitrification and associated soil N_2O emissions due to agricultural activities in a changing climate. *Current Opinion in Environmental Sustainability* 3(5): 389-395.

Dangal, S. R. S., Tian, H., Xu, R., Chang, J., Canadell, J. G., Ciais, P., Pan, S., Yang, J. & Zhang, B. (2019). Global nitrous oxide emissions from pasturelands and rangelands: Magnitude, spatiotemporal patterns, and attribution. *Global Biogeochemical Cycles* 33(2): 200-222.

de Klein, C. A. M., van der Weerden, T. J., Luo, J., Cameron, K. C. & Di, H. J. (2020). A review of plant options for mitigating nitrous oxide emissions from pasture-based systems. *New Zealand Journal of Agricultural Research* 63(1): 29-43.

Dietz, M., Machill, S., Hoffmann, H. C. & Schmidtke, K. (2013). Inhibitory effects of Plantago lanceolata L. on soil N mineralization. *Plant and Soil* 368(1): 445-458.

Du, Y., Guo, X., Li, J., Liu, Y., Luo, J., Liang, Y. & Li, T. (2022). Elevated carbon dioxide stimulates nitrous oxide emission in agricultural soils: A global meta-analysis. *Pedosphere* 32(1): 3-14.

Gardiner, C. A., Clough, T. J., Cameron, K. C., Di, H. J. & Edwards, G. R. (2020). Ruminant urine patch nitrification and N2O flux: effects of urine aucubin rate in a laboratory trial. *New Zealand Journal of Agricultural Research* 63(1): 65-72.

IPCC (2014).In *AR5, Climate Change Mitigation. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*(Eds O. Edenhofer, R. Pichs-Madruga, Y. Sokona, E. Farahani, S. Kadner, K. Seyboth, A. Adler, I. Baum, S. Brunner, P. Eickemeier, B. Kriemann, J. Savolainen, S. Schlömer, C. von Stechow, T. Zwickel and J. C. Minx). Cambridge, United Kingdom and New York, NY. Available at http://www.ipcc.ch/report/ar5/wg3/: Cambridge University Press,.

IPCC (2021).Summary for policymakers. In *Climate Change 2021: the Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*, 3-32 (Eds V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. B. R. Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, R. Yu and B. Zhou). Cambridge University Press, Cambridge, United Kingdom and New York, USA.

Kim, H. Y., Lieffering, M., Miura, S., Kobayashi, K. & Okada, M. (2001). Growth and nitrogen uptake of CO_2-enriched rice under field conditions. *The New Phytologist* 150(2): 223-229.

Luo, J., Balvert, S. F., Wise, B., Welten, B., Ledgard, S. F., de Klein, C. A. M., Lindsey, S. & Judge, A. (2018). Using alternative forage species to reduce emissions of the greenhouse gas nitrous oxide from cattle urine deposited onto soil. *Science of The Total Environment* 610-611: 1271-1280.

Newton, P. C. D., Clark, H. & Edwards, G. R. (2001).The effect of elevated atmospheric CO2 on grazed grasslands. In *Structure and Function in Agroecosystem Design and Management*, 297-311: CRC Press.

Newton, P. C. D., Lieffering, M., Parsons, A. J., Brock, S. C., Theobald, P. W., Hunt, C. L., Luo, D. & Hovenden, M. J. (2014). Selective grazing modifies previously anticipated responses of plant community composition to elevated CO_2 in a temperate grassland. *Global Change Biology* 20(1): 158-169.

Ravishankara, A. R., Daniel, J. S. & Portmann, R. W. (2009). Nitrous oxide (N_2O): The dominant ozone-depleting substance emitted in the 21st century. *Science* 326(5949): 123-125.
Ross, D. J., Newton, P. C. D., Tate, K. R. & Luo, D. (2013). Impact of a low level of CO_2 enrichment on soil carbon and nitrogen pools and mineralization rates over ten years in a seasonally dry, grazed pasture. *Soil Biology and Biochemistry* 58: 265-274.
Simon, P. L., de Klein, C. A. M., Worth, W., Rutherford, A. J. & Dieckow, J. (2019). The efficacy of *Plantago lanceolata* for mitigating nitrous oxide emissions from cattle urine patches. *Science of The Total Environment* 691: 430-441.
Stiling, P., Moon, D., Rossi, A., Forkner, R., Hungate, B. A., Day, F. P., Schroeder, R. E. & Drake, B. (2013). Direct and legacy effects of long-term elevated CO2 on fine root growth and plant–insect interactions. *New Phytologist* 200(3): 788-795.
Zhong, L., Bowatte, S., Newton, P. C. D., Hoogendoorn, C. J., Li, F. Y., Wang, Y. & Luo, D. (2015). Soil N cycling processes in a pasture after the cessation of grazing and CO_2 enrichment. *Geoderma* 259-260: 62-70.
Zhong, L., Bowatte, S., Newton, P. C. D., Hoogendoorn, C. J. & Luo, D. (2018). An increased ratio of fungi to bacteria indicates greater potential for N2O production in a grazed grassland exposed to elevated CO2. *Agriculture, Ecosystems & Environment* 254: 111-116.