University of São Paulo
"Luiz de Queiroz" College of Agriculture

Biochar use in agriculture as a nature-based solution: factors influencing N_2O emissions in tropical soils

Fernanda Palmeira Gabetto

Dissertation presented to obtain the degree of Master in Science. Area: Soil and Plant Nutrition

Piracicaba
2023

Fernanda Palmeira Gabetto
Agronomic Engineer

Biochar use in agriculture as a nature-based solution: factors influencing N_2O emissions in tropical soils

versão revisada de acordo com a Resolução CoPGr 6018 de 2011

Advisor:
PhD. **JOÃO LUÍS NUNES CARVALHO**

Dissertation presented to obtain the degree of Master in Science. Area: Soil and Plant Nutrition

Piracicaba
2023

Dados Internacionais de Catalogação na Publicação

DIVISÃO DE BIBLIOTECA - DIBD/ESALQ/USP

Gabetto, Fernanda Palmeira

Biochar use in agriculture as a nature-based solution: factors influencing N2O emissions in tropical soils / Fernanda Palmeira Gabetto. – – versão revisada de acordo com a Resolução CoPGr 6018 de 2011. – – Piracicaba, 2023.

91 p.

Dissertação (Mestrado) – – USP / Escola Superior de Agricultura “Luiz de Queiroz”.

1. Bio-carvão 2. Grupos funcionais de superfície 3. Genes funcionais 4. GHG I. Título

AGRADECIMENTOS

O desenvolvimento deste trabalho jamais seria possível sem a ajuda dessas pessoas, às quais eu dedico os meus mais sinceros agradecimentos.

Ao meu orientador João Luís Nunes Carvalho, por toda a paciência no ensino e pelas contribuições intelectuais, as quais foram fundamentais para que eu pudesse evoluir como profissional e concluir o mestrado de maneira tranquila e satisfeita com o meu trabalho.

À Julia Barra e Sarah Tenelli, pela valiosa contribuição intelectual com seus comentários, sugestões, revisões e suporte, que foram inquestionavelmente importantes para a conclusão desta pesquisa

Ao Leandro Carolino e José Eliseu Júnior, pela disponibilidade em ajudar em tudo o que foi necessário para os experimentos, pelas idas ao campo para coleta de solo e por todas as contribuições e ensinamentos essenciais para o desenvolvimento desta pesquisa. Sem vocês, meu tempo no CNPEM não seria o mesmo.

Aos meus amigos do CNPEM, Aline Oliveira, Sarah Tenelli, Thais Pessoa, Marcelo Pimentel e Marcela Consenza, pela parceria no dia a dia da pesquisa. Trabalhar em um projeto nunca é fácil, mas compartilhar risadas, angústias, certezas e incertezas com vocês tornou esse processo muito mais feliz e leve.

Às minhas avós, Rosa Palmeira e Heloísa Lopes, por todo o amor e carinho dedicados à minha criação. Saibam que me considero uma pessoa muito sortuda por ter tanto de vocês em mim.

Aos meus pais, Láida Palmeira e Mário Gabetto, todo o meu amor pelo apoio constante e fundamental, que me permitiu aproveitar todas as oportunidades que surgiram em meu caminho.

A toda a minha família, tios, primos e padrinhos, por torcerem tanto por mim e serem minha base. Vocês são exemplos de pessoas que sempre levo comigo e que me fazem lembrar que nunca estou sozinha.

Aos colegas e professores do Programa de Pós-Graduação em Solos e Nutrição de Plantas da ESALQ/USP. O caminho até o mestrado não foi simples, mas o convívio com vocês na universidade tornou tudo extremamente proveitoso e enriquecedor para minha experiência profissional e acadêmica.

A toda equipe do CNPEM que direta ou indiretamente contribuiu com este trabalho, em especial à Joice Gomes e Mathias Strauss do LNNANO pela imensa disponibilidade e ajuda nas análises de caracterização química do biochar. Aos membros do LNBR Aline Tieppo, Juliana

Velasco e Luciana Fender pela ajuda e orientação na realização das análises de microbiologia; e a Lucia Wolf, Karina Pontes e Daniele Santoro pela assistência nas análises de gases.

Aos membros do grupo SOHMA, Victória, Marta, Larissa, Bia, Bruna e Borba, pelas conversas e companhia durante minhas idas à ESALQ.

Aos membros do no Instituto Agronômico de Campinas (IAC) que me ajudaram nas com as coletas de solo, análises de nitrogênio e de gases realizadas no Instituto Agronômico de Campinas (IAC), em especial ao Gabriel, Bruna Oliveira e Maria Carolina pela contribuição durante o processo.

A todos os amigos que fiz em Barão Geraldo, em especial Thales, Iza, Dalton, Peixe, Ste, Clone, Pan, e Isa. Se hoje posso chamar esse lugar de casa, é devido à presença e companhia de vocês. Obrigada por dividirem o dia-a-dia, muitas vezes mesmo que corrido, comigo.

A todas as pessoas que tenho sorte de ter a amizade, Bruna, Bruno, Júlia, Laís, Lari e Gabi. Sempre serei grata por ter encontrado vocês e vocês terem me encontrado em um mundo onde é fácil a gente se perder. Amo vocês

Aos meus colegas e amigos da Rural, Gabriel, Ana, Andressa, Amanda, Cyndi, Diego, Júlia, Caio, Mel, Durval, e Anthony. Apesar da distância ser maior agora, a certeza de que estarei sempre torcendo pelo sucesso de vocês permanece. Obrigada por serem pessoas incríveis e por fazerem parte da minha vida.

À FAPESP, Fundação de Amparo à Pesquisa do Estado de São Paulo, pela concessão da bolsa de treinamento técnico (processo 21/02691-7) vinculada ao projeto FAPESP (processo 16/50403-2); e a bolsa de mestrado (processo 21/11995-0), que tornaram possível minha dedicação exclusiva ao desenvolvimento deste projeto.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

Muito obrigada a todos.

EPIGRAPH

"True pleasure lies not in discovering truth,
but in searching for it."

Leon Tolstoy, Anna Karenina

SUMMARY

RESUMO

Uso de biochar na agricultura como uma solução baseada na natureza: fatores influenciando as emissões de N_2O em solos tropicais

A aplicação de biochar no solo tem ganhado atenção devido aos seus benefícios associados ao sequestro de carbono (C) do solo e à mitigação das emissões de óxido nitroso (N_2O). Embora o efeito de redução da emissão de N_2O em resposta ao uso de biochar tenha sido observado em diversos estudos, a explição da causa e efeito continua desafiadora. Além dessa lacuna no conhecimento, há uma escassez de dados obtidos em ambientes tropicais. Desta forma, este estudo foi desenvolvido para avaliar o efeito da aplicação de biochar nas emissões de N_2O do solo em condições tropicais, e como diferentes fatores podem influenciar esta resposta. O primeiro experimento foi realizado para avaliar os efeitos da conversão da palha de cana-de-açúcar em biochar, comparando solos cobertos por palha com a aplicação de duas doses (5 e 10 Mg ha^{-1}) de biochar de palha. Em adição, se buscou desvendar como o biochar pode afetar estas emissões por meio da quantificação de genes funcionais relacionados ao ciclo do N (AOA, AOB, *nirK*, *nirS*, *nosZ*). A aplicação de biochar no solo diminuiu as emissões de N_2O em 73% em comparação com solos cobertos com palha. No entanto, esta redução foi relacionada ao aumento das emissões causado pela presença da palha, uma vez que a aplicação de biochar teve emissões semelhantes ao tratamento com uso de apenas fertilizante nitrogenado. As emissões de N_2O sob cobertos com palha teve uma forte interação positiva com a abundância relativa de AOB, que pode ser fonte significativa de N_2O pela nitrificação em solos tropicais. No entanto, a aplicação de biochar nas doses 5 e 10 Mg ha^{-1} demonstrou ser insuficiente para a mitigação de N_2O, portanto uma dose de 20 Mg ha^{-1} foi adotada no experimento seguinte. No segundo experimento, buscou-se determinar a influência de diferentes tipos de biochar produzidos a partir da palha (PB) e bagaço (BB) da cana-de-açúcar (*Saccharum* spp.), e resíduos de *Pinus* spp. (PB) e *Eucalyptus* spp. (EB). Para uma caracterização físico-química mais precisa, as amostras de foram analisadas por microscopia eletrônica de varredura (MEV), espectroscopia de raios-X por energia dispersiva (EDS) e espectroscopia de fotoelétrons excitados por raios X (XPS). Neste experimento, a capacidade do biochar em suprimir as emissões de N_2O derivadas do uso de fertilizantes nitrogenados foi confirmada. Porém a magnitude dessa capacidade de mitigação variou com a biomassa da matéria-prima, onde SB, BB, PB, EB diminuiram as emissões de N_2O do solo em 50, 35, 35 e 25%, respectivamente. Entre os tratamentos avaliados, apenas SB expressou uma maior capacidade de reduzir as emissões de N_2O do que EB, que também apresentou uma maior proporção de grupos funcionais hidroxila/éter (C−O) em sua superfície. Além disto, biochars produzidos de resíduos florestais apresentaram maiores teores de C em sua superfície, resultando em maiores níveis de C no solo. A aplicação de PB foi a melhor opção entre as biomassas florestais, pois reduziu as emissões de N_2O do solo para níveis semelhantes a SB e BB, ao mesmo tempo em que promoveu maior aporte de C no solo. Com isto, o uso de biochar para aplicação no solo pode ser considerado uma relação ganha-ganha, uma vez que aumenta os estoques de C do solo enquanto diminui as emissões de N_2O. No entanto, a magnitude dessa resposta sob condições tropicais depende da matéria prima utilizada e dose de aplicação do biochar. O presente trabalho contribui para elucidar os efeitos da aplicação de biochar no solo nas emissões de N_2O em ambientes tropicais e fornece resultados à futuros projetos que visam incluir a prática como uma solução baseada na natureza.

Palavras-chave: Bio-carvão, Grupos funcionais de superfície, Genes funcionais, GEE, Estoque de C do solo

ABSTRACT

Biochar use in agriculture as a nature-based solution: factors influencing N_2O emissions in tropical soils

The use of biochar as a soil amendment has recently gained attention due to its benefits associated with soil carbon (C) sequestration and mitigation of nitrous oxide (N_2O) emissions. Although several studies have observed soil N_2O emissions decrease in response to biochar application, explaining the cause-and-effect relationship remains challenging. In addition to this knowledge gap, there is a shortage of data obtained in tropical environments. In this context, this study was developed to assess the effect of biochar application on soil N_2O emissions in tropical conditions and how different factors can influence this response. Therefore, the first experiment was conducted to evaluate the effects of converting sugarcane straw into biochar, comparing straw-covered soils with the two application rates (5 and 10 Mg ha^{-1}) of straw-based biochar. In addition, we also focused on unraveling how biochar might affect these emissions through the quantification of N-related functional genes (AOA, AOB, *nirK*, *nirS*, *nosZ*). Our results revealed that applying sugarcane straw-based biochar to the soil decreased N_2O emissions by 73% compared to straw-covered soils. However, this reduction was due to the N_2O emissions increase caused by the straw presence, as the biochar application at both 5 and 10 Mg ha^{-1} rates exhibited similar results to the application of N fertilizer alone. The rise in N_2O emissions under soils covered with straw had a strong positive interaction with the AOB relative abundance, which seems to be a significant N_2O source from nitrification under tropical soils. Nevertheless, the biochar application rates of 5 and 10 Mg ha^{-1} appeared to be insufficient for N_2O mitigation; therefore, a 20 Mg ha^{-1} rate was adopted for the following experiment. In the second experiment, we sought to determine the influence of different types of biochar produced from sugarcane (*Saccharum* spp.) straw (SB) and bagasse (BB), together with *Pinus* spp. and *Eucalyptus* spp. residues on soil N_2O emissions. For a more precise physicochemical characterization, the different types of biochar were also examined through scanning electron microscopy (SEM), dispersive X-ray spectroscopy (EDS), and X-ray photoelectron spectroscopy (XPS). In this experiment, we observed the biochar capacity to suppress N_2O emissions driven by N fertilizer use. Our results showed that the magnitude of this mitigation capacity varied with the feedstock biomass, where SB, BB, PB, and EB decreased soil N_2O emissions by 50, 35, 35, and 25%, respectively. Among the evaluated treatments, only SB expressed a higher capacity to decrease N_2O emissions than EB, which also had the highest share of hydroxyl/ether (C−O) functional groups on its surface. Furthermore, biochars from forestry residues had more C in its surface composition, resulting in higher soil C contents. The application of PB was the best option among the forestry biomasses, as it decreased soil N_2O emissions to levels similar to SB and BB while promoting higher soil C input. Our results validate that the biochar use as a soil amendment can be considered a win-win strategy, as it enhances soil C stocks while decreasing N_2O emissions. However, the magnitude of this response relies on the biochar feedstock material and application rate under tropical conditions. Our study contributes to elucidating the effects of biochar application to soil on N_2O emissions in tropical environments and provides data for future projects that aim to include the practice as a nature-based solution.

Keywords: Black carbon, Surface functional groups, Functional genes, GHG, Soil carbon stock

1. GENERAL INTRODUCTION

The recent report published by the Intergovernmental Panel on Climate Change (IPCC, 2021) points out that the rise in atmospheric concentrations of greenhouse gases (GHG) due to anthropogenic activities is the main driver of global warming. In an effort to mitigate climate change, the Paris Agreement was reached in 2015 to limit the average temperature increase to 2°C by the end of the 21st century. For this, 153 countries have submitted their nationally determined contributions (NDCs) documents, compromising to establish and achieve sustainable long-term targets to lower GHG emissions in the atmosphere (Meinshausen et al., 2022). In NDCs last update of Brazil in the Glasgow Climate Pact 2021, the country committed to reducing 50 % of its emissions by 2030 compared with 2005 levels (UNFCCC, 2022). Inside the NDCs, 66% of the parties included nature-based solutions (NBS) to help achieve net-zero targets. (Seddon et al., 2019). Nature-based solutions can be defined as solutions inspired by nature, which provide environmental, social, and economic benefits, enhances biodiversity, and supports ecosystem services. Hence, NBS projects include practices promoting carbon neutrality and reducing GHG emissions.

Brazil is the 6th major contributor of GHG emissions worldwide (IPCC, 2021), and consequently, significant efforts to mitigate domestic emissions are necessary to achieve the proposed goals. Agriculture accounts for 27% of total national GHG emissions in Brazil and is responsible for 87% of total N_2O emissions. (MCTI, 2020) Moreover, national N_2O emissions increased by 120% between 1980-2016 (Tian et al., 2020). N_2O is a GHG with a global warming potential 273 times higher than CO_2, with a significant persistence time in the atmosphere of 109 years (Foster et al., 2021). Although soil N_2O emissions occur under natural conditions, anthropogenic activities have intensified this process (Hirsch et al., 2006). For this reason, modern agriculture represents the main anthropogenic source of N_2O since it relies on the application of reactive forms of N to attend to the nutritional demands of crops (Montzka et al., 2011).

Mineral N forms are supplied to the soil by N fertilizers (synthetic and organic), which increases the concentrations of available N that can be lost through nitrification and denitrification, two of the main microbial pathways related to N_2O production (Smith et al., 2019). These routes are influenced by specific microorganisms involved in the N biogeochemical cycle in soil. In the nitrification pathway, a critical control step is the ammonium (NH_4^+) oxidation to hydroxylamine by ammonia-oxidizing bacteria (AOB) and archaea (AOA) (Stein,

2020). During denitrification, N forms such as nitrate (NO_3^-) and nitrite (NO_2^-), are subsequently reduced to form gaseous of nitric oxide (NO), N_2O, which can further be reduced to dinitrogen gas (N_2) (Hallin et al., 2018). The N_2O reduction to N_2 is a sensitive step mediated by microorganisms that encode the *nosZ* gene, being the only known biological sink for the GHG gas (Conthe et al., 2019). The intensity of these transformations is controlled by the organic or inorganic N inputs, available soil C, microbial communities, and abiotic conditions (e.g., soil moisture and oxygen levels) (Hallin et al., 2018).

The recycling of agroindustry residues in agricultural soils has been recognized as a sustainable practice and should be encouraged as part of the circular economy (Carvalho et al., 2021). In general, recycling of organic residues in agricultural soils has the benefit of increasing carbon stocks (Tenelli et al., 2021) but also tends to increase N_2O emissions (Carmo et al., 2013; Gonzaga et al., 2019). Several studies have highlighted that the addition of crop and industrial residues in agricultural soils creates ideal conditions for N_2O formation, such as higher soil moisture, easily degradable C, and intense microbial activity (Carvalho et al., 2017; Gonzaga et al., 2019; Pinheiro et al., 2019). More recently, studies have indicated that biochar addition in soils is outside this rule, representing a strategy in terms of reducing N_2O emissions (Huang et al., 2023; Iqbal et al., 2023; Paustian et al., 2016).

Biochar is a product from pyrolysis (a thermochemical conversion) of lignocellulosic biomasses to produce bio-oil (liquid), biogas (gaseous), and biochar (solid) (Guimarães et al., 2023). Through pyrolysis, organic biomasses are degraded by high temperatures combined with zero or low levels of oxygen (Kambo & Dutta, 2015; Wang & Wang, 2019), where the process conditions, together with the feedstock, will influence the formation of the product, as well as its physicochemical characteristics (Ippolito et al., 2020; Li et al., 2023). Overall, the main destination of the subproducts of the pyrolytic process of lignocellulosic biomass is presented in Figure 1. Bio-oil is mainly used to produce biorenewables, like aviation biofuels and other biochemicals with high-added value products (Deuber et al., 2023). Biogas can be used for power generation, while biochar use is mainly linked to use as a soil amendment. Several reports point out the potential to be used as a soil amendment to increase the environmental sustainability of the supply chain and bring benefits for crop production (Blanco-Canqui, 2021; Das et al., 2022; Lehmann, 2007).

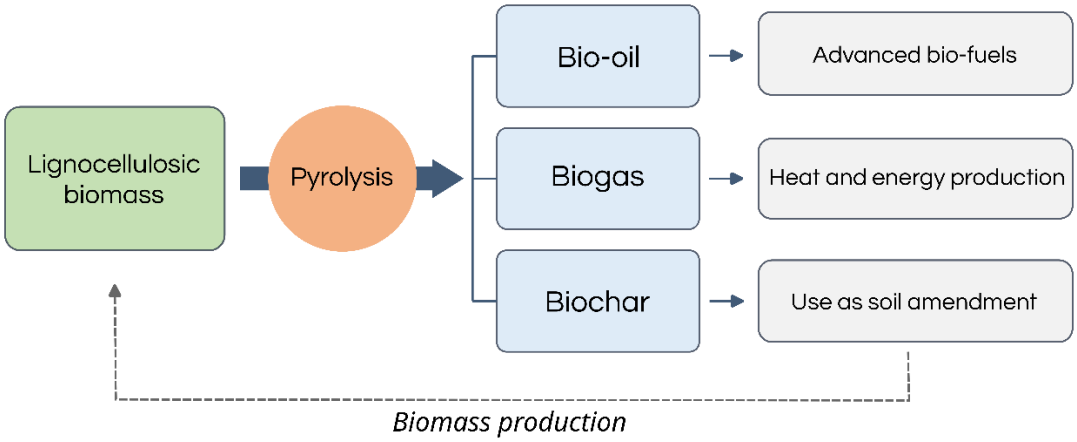

Figure 1. Simplified scheme of the main products resulting from the pyrolysis of lignocellulosic biomass and potential uses of these products.

Biochar is well known for its characteristics, such as high specific surface area, carbonized nature, high porosity, and ash content. Regarding its chemical composition, around 97% of the C found in biochar is in recalcitrant forms due to the pyrolysis process (Wang et al., 2016). The high temperatures of the process transform labile C into more chemically stable aromatic structures, thus increasing the material resistance against microbial degradation in soil (Chacón et al., 2017). Because of this, the C present in the biochar structure persists for more time in the soil, and 90% of the total C can be incorporated into stable organic matter in the long term (Tozzi et al., 2019). Application of biochar to the soil can bring positive economic aspects for agricultural production, such as increased crop productivity and soil health, thereby influencing chemical, physical and biological parameters (Alghamdi, 2018; Jindo et al., 2020; Lehmann et al., 2011). The general worldwide decrease seen for N_2O emissions caused by biochar application (Kaur et al., 2023) can be influenced by a series of abiotic and biotic variables, such as feedstock material, application rates, and environmental factors (e.g., temperature, and soil moisture) (Cayuela et al., 2013; Li et al., 2019; Lyu et al., 2022). This variety of external and internal factors influencing the response of the soil-plant-atmosphere system to biochar application becomes a challenge to foresee the results of this interaction.

Today in Brazil, residual biomass from agriculture and forestry is considered a feasible option to be used as feedstock material for biochar production since it is a low-cost option available in high quantities (Cervi et al., 2021; Roozen, 2015). Among the highest available residual agricultural biomasses found in Brazil, the sugarcane crop stands out as the largest global producer, responsible for 38% of global production (FAO, 2023). Furthermore, the sugarcane

crop management results in large quantities of organic residues (i.e., straw, bagasse, filter cake), as the residual biomass represents two-thirds of all sugarcane biomass produced (Buckeridge et al., 2012). Similarly, forestry residues are among the highest available lignocellulosic biomasses produced in Brazil (IPEA, 2012). From the total area destined for forestry, eucalyptus (*Eucalyptus grandis*) production represents 75% of the area, while pine (*Pinus* spp.) is the second largest cultivated species in 19% of the total area (IBA, 2022). In this scenario, the use of straw, bagasse, and forestry residues are already explored as alternative feedstock materials for bioenergy by current research (Deuber et al., 2023; Guimarães et al., 2023; Negrão et al., 2021). The reuse of these lignocellulosic biomasses might represent a potentially cost-effective option for biochar production. However, using different types of biomass can affect the physicochemical properties of biochar, which can alter its effect on the soil-plant-atmosphere system (Huang et al., 2023; Ippolito et al., 2020).

Despite the significant advances seen in recent years, the mechanisms by which biochar reduces N_2O emissions are poorly understood, and there is no consensus on how it affects N_2O production and consumption in different edaphoclimatic conditions. Some hypotheses have been investigated regarding its possible effects on soil parameters, such as increasing pH and porosity, microbiome interaction, the release of toxic/inhibitory compounds for N-producing microorganisms, and modification in soil mineral N dynamics (Case et al., 2012; Song et al., 2019; Spokas et al., 2010; Xu et al., 2020). Recent studies using molecular biology techniques have evaluated the capacity of biochar to modify or inhibit biological N_2O production routes in agricultural soils (Fan et al., 2020; Liu et al., 2021; Xu et al., 2020). However, albeit the increase in recent inquiries about how biochar influences soil N_2O emissions, these have been conducted mainly in regions under temperate climate conditions, which do not represent the prevailing conditions in the tropics (Agegnehu et al., 2017; Huang et al., 2023).

In this context, the present study was based on the following hypotheses: i) the conversion of sugarcane straw into biochar and its application in agricultural soils represents a strategy to reduce N_2O emissions; ii) biochar from different biomasses has different capacities to reduce soil N_2O emissions; iii) changes in N_2O emissions due biochar application are directly associated with soil microbial community involved in the steps of nitrification and denitrification.

The main objective of this study was to evaluate the impact of the application of different types of biochar on N_2O emissions from soils under tropical conditions and analyze possible variables influencing this response. In order to achieve this objective, the following specific goals were established: a) compare the effects on N_2O emissions of converting sugarcane

straw into biochar with straw-covered soils, evaluating its relationship with soil chemical attributes and functional genes involved the soil N cycle; b) determine the effects of applying biochar derived from different lignocellulosic biomasses available in Brazil (sugarcane straw and bagasse, and pine and eucalyptus biomass on soil N_2O emissions, and correlate with soil and biochar attributes.

References

Agegnehu, G., Srivastava, A. K., & Bird, M. I. (2017). The role of biochar and biochar-compost in improving soil quality and crop performance: A review. *Applied Soil Ecology*, *119*(June), 156–170. https://doi.org/10.1016/j.apsoil.2017.06.008

Alghamdi, A. G. (2018). Biochar as a potential soil additive for improving soil physical properties—a review. *Arabian Journal of Geosciences*, *11*(24). https://doi.org/10.1007/s12517-018-4056-7

Blanco-Canqui, H. (2021). Does biochar improve all soil ecosystem services? *GCB Bioenergy*, *13*(2), 291–304. https://doi.org/10.1111/gcbb.12783

Buckeridge, M. S., de Souza, A. P., Arundale, R. A., Anderson-Teixeira, K. J., & Delucia, E. (2012). Ethanol from sugarcane in Brazil: A "midway" strategy for increasing ethanol production while maximizing environmental benefits. *GCB Bioenergy*, *4*(2), 119–126. https://doi.org/10.1111/j.1757-1707.2011.01122.x

Carvalho, J. L. N., Nogueirol, R. C., Menandro, L. M. S., Bordonal, R. de O., Borges, C. D., Cantarella, H., & Franco, H. C. J. (2017). Agronomic and environmental implications of sugarcane straw removal: a major review. *GCB Bioenergy*, *9*(7), 1181–1195. https://doi.org/10.1111/gcbb.12410

Case, S. D. C., McNamara, N. P., Reay, D. S., & Whitaker, J. (2012). The effect of biochar addition on N 2O and CO 2 emissions from a sandy loam soil - The role of soil aeration. *Soil Biology and Biochemistry*, *51*, 125–134. https://doi.org/10.1016/j.soilbio.2012.03.017

Cayuela, M. L., Sánchez-Monedero, M. A., Roig, A., Hanley, K., Enders, A., & Lehmann, J. (2013). Biochar and denitrification in soils: When, how much and why does biochar reduce N2O emissions? *Scientific Reports*, *3*(Experiment 2), 1–7. https://doi.org/10.1038/srep01732

Cervi, W. R., Lamparelli, R. A. C., Gallo, B. C., de Oliveira Bordonal, R., Seabra, J. E. A., Junginger, M., & van der Hilst, F. (2021). Mapping the environmental and techno-economic potential of biojet fuel production from biomass residues in Brazil. *Biofuels, Bioproducts and Biorefining*, *15*(1), 282–304. https://doi.org/10.1002/bbb.2161

Chacón, F. J., Cayuela, M. L., Roig, A., & Sánchez-Monedero, M. A. (2017). Understanding, measuring and tuning the electrochemical properties of biochar for environmental applications. *Reviews in Environmental Science and Biotechnology*, *16*(4), 695–715. https://doi.org/10.1007/s11157-017-9450-1

Conthe, M., Lycus, P., Arntzen, M., Ramos da Silva, A., Frostegård, Å., Bakken, L. R., Kleerebezem, R., & van Loosdrecht, M. C. M. (2019). Denitrification as an N2O sink. *Water Research*, *151*, 381–387. https://doi.org/10.1016/j.watres.2018.11.087

Das, S., Chatterjee, S., & Rajbanshi, J. (2022). Responses of soil organic carbon to conservation practices including climate-smart agriculture in tropical and subtropical regions: A meta-analysis. *Science of The Total Environment*, *805*, 150428. https://doi.org/10.1016/j.scitotenv.2021.150428

Deuber, R. de S., Bressanin, J. M., Fernandes, D. S., Guimarães, H. R., Chagas, M. F., Bonomi, A., Fregolente, L. V., & Watanabe, M. D. B. (2023). Production of Sustainable Aviation Fuels from Lignocellulosic Residues in Brazil through Hydrothermal Liquefaction: Techno-Economic and Environmental Assessments. *Energies*, *16*(6), 2723. https://doi.org/10.3390/en16062723

Fan, C., Duan, P., Zhang, X., Shen, H., Chen, M., & Xiong, Z. (2020). Mechanisms underlying the mitigation of both N2O and NO emissions with field-aged biochar in an Anthrosol. *Geoderma*, *364*(January), 114178. https://doi.org/10.1016/j.geoderma.2020.114178

FAO, & Food and Agriculture Organization of the United Nations. (2023). *FAOSTAT – Production – Crops: Sugarcane*. http://wwwfaoorg/faostat/en/

Foster, P., Storelvmo, T., Armour, K., Collins, W., Dufresne, J. L., Frame, D., Lunt, D. J., Mauritsen, T., Palmer, M. D., Watanabe, M., Wild, M., & Zhang, H. (2021). The Earth's Energy Budget, Climate Feedbacks, and Climate Sensitivity. In 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, & B. Zhou (Eds.), *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 923–1054). Cambridge University Press.

Gonzaga, L. C., Zotelli, L. do C., de Castro, S. G. Q., de Oliveira, B. G., Bordonal, R. de O., Cantarella, H., & Carvalho, J. L. N. (2019). Implications of Sugarcane Straw Removal for Soil Greenhouse Gas Emissions in São Paulo State, Brazil. *Bioenergy Research*, *12*(4), 843–857. https://doi.org/10.1007/s12155-019-10006-9

Guimarães, H. R., Marcon Bressanin, J., Lopes Motta, I., Ferreira Chagas, M., Colling Klein, B., Bonomi, A., Maciel Filho, R., & Djun Barbosa Watanabe, M. (2023). Decentralization of sustainable aviation fuel production in Brazil through Biomass-to-Liquids routes: A techno-economic and environmental evaluation. *Energy Conversion and Management*, *276*(November 2022). https://doi.org/10.1016/j.enconman.2022.116547

Hirsch, A. I., Michalak, A. M., Bruhwiler, L. M., Peters, W., Dlugokencky, E. J., & Tans, P. P. (2006). Inverse modeling estimates of the global nitrous oxide surface flux from 1998-2001. *Global Biogeochemical Cycles*, *20*(1), 1–17. https://doi.org/10.1029/2004GB002443

Huang, Y., Tao, B., Lal, R., Lorenz, K., Jacinthe, P. A., Shrestha, R. K., Bai, X., Singh, M. P., Lindsey, L. E., & Ren, W. (2023). A global synthesis of biochar's sustainability in climate-smart agriculture - Evidence from field and laboratory experiments. *Renewable and Sustainable Energy Reviews*, *172*(November 2022), 113042. https://doi.org/10.1016/j.rser.2022.113042

IBA - Indústria Brasileira de Árvores. (2022). *Annual Report 2022*. https://www.iba.org/datafiles/publicacoes/relatorios/relatorio-anual-iba2022-compactado.pdf

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

IPEA - Instituto de Pesquisas Aplicadas. (2012). *Diagnóstico dos Resíduos Orgânicos do Setor Agrossilvopastoril e Agroindústrias Associadas*. Relatório de Pesquisa.

Ippolito, J. A., Cui, L., Kammann, C., Wrage-Mönnig, N., Estavillo, J. M., Fuertes-Mendizabal, T., Cayuela, M. L., Sigua, G., Novak, J., Spokas, K., & Borchard, N. (2020). Feedstock choice, pyrolysis temperature and type influence biochar characteristics: a comprehensive meta-data analysis review. *Biochar*, *2*(4), 421–438. https://doi.org/10.1007/s42773-020-00067-x

Iqbal, S., Xu, J., Khan, S., Worthy, F. R., Khan, H. Z., Nadir, S., & Ranjitkar, S. (2023). Regenerative fertilization strategies for climate-smart agriculture: Consequences for greenhouse gas emissions from global drylands. *Journal of Cleaner Production*, *398*(February). https://doi.org/10.1016/j.jclepro.2023.136650

Jindo, K., Audette, Y., Higashikawa, F. S., Silva, C. A., Akashi, K., Mastrolonardo, G., Sánchez-Monedero, M. A., & Mondini, C. (2020). Role of biochar in promoting circular economy in the agriculture sector. Part 1: A review of the biochar roles in soil N, P and K cycles. *Chemical and Biological Technologies in Agriculture*, *7*(1), 1–12. https://doi.org/10.1186/s40538-020-00182-8

Kambo, H. S., & Dutta, A. (2015). A comparative review of biochar and hydrochar in terms of production, physico-chemical properties and applications. *Renewable and Sustainable Energy Reviews*, *45*, 359–378. https://doi.org/10.1016/j.rser.2015.01.050

Kaur, N., Kieffer, C., Ren, W., & Hui, D. (2023). How much is soil nitrous oxide emission reduced with biochar application? An evaluation of meta-analyses. *GCB Bioenergy*, *15*(1), 24–37. https://doi.org/10.1111/gcbb.13003

Lehmann, J. (2007). Bio-energy in the black. *Frontiers in Ecology and the Environment*, *5*(7), 381–387. https://doi.org/10.1890/1540-9295(2007)5[381:BITB]2.0.CO;2

Lehmann, J., Rillig, M. C., Thies, J., Masiello, C. A., Hockaday, W. C., & Crowley, D. (2011). Biochar effects on soil biota - A review. *Soil Biology and Biochemistry*, *43*(9), 1812–1836. https://doi.org/10.1016/j.soilbio.2011.04.022

Li, L., Long, A., Fossum, B., & Kaiser, M. (2023). Effects of pyrolysis temperature and feedstock type on biochar characteristics pertinent to soil carbon and soil health: A meta-analysis. *Soil Use and Management*, *39*(1), 43–52. https://doi.org/10.1111/sum.12848

Li, S., Harris, S., Anandhi, A., & Chen, G. (2019). Predicting biochar properties and functions based on feedstock and pyrolysis temperature: A review and data syntheses. *Journal of Cleaner Production*, *215*, 890–902. https://doi.org/10.1016/j.jclepro.2019.01.106

Liu, X., Shi, Y., Zhang, Q., & Li, G. (2021). Effects of biochar on nitrification and denitrification-mediated N2O emissions and the associated microbial community in an agricultural soil. *Environmental Science and Pollution Research*, *28*(6), 6649–6663. https://doi.org/10.1007/s11356-020-10928-4

Lyu, H., Zhang, H., Chu, M., Zhang, C., Tang, J., Chang, S. X., Mašek, O., & Ok, Y. S. (2022). Biochar affects greenhouse gas emissions in various environments: A critical review. *Land Degradation & Development*, *January 2021*, 1–16. https://doi.org/10.1002/ldr.4405

MCTI - Ministry of Science, T. and I. (2020). *Fourth National Communication of Brazil to the UNFCC*. https://www.gov.br/mcti/pt-br/centrais-de-conteudo/publicacoes-mcti/quarta-comunicacao-nacional-do-brasil-a-unfccc/executive_summary-_4nc_brazil_web.pdf

Meinshausen, M., Lewis, J., McGlade, C., Gütschow, J., Nicholls, Z., Burdon, R., Cozzi, L., & Hackmann, B. (2022). Realization of Paris Agreement pledges may limit warming just below 2 °C. *Nature*, *604*(7905), 304–309. https://doi.org/10.1038/s41586-022-04553-z

Montzka, S. A., Dlugokencky, E. J., & Butler, J. H. (2011). Non-CO 2 greenhouse gases and climate change. *Nature*, *476*(7358), 43–50. https://doi.org/10.1038/nature10322

Negrão, D. R., Grandis, A., Buckeridge, M. S., Rocha, G. J. M., Leal, M. R. L. V., & Driemeier, C. (2021). Inorganics in sugarcane bagasse and straw and their impacts for bioenergy and biorefining: A review. *Renewable and Sustainable Energy Reviews*, *148*(September 2020), 111268. https://doi.org/10.1016/j.rser.2021.111268

Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P., & Smith, P. (2016). Climate-smart soils. *Nature*, *532*(7597), 49–57. https://doi.org/10.1038/nature17174

Pinheiro, P. L., Recous, S., Dietrich, G., Weiler, D. A., Schu, A. L., Bazzo, H. L. S., & Giacomini, S. J. (2019). N2O emission increases with mulch mass in a fertilized sugarcane cropping system. *Biology and Fertility of Soils*, *55*(5), 511–523. https://doi.org/10.1007/s00374-019-01366-7

Roozen, A. (2015). *Availability of sustainable lignocellulosic biomass residues in Brazil for export to the EU* [Master Thesis]. Utrecht University.

Seddon, N., Sengupta, S., García-Espinosa, M., Hauler, I., Herr, D., & Rizvi, A. R. (2019). *Nature-based Solutions in Nationally Determined Contributions: Synthesis and recommendations for enhancing climate ambition and action by 2020*.

Smith, P. , J., Nkem, K., Calvin, D., Campbell, F., Cherubini, G., Grassi, V., V. Korotkov, A. L., Hoang, S., Lwasa, P., McElwee, E., Nkonya, N., Saigusa, J.-F., & Soussana, M. A. T. (2019). *Interlinkages between Desertification, Land Degradation, Food Security and GHG fluxes: 3 synergies, trade-offs and Integrated Response Options. s. In: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems*. Raphael Slade. https://www.ipcc.ch/site/assets/uploads/sites/4/2019/11/09_Chapter-6.pdf

Song, Y., Li, Y., Cai, Y., Fu, S., Luo, Y., Wang, H., Liang, C., Lin, Z., Hu, S., Li, Y., & Chang, S. X. (2019). Biochar decreases soil N2O emissions in Moso bamboo plantations through decreasing labile N concentrations, N-cycling enzyme activities and nitrification/denitrification rates. *Geoderma*, *348*(April), 135–145. https://doi.org/10.1016/j.geoderma.2019.04.025

Spokas, K. A., Baker, J. M., & Reicosky, D. C. (2010). Ethylene: Potential key for biochar amendment impacts. *Plant and Soil*, *333*(1), 443–452. https://doi.org/10.1007/s11104-010-0359-5

Stein, L. Y. (2020). The Long-Term Relationship between Microbial Metabolism and Greenhouse Gases. *Trends in Microbiology*, *28*(6), 500–511. https://doi.org/10.1016/j.tim.2020.01.006

Tian, H., Xu, R., Canadell, J. G., Thompson, R. L., Winiwarter, W., Suntharalingam, P., Davidson, E. A., Ciais, P., Jackson, R. B., Janssens-Maenhout, G., Prather, M. J., Regnier, P., Pan, N., Pan, S., Peters, G. P., Shi, H., Tubiello, F. N., Zaehle, S., Zhou, F., … Yao, Y. (2020). A comprehensive quantification of global nitrous oxide sources and sinks. *Nature*, *586*(7828), 248–256. https://doi.org/10.1038/s41586-020-2780-0

Tozzi, F. V. D. N., Coscione, A. R., Puga, A. P., Carvalho, C. S., Pellegrino Cerri, C. E., & de Andrade, C. A. (2019). Carbon stability and biochar aging process after soil application. *Horticulture International Journal*, *3*(6), 320–329. https://doi.org/10.15406/hij.2019.03.00148

UNFCCC - United Nations Framework Convention on Climate Change. (2022). *Brazil First NDC - Second update*. https://unfccc.int/documents/497364

Wang, J., & Wang, S. (2019). Preparation, modification and environmental application of biochar: A review. *Journal of Cleaner Production*, *227*, 1002–1022. https://doi.org/10.1016/j.jclepro.2019.04.282

Wang, J., Xiong, Z., & Kuzyakov, Y. (2016). Biochar stability in soil: Meta-analysis of decomposition and priming effects. *GCB Bioenergy*, *8*(3), 512–523. https://doi.org/10.1111/GCBB.12266

Xu, X., He, C., Yuan, X., Zhang, Q., Wang, S., Wang, B., Guo, X., & Zhang, L. (2020). Rice straw biochar mitigated more N2O emissions from fertilized paddy soil with higher water content than that derived from ex situ biowaste. *Environmental Pollution*, *263*. https://doi.org/10.1016/j.envpol.2020.114477

2. CONVERSION OF SUGARCANE STRAW TO BIOCHAR: IMPLICATIONS ON N_2O EMISSIONS AND MICROBIAL COMMUNITY

Abstract

The use of sugarcane (*Saccharum* spp.) straw has been considered as a management strategy due to its potential as a feedstock for bioenergy production. One of the by-products from the pyrolysis of sugarcane straw biomass is biochar, a C-rich material that has been studied for its role in suppressing soil greenhouse gas emissions driven by N fertilizers. However, understanding the magnitude of mitigation and how biochar influences soil N_2O emissions remains limited. In this scenario, the focus of this study was to assess the effects of the application of sugarcane straw-based biochar on N_2O emissions from tropical soils. We also explored if the effect of biochar on N_2O formation is associated with changes in the functional genes related to the soil N-cycle. To achieve those goals, a greenhouse pot experiment with sugarcane plants was conducted for 60 days. Treatments consisted of five treatments: control (CTR), N fertilizer (NF), NF + 15 Mg ha^{-1} straw (NF+S); NF + 5 Mg ha^{-1} biochar (NF+B5); and NF + 10 Mg ha^{-1} biochar (NF+B10). Our results showed that N_2O emissions under NF+S soils were 73% higher than those for biochar treatments (NF+B5 and NF+B10). However, biochar did not decrease N_2O emissions driven by N fertilizer application at any level of application rates. Plants grown under NF+S increased biomass production, although this gain was not enough to compensate for gas emissions since N_2O emission intensity from NF+B5 and NF+B10 were 63% and 62% lower, respectively. The straw material also immobilized N-NH_4^+, whereas both biochar treatments had the same effect on soil N-NH_4^+ dynamics as NF. Biochar application increased soil C, where NF+B10 had values 11% higher than NF. Overall, the ammonia-oxidizing bacteria (AOB) relative abundance in soils amended with biochar and straw were higher than in the NF treatment. During the highest emission period the nitrification step was the main microbial pathway process controlling the N_2O fluxes, as pointed out by the strong positive correlation with AOB *amoA* gene seen in the the canonical discriminant analysis. Conversely, denitrification did appears to influence N_2O emissions under tropical soils, as *nirK* and *nirS* did not correlated with N_2O emissions. Biochar application at a 5 Mg ha^{-1} rate increased the *nosZ* relative abundance by the end of the experiment compared with NF+S and CTR. However, the changes in the *nosZ* relative abundance caused by treatments did not decreased N_2O fluxes. Thus, our results suggest that straw-based biochar application is a promising strategy to reduce N_2O emissions compared to sugarcane straw maintained on the soil surface in sugarcane fields.

Keywords**:** straw removal; marker genes; GHG emissions; N dynamic; tropical soils.

2.1. Introduction

To ensure the energy supply demands of an increasing world population (UN DESA, 2022), the use of non-fossil alternatives is a fundamental key to reducing greenhouse gas (GHG) emissions and limiting global warming. Brazil is the second largest biofuel producer in the world, only behind the USA (FAO, 2021), with a production based mostly on sugarcane (*Saccharum* spp.) cultivation (EPE, 2022). As a result of this high demand, Brazil occupies a leading position as a sugarcane producer, with an estimated area of 8.4 million ha utilized for its production (CONAB, 2023). Biofuels made from sugarcane stalk biomass stand out for their sustainability since their use as a gasoline replacement can significantly decrease GHG emissions (Cavalett et al., 2013; Wang et al., 2014).

Another environment-friendly option recently explored is the second-generation biofuels produced from lignocellulosic biomass residues. They represent an alternative to reduce the competition between the energy and food sectors, as the sugarcane stalk can be utilized for both sugar and ethanol production (Dias et al., 2018; Thompson & Meyer, 2013). Among the gases emitted by the sugarcane biofuel supply chain, nitrous oxide (N_2O) is one of the main contributors to the emissions linked with the activity (Pereira et al., 2019). Although N_2O emissions are lower than carbon dioxide (CO_2), this gas is capable of absorbing 273 times more radiation than CO_2, remaining in the atmosphere for over 109 years (Foster et al., 2021). Due to its contribution to global warming, continued increases in N_2O emissions, as seen in the last years, may affect the proposed sustainability goals to limit the increase in the global temperature by 2°C until the end of the century (Meinshausen et al., 2022). It is estimated that approximately 44% of the total GHG emissions from the production of one liter of sugarcane ethanol can be attributed to the use of synthetic or organic nitrogen (N) in agricultural soils (Carvalho et al., 2021). As soils are a natural N_2O source, N inputs from fertilizers influence the magnitude of gas formation, which makes direct emissions from agricultural soils a major contributor to anthropogenic N_2O emissions (FAO, 2021; Tian et al., 2020).

The forms of N added via fertilizers (i.e., ammonium ($N\text{-}NH_4^+$) and nitrate ($N\text{-}NO_3^-$)) are available in the soil solution and can be metabolized by soil microorganisms, thereby intensifying the N_2O formation by biotic processes. Among the many N transformations undergoing in soil, nitrification and denitrification process are considered the main biotic pathways for the N_2O formation (Figure 2) (Stein, 2020). Since these are the major routes for N_2O production, current studies focus on the analysis of microorganisms containing specific functional genes (Harter et al., 2014; Morales et al., 2010). These genes are responsible for

encoding enzymes accountable for catalyzing N transformation processes in the soil. In the nitrification process, the main drivers of N_2O emissions are ammonia-oxidizing archaea (AOA) and bacteria (AOB) containing the *amoA* (Stein, 2019). Similarly, the nitrite (NO_2^-) reduction to nitric oxide (NO) is a key step to denitrification as it transforms mineral N into a gas form by microorganisms containing the copper nitrite reductase (*nirK*) and iron nitrite reductase (*nirS)* genes (Kuypers et al., 2018). In the last steps of denitrification, the N_2O produced by NO reduction can be utilized as an electron receptor by n*osZ*-containing microorganisms, transforming it into dinitrogen gas (N_2) by the enzyme nitrous oxide reductase (Stein & Klotz, 2016). The reduction of N_2O to N_2 is the only known biological sink for N_2O. Thus, the processes that influence this step can lead to changes in the GHG consumption/emission patterns. In agreement with this idea, several reports point out that these genes can respond significantly to the input of organic residues, having its response often linked to the patterns of N_2O formation and consumption (Lourenço et al., 2018; Wan et al., 2023).

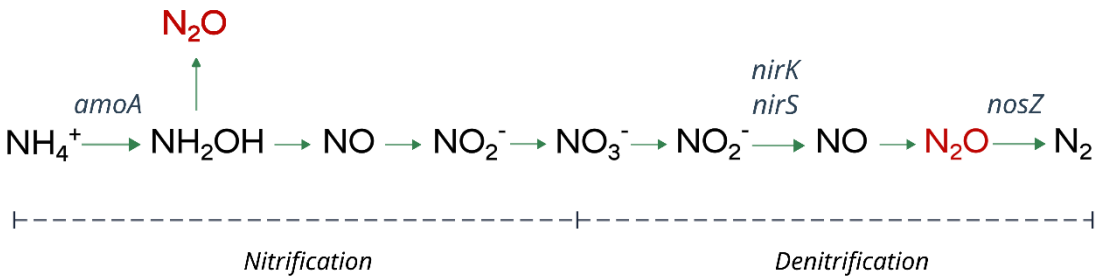

Figure 2. Overview of the N transformations in soil mediated by microorganisms that encode ammonia monooxygenase (*amoA*), nitrite reductase (*nirK* and *nirS*), and nitrous oxide reductase (*nosZ*) enzymes (Brady & Weil, 2016; Stein, 2019).

One residue that has been considered as a feedstock for second-generation bioenergy is sugarcane straw since it is produced in high quantities and can be an economically feasible option (Cervi et al., 2021; Okuno et al., 2019). However, straw-removal operations can increase GHG emissions in the life-cycle analyses of biofuels, which can hamper the sustainability of the product. A possible pathway to decrease GHG emissions associated with this process is the use of biochar as a soil amendment (Lefevebre et al., 2021), as the material is obtained together with bio-oil through the pyrolysis of organic biomasses. In the pyrolisys, the high temperatures and low or zero oxygen levels transforms the organic materials, producing bio-oil, biochar, and bio-gas (Kambo & Dutta, 2015; Kan et al., 2016; Wang & Wang, 2019). Biochar is a carbonized material rich in aromatic carbon (C) that has been utilized for soil application for its benefits

related to C sequestration and N_2O mitigation potential (Blanco-Canqui, 2021). Once applied to the soil, biochar can persist for years, as seen in Terra preta soils, where the material is still present after being added by human activity centuries ago (Kern et al., 2019).

Biochar use as a soil amendment can influence soil N_2O formation by increasing it, lowering, or having neutral effects, as pointed out by several meta-analyses (Borchard et al., 2019; Kaur et al., 2023; Woolf et al., 2021). However, the effects of biochar on N_2O emission mitigation are not always consistent, as demonstrated by the low responses to biochar application in some of the evaluated papers by Kaur et al.(2023). One of the proposed mechanisms by which biochar can influence soil N_2O fluxes is by controlling the soil microbial sources and sink, marked by functional genes (Harter et al., 2014; Zhang et al., 2021). In a scenario where the straw is removed for bioenergy production, the biochar obtained by the thermal conversion process can return to croplands as a management strategy to mitigate N_2O emissions. However, the biochar impacts on N_2O fluxes are dynamic, and there is no consensus on the mechanisms that influence the gas emissions. Furthermore, most studies that evaluated the biochar impact on N_2O emissions have been conducted in temperate climate conditions, thus creating a knowledge gap for tropical environments.

Based on this scenario, we hypothesized that soil application of straw-derived biochar would result in lower N_2O emissions than keeping the straw in its natural form above the soil surface. Therefore, the objectives of this study were to: a) assess the N_2O emission response to the application of sugarcane straw-derived biochar compared with straw-covered soils, b) analyze the interaction between N_2O emissions induced by biochar and straw with changes in N-related functional genes. To the best of our knowledge, this is the first study to evaluate the effects of biochar on the relationship between N_2O emissions and N-related functional genes in Brazil.

2.2. Material and Methods

2.2.1. Experimental design

A 60-day pot experiment with sugarcane plants was conducted in a greenhouse environment to test our hypothesis. Plants were grown in pots with a volume capacity of 9 L (20 cm x 22 cm x 27 cm); each one was filled up with 8.3 kg of dry soil. Pre-sprouted sugarcane seedlings (variety RB 985476) were utilized for propagation. The soil used in the experiment was classified as an Ferrasol (IUSS, 2022) sampled from a commercial sugarcane field (47°19' W, 22°44' S) located in São Paulo State, Brazil. Soil samples were collected from the surface layer (0-

20 cm). Soil samples were collected prior to the beginning of the experiment in order to proceed to obtaing the soil physicochemical characteristics (Table 1). Soil chemical analyses were performed according to Raij et al. (2001), and soil particle-size analysis was done according to Dane & Topp (2020).

Table 1. Physicochemical properties of the soil utilized in the 60-day greenhouse experiment.

Parameters	**Values**
pH ($CaCl_2$)	6.5
Organic Matter (g dm^{-3})	35.7
P (mg dm^{-3})	239.2
S (mg dm^{-3})	8.0
K ($mmol_c$ dm^{-3})	0.9
Ca ($mmol_c$ dm^{-3})	77.9
Mg ($mmol_c$ dm^{-3})	32.2
Al ($mmol_c$ dm^{-3})	< 0.1
H+Al ($mmol_c$ dm^{-3})	13.3
B (mg dm^{-3})	0.2
Cu (mg dm^{-3})	2.65
Fe (mg dm^{-3})	47.0
Mn (mg dm^{-3})	8.32
Zn (mg dm^{-3})	4.07
Sand (g kg^{-1})	474.5
Silt (g kg^{-1})	117.2
Clay (g kg^{-1})	408.2
Soil texture	Clayey

The study was arranged in a completely randomized experimental design with five treatments and five replicates each. The treatments consisted of unfertilized soil (CTR); soil with N fertilizer application (NF); NF with 15 Mg ha^{-1} sugarcane straw application (NF+S); NF with 5 Mg ha^{-1} biochar application (NF+B5); and NF with 10 Mg ha^{-1} biochar application (NF+B10). The straw rate was chosen based on mean straw production in sugarcane fields in Brazil (10-20 Mg ha^{-1}) (Menandro et al., 2017). For the biochar rate of 5 Mg ha^{-1}, a mean production rate of 30-35% was considered for sugarcane straw biochar during pyrolysis (Abbruzzini, 2015; Lefebvre et al., 2020). Based on this, the 10 Mg ha^{-1} rate was chosen to observe the effects of doubling the previous rate, since most studies about the biochar effects on N_2O emissions are performed with biochar inputs higher than 5 Mg ha^{-1}. Ammonium sulfate was applied on the soil surface at a rate

of 2.5 g N kg^{-1} in all treatments with N fertilizer addition at day zero of the experiment. All treatments received 1.6 g P_2O_5 kg^{-1} and 2.5 g K_2O kg^{-1} through single superphosphate and potassium chloride, respectively.

To prepare the NF+B5 and NF+B10 treatments, 21 and 42 g of biochar were thoroughly homogenized with the soil respectively, while 62 g of straw was placed on the soil surface to emulate field conditions. All rates were calculated on a dry soil weight basis. To stabilize microbial activity, the pots were pre-incubated for 13 days prior to the start of the experiment. For that, the soil was added to the pots, and the respective biochar and straw treatments were set. All rates were calculated on a dry soil weight basis. Throughout the experiment, soil water content was maintained at 60% of the water field pore space (WFPS) based on soil moisture measurements using the sensor TEROS 10 (Meter), which was calibrated in advance for the soil in use

2.2.2. Biochar production and physicochemical properties

Biochar was produced with sugarcane straw as feedstock (Bioware, Campinas, Brazil). The feedstock material was left to dry until it reached ~10% moisture, and then it was crushed and sieved into a 5-mesh particle size. With the feedstock material prepared, it was fed to a fluidized bed fast-pyrolysis reactor at a 60 kg h^{-1} rate and 450°C temperature. The chemical composition characteristics of sugarcane straw biochar is shown in Table 2. More details about pyrolysis conditions and biochar characterization analysis can be found in Borges et al. (2020).

Table 2. Chemical properties of sugarcane straw biochar and sugarcane straw.

Parameters	Biochar	Sugarcane Straw
Density (g cm^{-3})	0.1	0.2
pH ($CaCl_2$)	7.5	4.2
CEC ($cmol_c$ kg^{-1})	37	24
C:N	104	97
Ash (g kg^{-1})	340	465
C (g kg^{-1})	519	485
N (g kg^{-1})	5	5
P (g kg^{-1})	2	2
K (g kg^{-1})	7	3
Ca (g kg^{-1})	8	4
Mg (g kg^{-1})	3	1
S (g kg^{-1})	0.4	0.3
B (mg kg^{-1})	12	3
Zn (mg kg^{-1})	67	31
Cu (mg kg^{-1})	30	7
Mn (mg kg^{-1})	479	110
Fe (mg kg^{-1})	9634	2583

CEC: Cation exchange capacity.

2.2.3. GHG sampling and measurements

For gas sampling, mini-PVC static chambers (diameter: 5 cm, height: 15 cm) were placed in each replicate. The chambers were sealed through lids with septa only during the gas sampling period, with air samples being collected from the chamber headspace (117.8 cm^3). Gas samples (20 mL) were collected at 0, 15, and 30 minutes after the lid was closed by using a syringe during the morning period (10:00 h to 12:00 h). A reflux with the syringe was performed prior to sample collection to homogenize the atmosphere within the headspace. Sample collection was perfomed six days a week during the first 14 days, 3 days a week for the following 3 weeks, and 2 times a week in the last four weeks of the experiment. Gas samples were immediately stored in vacuumed Exetainer vials (12 mL; Labco Inc., UK). Samples were injected in a Shimadzu gas chromatograph (GC 2014, Japan), equipped with an electron capture detector (ECD) to quantify N_2O, and a hydrogen flame ionization detector (FID) to quantify CO_2 and CH_4. The column temperature was maintained at 80°C, while ECD and FID temperatures were

set at 325°C and 250°C, respectively. Daily GHG fluxes were calculated through linear extrapolation methodology based on Jantalia et al. (2008) using the following equation:

$$N_2O_{fluxes} = P \times V \times T \times \left(\frac{\Delta C}{\Delta t}\right) \times A^{-1} \times \frac{m}{V_m}$$

where P is the atmospheric pressure (atm), V is the empty headspace volume (L), T is the air temperature (K), $\Delta C/\Delta t$ is the concentration change rate inside the chamber during the period during which it is closed, A is the soil surface area covered by the chamber (m^2), and m/V_m is the transformation constant of N_2O into N which considers the molecular weight of N_2O (m) by the gas molar volume (V_m). Based on the daily GHG emissions previously calculated, the cumulated gas emissions were estimated by linear interpolation between two sequential samplings over 60 days.

2.2.4. Soil total C, total N, and mineral N analysis

Soil mineral N concentrations were obtained from soil samples collected at a depth of 5 cm from the pot surface from all experimental units, on four representative sampling days (3, 11, 39, and 60 after N fertilizer application). For this, 20 grams of soil were stored in a plastic bag and then maintained in a fridge at -5°C until further use. The samples were then extracted by using 50 mL of a 2 M KCl solution. From the extracted solution, ammonium ($N\text{-}NH_4^+$) and nitrate ($N\text{-}NO_3^-$) were quantified through the steam distillation method (Keeney & Nelson, 2015). In order to convert the obtained N concentrations to a dry-weight basis (mg kg^{-1}), soil samples (10 g) were weighed before and after being air dried at 105°C until reaching a constant weight to determine the soil moisture content. Soil total C and N concentrations were determined from samples collected at the end of the experiment. Soil samples were air-dried, and then crushed and passed through a 150 mm sieve before C and N were quantified through dry combustion.The quantification was performed through dry combustion utilizing the TruSpec CHN elemental analyzer (LECO - St. Joseph, MI, USA).

2.2.5. Soil DNA extraction and real-time quantitative PCR

Soil sampling for microbiological analysis was taken at a depth of 3 cm from the pot surface during four periods of time (on days 3, 11, 39, and 60 after N fertilizer application). The

amount of soil collected in each pot was enough to fill a 2 ml Eppendorf tube, and these soil samples were stored at a temperature of -80°C until further use. Afterward, soil subsamples of 0.300 mg had their DNA extracted with the use of the FastDNA SPIN Kit for Soil (MP Biomedicals - Santa Ana, CA, USA), following the instructions of the manufacturer. The quality of the extracted DNA was checked through agarose gel electrophoresis 0.8% (w/v), and concentrations of samples were determined by the absorbance reading at ratios 230, 260, and 280 nm (NanoDrop 2000c, ThermoFisher Scientific, Waltham, MA, USA).

Quantitative polymerase chain reaction (qPCR) was performed to quantify functional marker genes related to total bacteria abundance (*16S*), and genes responsible to encode archaeal (AOA) and bacterial (AOB) ammonia monooxygenase (*amoA*), nitrite reductase (*nirK* and *nirS*), and nitrous oxide reductase (*nosZ*) enzymes. The qPCR was realized in the StepOne PlusTM Real-Time PCR System (Applied Biosystems) equipment. For the analysis, the mixture reaction contained 2 μL of the DNA template, 5 μL of SYBRGreen PCR Master Mix (Applied Biosystems, Waltham, MA, USA), 1 μL of forward primer (5 μM), 1 μL of reverse primer (5 μM), and 1 μL water to complete a final reaction volume of 10 μL. Information about real-time qPCR conditions and primers can be found on Table S1.

Data obtained from the qPCR was utilized to calculate the relative target gene abundance, where absolute gene values were normalized to the total amount of bacteria quantified in each sample. For this operation, the total copies quantified by each gene were divided by the total 16S rRNA gene copies.

2.2.6. Biomass production and N_2O emission intensity

All sugarcane plants were harvested on the 60th day with a knife and cut at the bottom for the aboveground biomass production measurements. The sampled biomass was reduced into smaller pieces through a grinder. For fresh belowground biomass measurement, the plant roots were collected, washed for soil removal, and weighed. All the fresh material was air dried at 60°C until no variation in the weight was observed. At this point, the plant material was weighed again on a digital scale to obtain the aboveground and belowground dry biomass.

The N_2O emission intensity (EI) is a parameter that expresses the ratio between the amount of N_2O emitted in a CO_2 equivalent basis, per yield production unit, calculated by the following equations (1) and (2):

$$CO_2\ eq\ emission\ (g\ pot^{-1}) = \frac{Cumulative\ N_2O\ (mg\ m^2) \times pot\ surfice\ area\ (m^2) \times 273}{1000} \quad (1)$$

$$EI_{N_2O} = \frac{CO_2\ eq\ emission\ (g\ pot^{-1})}{Aboveground\ biomass\ production\ (kg\ \ pot^{-1})} \quad (2)$$

where CO_2 eq emission (g pot^{-1}) denotes the N_2O quantity emissions, and aboveground biomass is expressed on a dry basis (kg pot^{-1}). For gas conversion into CO_2 equivalent (CO_2-eq), 273 was considered as the global warming potential from N_2O (Foster et al., 2021).

2.2.7. Statistical analysis

Data were analyzed using the R software (R Core Team, 2022). Response variables that were only measured at the end of the experiment or analyzed as cumulative responses (i.e., cumulative N_2O, emission intensity, aboveground biomass, and belowground biomass) were submitted to a one-way analysis of variance (ANOVA). Mean comparisons were performed using the *emmeans* package with Tukey adjustment at α=0.05 (Lenth, 2022). A repeated-measures model was performed to test the response of N mineralization and functional gene abundance to treatments using the *nlme* package (Pinheiro et al., 2022; Pinheiro & Bates, 2000). Treatment and DAF were set as fixed effects, and the interaction among replicates and treatment as random effects.

The addition of a heteroscedasticity group and covariance structures were evaluated through the improvement in the heterogeneity and normality of the residuals. The goodness of fit between the simple and more complex models was assessed according to the AIC fit statistic (Burnham & Anderson, 2002). As mineralization affects N availability over time, the DAF was incorporated as the a priori known within-group heteroscedasticity structure to account for unequal variances (*varIdent*, *nlme*) in the analysis of soil N-NO_3^- and N-NH_4^+ (Pinheiro et al. 2000). Adding a grouping structure did not improve the model fit for gene expression and the addition of covariance structures among measurements obtained in a same experimental unit did not improve the overall fit of the model for all response variables (Piepho et al., 2003; WoTolfinger, 1996).

The canonical discriminant analysis (CDA) was used as a dimension-reduction approach to determine the linear combination of the measured variables that maximized the differences between treatments and DAF (Arnold et al., 2019). The CDA derives canonical discriminant

functions that have the highest possible multiple correlations with groups and summarizes the among-class variation (Vaylay & van Santen, 2002). For the CDA obtention, the *candisc* package was utilized to facilitate the differentiation of groups by considering the interrelationships of the independent variables (treatments) based on the correlations among all variables measured (Friendly & Fox, 2021).

2.3. Results

2.3.1. GHG fluxes and cumulative emissions

Overall, NF+S presented emission peaks superior to other treatments over the experiment period (Figure 3 A). All treatments with N fertilizer presented high emission peaks between 12 and 16 days after fertilization (DAF), except for NF+B10 which happened on the 6th DAF. Among those treatments, peak daily means N_2O emission varied from 26 mg N m^{-2} day^{-1} under NF to 82 mg N m^{-2} day^{-1} under NF+S. Moreover, the biochar treatments (NF+B5 and NF+B10) had peak emissions of 27 and 28 mg N m^{-2} day^{-1}, respectively. No peaks were observed under CTR, with the higher daily emission of 2.1 mg N m^{-2} day^{-1} happening on the 2nd DAF. On the 40th day, N_2O emissions were near zero for all treatments, and this pattern was maintained until the end of the experiment.

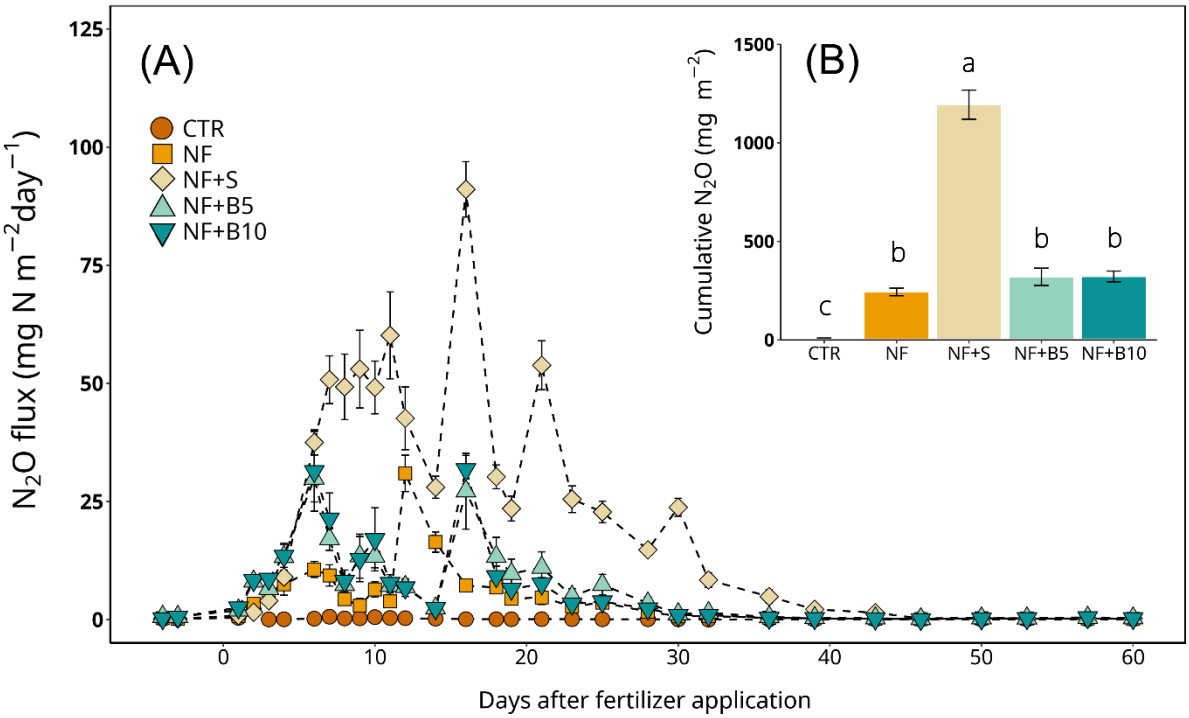

Figure 3. Temporal changes in N_2O flux (A), and cumulative N_2O (B) in response to soil only (CTR), N fertilizer application alone (NF), or combined with straw (NF+S), or variable biochar rates – 5 (NF+B5) and 10 Mg ha^{-1} (NF+B10), over a 60-day greenhouse pot experiment. Data (mean ± SE, n=5) followed by different letters denote significant differences between treatments by Tukey's test ($p < 0.05$).

Cumulative N_2O emission was significantly affected by N fertilizer and organic residues (Table S2; p <0.05). The NF+B5 and NF+B10 showed no differences concerning cumulative N_2O, totaling 320 and 322 mg m^{-2}, respectively (Figure 3 B). Furthermore, both biochar rates had cumulative N_2O emission 73% lower than NF+S (1194 mg m^{-2}). Despite this reduction, the biochar treatments registered emissions 1.3 folds higher than the mean observed under NF (243 mg m^{-2}). However, this increase was not significant (p<0.05). Besides, N fertilizer addition increased by 30-folds the cumulative N_2O emissions compared to CTR (8 mg m^{-2}). Carbon dioxide (CO_2) and methane (CH_4) fluxes presented temporal variation throughout the experiment (Figure S1 A, C). However, cumulative CO_2 and CH_4 were not influenced by the treatments (Figure S1 B, D).

2.3.2. Dynamics of soil mineral N concentration

Significant differences among treatments were observed through measures in time analysis (MANOVA) for soil N-NH_4^+ and N-NO_3 (Table S2). All treatments showed a similar trend for N-NH_4^+ concentration during the experimental period, with soil concentration decreasing over time (Figure 4 A). At 3 DAF, all treatments had peak N-NH_4^+, with values ranging from 3.5 mg N kg^{-1} (CTR) to 1802 mg N kg^{-1} (NF+B5). The treatment NF+S had lower N-NH_4^+ availability at 3 and 11 DAF, presenting concentrations lower than NF. The values of N-NH_4^+ under NF+B5, NF+B10, and NF were similar throughout the experimental period. Soil N-NO_3^- concentration increase was observed at 11 DAF for most of the treatments, except for NF+S (Figure 4 B). The concentration of N-NO_3^- in NF+S treatment differed from the CTR only at 39 DAF, which, together with the biochar treatments, had the highest values. Soils under the NF+B5 and NF+NB10 treatments presented similar N-NH_4^- and N-NO_3^- values in all evaluated sampling points. Additionally, soil N mineral availability only differed between NF and biochar treatments in the N-NO_3^- concentration at the 39 DAF. At the end of the experiment, both N-NH_4^+ and N-NO_3^- values were similar and close to zero for all treatments.

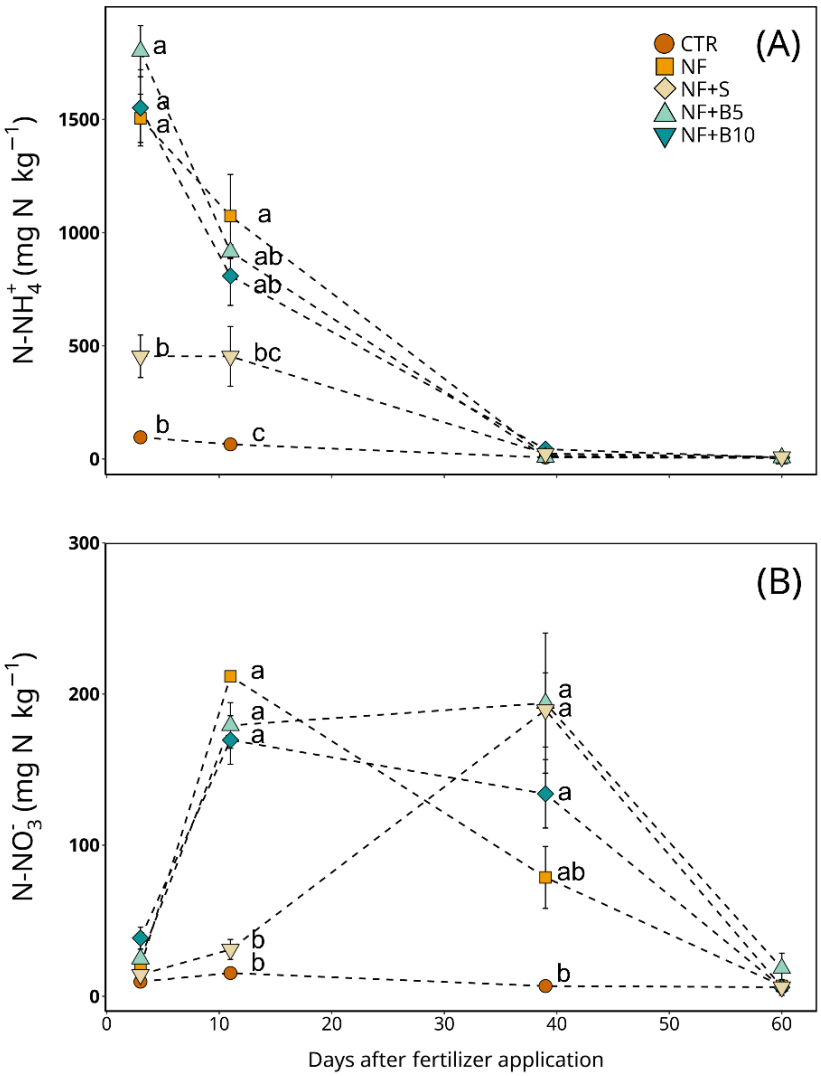

Figure 4. Soil ammonium N-NH_4^+ (A) and nitrate N-NO_3^- (B) concentration in response to soil only (CTR), N fertilizer application alone (NF), or combined with straw (NF+S), or variable biochar rates – 5 (NF+B5) and 10 Mg ha^{-1} (NF+B10), over a 60-day greenhouse pot experiment. Data (mean ± SE. *n*=4) followed by different letters denote significant differences between treatments by Tukey's test ($p < 0.05$).

2.3.3. Functional genes associated with the soil N cycle

Overall, functional gene abundance varied in response to the sampling period and treatments application (Figure 5; Table S3). Comparing the genes associated with the nitrification step at 3 and 11 DAF, AOA-*amoA* had a peak 2-fold higher than AOB-*amoA*. Moreover, AOA-*amoA* gene abundance decreased throughout the experiment, as AOB *amoA* genes increased. At 11 DAF, NF+S had the highest mean and increased the AOB-*amoA* gene by 90% compared to NF. Furthermore, the NF+S treatment had a higher relative abundance of AOB-amoA than all

treatments except NF+B10. For the 60th day, the AOB *amoA* genes relative abundance registered under the NF+S treatment was only higher than CTR, and all treatments with N fertilizer presented similar values.

Among the genes linked with nitrite reduction, the *nirS* relative abundance was superior to the *nirK* gene. The *nirK* gene relative abundance generally increased with time and peaked at 0.09%, as observed under NF on the 60th day, with CTR having the lowest result. Also at that period, NF had a higher *nirK* relative abundance than NF+S and NF+B10. No differences were observed between NF+S or NF+B10 *nirK* results. Concerning the treatment effects on the *nirS*, soils under NF+B5 presented a higher relative abundance of the gene than NF+S and CTR. On the 11th day, the relative abundance of *nosZ* was similar in all treatments containing N fertilizer, except for NF+B5. At 60 DAF, all biochar treatments had the same *nosZ* expression as NF, where only NF+B5 presented a higher value than NF+S.

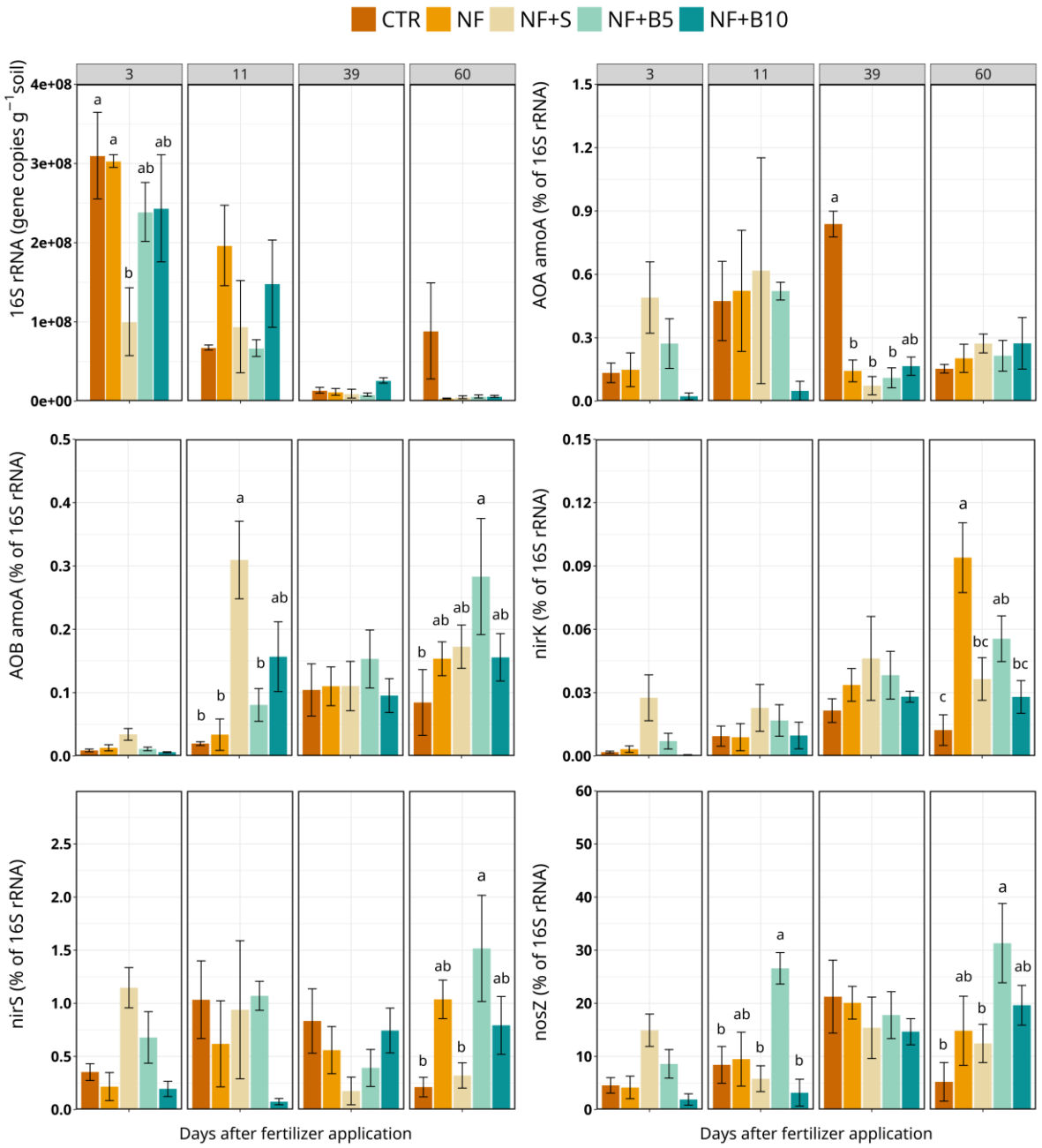

Figure 5. Gene abundance related to archaeal (AOA-*amoA*) and bacterial ammonium oxidation (AOB-*amoA*), denitrification (*nirK* and *nirS*), and complete denitrification (*nosZ*), relative to the 16S rRNA. Data (mean ± SE. *n*=4) obtained in response to soil only (CTR), N fertilizer application alone (NF), or combined with straw (NF+S) or variable biochar rates – 5 (NF+B5) or 10 Mg ha^{-1} (NF+B10). Data (mean ± SE. *n*=4) followed by different letters denote significant differences between treatments at the same period by Tukey's test ($p < 0.05$).

2.3.4. Correlations between N_2O emissions, soil mineral N availability, and functional genes abundance

A canonical discriminant analysis (CDA) was performed for different sampling days. Considering the first and second axis together, the analyzes explained 96,6% of the total variance on day 3; 96.7% on day 11; 82.8% on day 39, and 86.1% on day 60 (Figure 6). At 3 DAF, N_2O soil emissions were strongly correlated with soil N-NH_4^+ concentration, followed up by the N-NO_3^- levels (Figure 6 a). Conversely, all functional genes exhibited a weak correlation with the gas flux that day. At the 11 DAF, the strongest positive relationship with N2O fluxes was seen with the AOB-*amoA* gene, as shown by the first canonical variate (Figure 6 b). A negative correlation was seen between N_2O fluxes and the *nosZ* gene abundance in both axes (Figure 6 b). Moreover, n*irS* mean relative abundance negatively correlated with N_2O flux only at the first canonical variate, albeit less strongly than the one observed for *nosZ*. At the 39 DAF, N-NO_3^- showed a stronger positive correlation than soil N-NH_4^+ with N_2O emissions (Figure 6 c). Among the functional genes analyzed, *nirK* abundance had the strongest positive correlation with N_2O flux (Figure 6 c). As for the 60 DAF (Figure 6 d), AOA-*amoA* and AOB-*amoA* were the only functional genes to exhibit a relationship with N_2O on both axes, whereas *nirK* was the only functional gene that correlated negatively with N_2O on the first canonical variate. The CTR was primarily separated from the other (Figure 6 a,b,c), indicating that the effect of treatments was temporary. On the day of high N_2O emission (Figure 6 b), the NF+S was separated from the CTR and the other treatments, and N_2O emissions correlated AOB-*amoA* abundance.

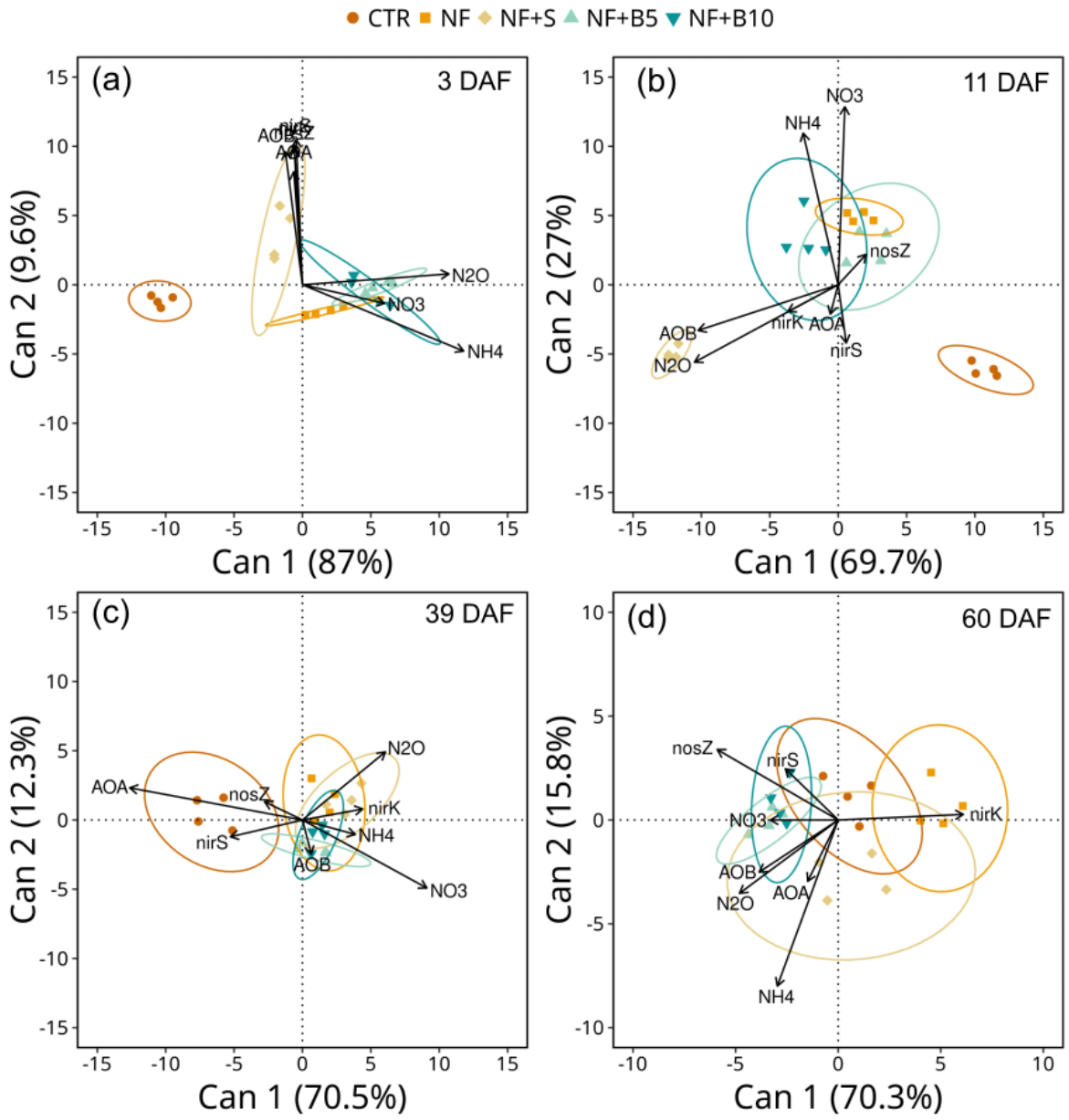

Figure 6. Canonical discriminant analysis for daily N_2O emission (N_2O), soil ammonium (NH_4), and nitrate (NO_3) concentrations; functional genes related to archaeal (AOA) and bacterial (AOB) ammonia oxidation, denitrification (nirK and nirS); and total denitrification (nosZ) on days 3 (a), 11 (b), 39 (c), and 60 (d) after N fertilizer application (DAF). Different ellipses indicate the grouping of different canonical means for treatments, and the response variables are represented by arrows. CAN1: canonical variable 1; CAN2: canonical variable 2.

2.3.5. Soil total C, N, and C/N ratio

The concentration of soil C and and C/N ratio values are presented in Table 3, where only total C showed differences among the treatments (Table S2). Soil C content varied from 23

under NF to 26 (g kg^{-1}) under NF+B10 (Figure 7). Both NF+B5 and NF+B10 had the highest soil C concentrations, albeit having similar values to CTR and NF+S (p<0.05). However, the biochar treatments were the only ones that presented a higher soil C value than NF. The NF+B5 and NF+B10 treatments showed increased soil C content by 6.8 and 11.6 % compared to NF. While the soils under NF+S treatment were similar to NF treatment. Soil total N values ranged from 1.81% to 1.95%, but no significant differences were identified among treatments (Table S4). As for the C/N ratio, values ranged from 13.1, under both NF and NF+S treatments, to 13.4 under NF+B10 (Table S4).

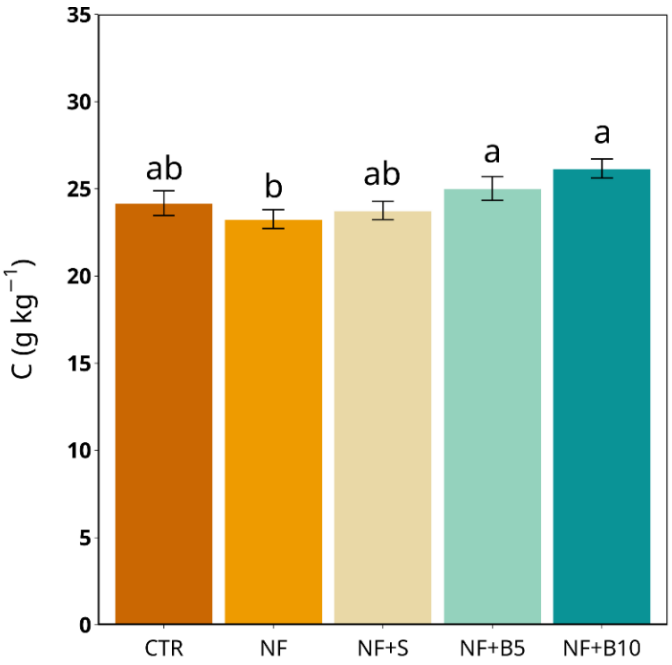

Figure 7. Soil total C concentration in response to N fertilizer application alone (NF) or combined with straw (NF+S) or variable biochar rates – 5 (NF+B5) or 10 Mg ha^{-1} (NF+B10), over a 60-day greenhouse pot experiment. Data (mean ± SE. n=5) followed by the same letters do not differ significantly by Tukey's test (p <0.05).

2.3.6. Biomass production and N_2O emission intensity

The mean results of aboveground biomass, belowground biomass, and emission intensity were different among treatments (Table 3). Higher above and belowground biomass was observed in NF+S. The treatments NF, NF+B5, and NF+B10 presented similar aboveground biomass, which was higher than CTR. The treatment NF+S also registered a high average N_2O

emission intensity. On average, the treatments NF+B5 and NF+B10 present an N_2O emission intensity 63% and 62% lower than the NF+S treatment, respectively. The NF treatment had similar emission intensity compared with biochar treatments.

Table 3. Above and belowground sugarcane biomass (dry basis), and N_2O emission intensity in response to N fertilizer application alone (NF) or combined with straw (NF+S) or variable biochar rates – 5 (NF+B5) or 10 Mg ha^{-1} (NF+B10), over a 60-day greenhouse pot experiment. Data (mean ± SE. *n*=4) followed by the same letters do not differ significantly by Tukey's test (p <0.05).

Treatment	Aboveground Biomass (g pot^{-1})	Belowground Biomass (g pot^{-1})	N_2O Emission Intensity (g CO_2 eq. kg $biomass^{-1}$)
CTR	25.7 ± 2.4 c	39.6 ± 4.5 b	5.0 ± 1.3 c
NF	45.1 ± 2.3 b	59.4 ± 9.2 b	83.8 ± 6.0 b
NF+S	65.0 ± 2.9 a	131.8 ± 18.1 a	288.3 ± 17.3 a
NF+B5	47.0 ± 1.7 b	61.7 ± 8.2 b	106.0 ± 13.4 b
NF+B10	47.2 ± 1.0 b	54.4 ± 6.5 b	107.4 ± 9.6 b

2.4. Discussion

The results obtained herein show that the application of straw-based biochar resulted in lower N_2O emissions compared to keeping sugarcane straw material on the surface which had the highest cumulative N_2O emissions (Figure 3). Although the application of both biochar rates induced a cumulative soil N_2O emission 73% lower than NF+S, it was not different from NF. Furthermore, the different rates of biochar application (5 and 10 Mg ha^{-1}) had no effect on soil N_2O emissions. This finding goes against what was found by Kaur et al. (2023), who observed that biochar application reduces by 10% – 50% the emissions of N_2O driven by N fertilizer addition. Nevertheless, it is important to highlight that besides the biochar impact on N_2O emissions being highly variable, the most significative mitigation results were associated with higher biochar rates than the ones used in this study.

Previous studies in tropical soils have contrasting results regarding the mitigation potential of biochar, with variations in the magnitude of N_2O emission suppression (Abbruzzini et al., 2019; Grutzmacher et al., 2018; Novais et al., 2017; Rittl et al., 2021), and some studies also finding neutral effects (Lu et al., 2020; Novais et al., 2017; Ramlow et al., 2019). In contrast to previous findings that observed that the N_2O mitigation effect increased with the biochar rate applied (Rittl et al., 2021), our results exhibited no relationship among these parameters. A

hypothesis for these contrasting might be the different experimental conditions and the low biochar rates used in our experiment (≤10 Mg ha^{1}), as the inconsistency of biochar capacity to mitigate N_2O fluxes can be attributed to these factors (Huang et al., 2023; Kaur et al., 2023).

Consistent with our findings, other studies concluded that the maintenance of a large amount of sugarcane straw on the soil surface results in high N_2O emissions (Carmo et al., 2013; Gonzaga et al., 2018; Vasconcelos et al., 2022). This effect of straw management on soil N_2O emissions is relatively well-documented. Currently, the primary method of sugarcane harvest in Brazil leaves a large amount of sugarcane straw on the agricultural field (Menandro et al., 2017), with this approach being reportedly associated with an increase in soil N_2O emissions when utilized along with N fertilizer input (Vargas et al., 2019; Vasconcelos et al., 2018). Soil N_2O emissions driven by this management strategy are likely associated with the addition of fresh organic residues, N fertilizer presence, and higher moisture content since the straw minimizes water loss to the atmosphere (Fracetto et al., 2017; Ruiz Corrêa et al., 2019). The maintenance of soil moisture can result in the formation of suboxic or anoxic microsites, as the low levels of oxygen caused by the water inside soil pores influence the magnitude of N_2O production (Davidson et al., 2000). Moreover, plant residues such as sugarcane straw can absorb water inside their tissue and exhibit intense microbial activity, thus forming significant hotspots for denitrification even in dry soils (Kravchenko et al., 2017).

Nevertheless, the alterations in soil driven by straw coverage can also influence sugarcane biomass production. This assumption is supported by the study of Carvalho et al. (2019), who observed through a meta-analysis study that different levels of straw removal harmed the sugarcane yield in some of the studied sites. Conversely, the biochar addition, in both rates, did not increase biomass production above the levels seen under NF. As biochar has direct effects on soil water and nutrient retention (Huang et al., 2021; Jesus et al., 2023; Jindo et al., 2020), it can enhance crop production when applied at high rates (Abbruzzini et al., 2019). But recent reports have highlighted that crop yield responses to biochar addition are not easily predicted, having no clear correlations with soil characteristics (Lehmann et al., 2021).

Despite the higher biomass production observed under straw-covered soils, those also presented the highest soil N_2O emission intensity (Table 3), indicating that more N_2O was emitted per unit of aboveground biomass. The emission intensity takes into consideration the amount of N_2O emitted on a CO_2 equivalent basis per unit of the agricultural product obtained, and it is considered to be an important indicator to evaluate the sustainability of the crop system (Campanha et al., 2019; Sainju, 2016). As biochar application can enhance crop productivity while

decreasing N_2O emissions, previous reports have pointed out its ability to reduce global warming potential (Liu et al., 2019), which was not confirmed by our study. However, it is essential to highlight that the calculated N_2O emission intensity does not consider C sequestration induced by the application of biochar and straw, which can change the GHG emission balance from a long-term perspective. Therefore, it fails to account for the soil organic C storage, which contributes to lower the GHG balance from the activity (Bhattacharyya et al., 2022; Guenet et al., 2021; Song et al., 2021).

The highest increase in soil C under treatments with biochar addition **Figure 7**can be attributed to the fact that although both sugarcane straw and biochar have significative C concentrations (Das et al., 2021; Ghodake et al., 2021; Menandro et al., 2017), the C present in the composition of biochar was already incorporated in the soil and, therefore, quantified by the analysis. On the other hand, the straw biomass carbon could only be incorporated into the soil after its decomposition transformations had taken since it was placed on the soil surface, which can take a long period of time (Cotrufo et al., 2015). Overall, even though biochar application at the rates 5 and 10 Mg ha^{-1} had the highest total C accumulation gains, no significant increases in the CO_2 cumulative emissions were observed, probably due to the highly recalcitrant and stable nature of the C present in the biochar composition (Tozzi et al., 2019). This indicates that the added C by the biochar is momentarily stable and is not lost in gaseous form to the atmosphere, which aligns with studies that have not observed an increase in cumulative CO_2 emissions caused by biochar addition (Jiang et al., 2016). Furthermore, it was observed that the soil C content decreased when only N fertilizer was applied, which reinforces the importance of crop residues for maintaining C stock levels in agricultural soils of tropical environments (Rasche & Diego, 2020; Tenelli et al., 2021).

Previous studies have pointed out the ability of biochar to retain available ions present in the soil solution, such as N-NH_4^+ and N-NO_3^-, which can be influenced by parameters such as feedstock material and pyrolisis temperature (Elkhlifi et al., 2023; Joseph et al., 2021). In contrast, our results showed no significant changes in N availability caused by biochar addition regardless of the application rate (Figure 4). The more prominent immobilization effect on N-mineral was registered under soils with straw addition (NF+S) due to its capacity to retain N (Carvalho et al., 2017) (Figure 6 a,b). This effect happens mainly by the presence of labile C components in the straw material (Vasconcelos et al., 2018), which can enhance soil microbial activity and favor N immobilization. A possible indication that biochar-N interaction was significant was that it presented higher N-NO_3^- levels on the 39 DAF (Figure 4 A). This late decrease in N-NO_3

concentration might be due to the added N was initially in the ammoniacal form. This shows the role that organic material plays in maintaining the soil N under forms available to be taken up by plants, which is a critical aspect in terms of crop nutrition (Otto et al., 2016; Castro et al., 2021). One possible explanation for our lack of results regarding the impact of biochar impact on N availability is the period of evaluation (60 days) since the ion adsorption ability can be more intense in field-aged biochar (Haider et al., 2020; Hagemann et al., 2017).

We expected to see a correlation between AOA-a*moA* genes as they registered a high relative abundance under treatments with high N_2O emissions, but this was not confirmed by the CDA. Instead, it seems that the primary pathway related to the N_2O emissions was associated with AOB organisms, as pointed out by the strong positive correlation (Figure 6 b). In line with our findings, previous studies also reported that under tropical soils, increases in soil N_2O emissions were mainly associated with AOB abundance (Grassmann et al., 2022). Our results also registered an increase in the AOB-*amoA* relative abundance under straw-covered soils, which also exhibited higher N_2O emissions (Figure 5). This result corroborates with previous works that have emphasized that AOB-*amoA* abundance is correlated with N_2O sugarcane soils under residues amendment, such as straw and vinasse (Lourenço et al., 2018; Soares et al., 2016). Although both the metabolism of AOA and AOB can influence soil N_2O production (Stein, 2020), previous reports mention that AOB can be responsible for up to 70% of the nitrification derived-N_2O emission (Liang & Robertson, 2021). Thus, it appears that the alterations in the populations of ammonia-oxidizing bacteria play a central role in controlling N_2O emissions in tropical soils.

Taxonomic shifts have been reported in *nirK* and *nosZ*-expressing communities under biochar-amended soils (Harter et al., 2017), possibly due to the material impacts on parameters that affect microbial metabolism, such as electron transfer properties, soil pH, oxygen levels, and nutrient availability (Chen et al., 2014; Choudhary et al., 2021; Kaur et al., 2023; Verheijen et al., 2019). However, we did not observe any significant responses until the end of the experiment for the *nirK, nirS,* and *nosZ* (Figure 5). Even at the sampling points where treatments influenced the relative abundance of denitrification-related genes, the CDA analysis did not exhibit any strong relationship between the relative abundance of the genes and N_2O fluxes (Figure 6). Possibly the late treatment effects on genes involved in denitrification, combined with the lack of correlation with N_2O fluxes, may be due to the denitrification pathway not being a significant source of N_2O emissions on tropical soils, where nitrification can play a major role (Pajares & Bohannan, 2016). This hypothesis is supported by the work of Grassmann et al. (2022), which also observed that

N_2O fluxes had a closer relationship with AOB than denitrification genes. Nevertheless, it is essential to mention that there are few specific data regarding biochar impacts on the N-related functional genes in tropical soils, which may represent a knowledge gap for results comparison.

Considering that the N_2O response to the biochar presence is complex and varies vastly with biotic and abiotic factors, studies such as this could help estimate the benefits that biochar application use can have on agriculture in tropical countries. However, it is essential to mention that even though straw removal can be an alternative to reduce N_2O emissions, it is worth considering that a certain quantity should remain in the field to guarantee that parameters such as nutrient cycling, soil conservation, and soil ecology are not being jeopardized (Cherubin et al., 2021; Menandro et al., 2019). Therefore, the several local studies that documented the ideal removal percentage for each agricultural field (Carvalho et al., 2019; Tenelli et al., 2021) should be considered. Those scientific reports have included specific factors from each area, such as biomass capacity production, soil carbon stocks, and texture. In addition, a possibility to be explored is the use of other lignocellulosic residual feedstock biomasses that are also considered cost-effective options for biochar production, such as forest residues from pine and eucalyptus (Cervi et al., 2021; Roozen, 2015). Therefore, future studies considering the use of biochar by agriculture as a nature-based solution should explore other types of feedstock biomass together with the ideal sugarcane straw removal rates that each area can support to mitigate N_2O emissions.

2.5. Conclusions

The use of straw-derived biochar did not decrease N_2O emissions driven by N-fertilizer addition, regardless of the application rate of 5 or 10 Mg ha^{-1}. However, the conversion of sugarcane straw into biochar followed by its use as soil amendment represents 73% less N_2O cumulative emissions than leaving the raw material above the soil surface. The straw presence also induced an increase in the relative abundance of *amoA*-containing bacteria, which indicates that the rise seen in N_2O emissions under these soils might be linked with the presence of AOB microorganisms. Conversely, the biochar influence on the genes related to the soil N-cycle did not correlate with N_2O emissions. To the best of our knowledge, this is the first study to evaluate the biochar impact on N_2O emissions under tropical environments. Therefore, our study helps to provide information about the biochar impact on N_2O emissions which can be used in future

life-cycle assessment analysis that analyses that aims to quantify the GHG balance under straw-removal for bioenergy production scenarios.

References

Abbruzzini, T. F. (2015). *The role of biochar on greenhouse gas offsets, improvement of soil attributes and nutrient use efficiency in tropical soils* [Universidade de São Paulo]. https://doi.org/10.11606/T.11.2015.tde-30092015-115437

Abbruzzini, T. F., Davies, C. A., Toledo, F. H., & Cerri, C. E. P. (2019). Dynamic biochar effects on nitrogen use efficiency, crop yield and soil nitrous oxide emissions during a tropical wheat-growing season. *Journal of Environmental Management*, *252*(September), 109638. https://doi.org/10.1016/j.jenvman.2019.109638

Bhattacharyya, S. S., Ros, G. H., Furtak, K., Iqbal, H. M. N., & Parra-Saldívar, R. (2022). Soil carbon sequestration – An interplay between soil microbial community and soil organic matter dynamics. *Science of the Total Environment*, *815*. https://doi.org/10.1016/j.scitotenv.2022.152928

Blanco-Canqui, H. (2021). Does biochar improve all soil ecosystem services? *GCB Bioenergy*, *13*(2), 291–304. https://doi.org/10.1111/gcbb.12783

Borchard, N., Schirrmann, M., Cayuela, M. L., Kammann, C., Wrage-Mönnig, N., Estavillo, J. M., Fuertes-Mendizábal, T., Sigua, G., Spokas, K., Ippolito, J. A., & Novak, J. (2019). Biochar, soil and land-use interactions that reduce nitrate leaching and N2O emissions: A meta-analysis. *Science of the Total Environment*, *651*, 2354–2364. https://doi.org/10.1016/j.scitotenv.2018.10.060

Borges, B. M. M. N., Strauss, M., Camelo, P. A., Sohi, S. P., & Franco, H. C. J. (2020). Re-use of sugarcane residue as a novel biochar fertiliser - Increased phosphorus use efficiency and plant yield. *Journal of Cleaner Production*, *262*, 121406. https://doi.org/10.1016/j.jclepro.2020.121406

Brady, N. C., & Weil, R. R. (2016). Nitrogen and Sulfur Economy of Soils. In *The nature and properties of soils* (15th ed., p. 1104). Pearson Education Limited.

Buresh, R., Austin, E., & Craswell, E. (1982). Analytical methods in15N research. *Fertilizer Research*, *3*(1), 37–62. https://doi.org/10.1007/BF01063408

Campanha, M. M., de Oliveira, A. D., Marriel, I. E., Gontijo Neto, M. M., Malaquias, J. V., Landau, E. C., de Albuquerque Filho, M. R., Ribeiro, F. P., & de Carvalho, A. M. (2019). Effect of soil tillage and N fertilization on N2O mitigation in maize in the Brazilian Cerrado. *Science of the Total Environment*, *692*, 1165–1174. https://doi.org/10.1016/j.scitotenv.2019.07.315

Carvalho, J. L. N., Menandro, L. M. S., de Castro, S. G. Q., Cherubin, M. R., Bordonal, R. de O., Barbosa, L. C., Gonzaga, L. C., Tenelli, S., Franco, H. C. J., Kolln, O. T., & Castioni, G. A. F. (2019). Multilocation Straw Removal Effects on Sugarcane Yield in South-Central Brazil. *Bioenergy Research*, *12*(4), 813–829. https://doi.org/10.1007/s12155-019-10007-8

Carvalho, J. L. N., Nogueirol, R. C., Menandro, L. M. S., Bordonal, R. de O., Borges, C. D., Cantarella, H., & Franco, H. C. J. (2017). Agronomic and environmental implications of sugarcane straw removal: a major review. *GCB Bioenergy*, *9*(7), 1181–1195. https://doi.org/10.1111/gcbb.12410

Carvalho, J. L. N., Oliveira, B. G., Cantarella, H., Chagas, M. F., Gonzaga, L. C., Lourenço, K. S., Bordonal, R. O., & Bonomi, A. (2021). Implications of regional N2O–N emission factors on sugarcane ethanol emissions and granted decarbonization certificates. *Renewable and Sustainable Energy Reviews*, *149*(July 2020). https://doi.org/10.1016/j.rser.2021.111423

Cavalett, O., Chagas, M. F., Seabra, J. E. A., & Bonomi, A. (2013). Comparative LCA of ethanol versus gasoline in Brazil using different LCIA methods. *International Journal of Life Cycle Assessment*, *18*(3), 647–658. https://doi.org/10.1007/s11367-012-0465-0

Cervi, W. R., Lamparelli, R. A. C., Gallo, B. C., de Oliveira Bordonal, R., Seabra, J. E. A., Junginger, M., & van der Hilst, F. (2021). Mapping the environmental and techno-economic potential of biojet fuel production from biomass residues in Brazil. *Biofuels, Bioproducts and Biorefining*, *15*(1), 282–304. https://doi.org/10.1002/bbb.2161

Cherubin, M. R., Bordonal, R. O., Castioni, G. A., Guimarães, E. M., Lisboa, I. P., Moraes, L. A. A., Menandro, L. M. S., Tenelli, S., Cerri, C. E. P., Karlen, D. L., & Carvalho, J. L. N. (2021). Soil health response to sugarcane straw removal in Brazil. *Industrial Crops and Products*, *163*(January). https://doi.org/10.1016/j.indcrop.2021.113315

CONAB— Companhia Nacional de Abastecimento. (2023). *Acompanhamento da safra brasileira de cana-de-açúcar. v. 11—Safra 2023/2024, no. 2—Primeiro Levantamento* (Vol. 11).

Cotrufo, M. F., Soong, J. L., Horton, A. J., Campbell, E. E., Haddix, M. L., Wall, D. H., & Parton, W. J. (2015). Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nature Geoscience*, *8*(10), 776–779. https://doi.org/10.1038/ngeo2520

Dai, L., Wang, Y., Liu, Y., Ruan, R., He, C., Yu, Z., Jiang, L., Zeng, Z., & Tian, X. (2019). Integrated process of lignocellulosic biomass torrefaction and pyrolysis for upgrading bio-oil production: A state-of-the-art review. *Renewable and Sustainable Energy Reviews*, *107*(January), 20–36. https://doi.org/10.1016/j.rser.2019.02.015

Dane, J. H., & Topp, C. G. (Eds). (2020). Methods of soil analysis, Part 4: Physical methods (1st ed.). John Wiley & Sons, Ltd.

Das, S. K., Ghosh, G. K., Avasthe, R. K., & Sinha, K. (2021). Compositional heterogeneity of different biochar: Effect of pyrolysis temperature and feedstocks. *Journal of Environmental Management*, *278*(P2), 111501. https://doi.org/10.1016/j.jenvman.2020.111501

Davidson, E. A., Keller, M., Erickson, H. E., Verchot, L. V., & Veldkamp, E. (2000). Testing a conceptual model of soil emissions of nitrous and nitric oxides. *BioScience*, *50*(8), 667–680. https://doi.org/10.1641/0006-3568(2000)050[0667:TACMOS]2.0.CO;2

Dias, M. O. S., Lima, D. R., & Mariano, A. P. (2018). Techno-Economic Analysis of Cogeneration of Heat and Electricity and Second-Generation Ethanol Production from Sugarcane. In *Advances in Sugarcane Biorefinery: Technologies, Commercialization, Policy Issues and Paradigm Shift for Bioethanol and By-Products*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-804534-3.00010-0

Elkhlifi, Z., Iftikhar, J., Sarraf, M., Ali, B., Saleem, M. H., Ibranshahib, I., Bispo, M. D., Meili, L., Ercisli, S., & Kayabasi, E. T. (2023). *Potential Role of Biochar on Capturing Soil Nutrients , Carbon Sequestration and Managing Environmental Challenges : A Review*.

EPE - Empresa de Pesquisa Energética. (2022). *Análise de Conjuntura dos Biocombustíveis – Ano 2021*.

Fan, C., Duan, P., Zhang, X., Shen, H., Chen, M., & Xiong, Z. (2020). Mechanisms underlying the mitigation of both N2O and NO emissions with field-aged biochar in an Anthrosol. *Geoderma*, *364*(January), 114178. https://doi.org/10.1016/j.geoderma.2020.114178

FAO. (2021). *FAOSTAT Emissions Database*.

Foster, P., Storelvmo, T., Armour, K., Collins, W., Dufresne, J. L., Frame, D., Lunt, D. J., Mauritsen, T., Palmer, M. D., Watanabe, M., Wild, M., & Zhang, H. (2021). The Earth's Energy Budget, Climate Feedbacks, and Climate Sensitivity. In 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, & B. Zhou (Eds.), *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 923–1054). Cambridge University Press.

Ghodake, G. S., Shinde, S. K., Kadam, A. A., Saratale, R. G., Saratale, G. D., Kumar, M., Palem, R. R., AL-Shwaiman, H. A., Elgorban, A. M., Syed, A., & Kim, D. Y. (2021). Review on biomass feedstocks, pyrolysis mechanism and physicochemical properties of biochar: State-of-the-art framework to speed up vision of circular bioeconomy. *Journal of Cleaner Production*, *297*, 126645. https://doi.org/10.1016/j.jclepro.2021.126645

Gonzaga, L. C., Carvalho, J. L. N., Oliveira, B. G. de, Soares, J. R., & Cantarella, H. (2018). Crop residue removal and nitrification inhibitor application as strategies to mitigate N2O emissions in sugarcane fields. *Biomass and Bioenergy*, *119*(September), 206–216. https://doi.org/10.1016/j.biombioe.2018.09.015

Grassmann, C. S., Mariano, E., Diniz, P. P., Borges, B. M. F., Borges, C. D., Tsai, S. M., & Rosolem, C. A. (2022). Functional N-cycle genes in soil and N2O emissions in tropical grass-maize intercropping systems. *Soil Biology and Biochemistry*, *169*(April), 108655. https://doi.org/10.1016/j.soilbio.2022.108655

Grutzmacher, P., Puga, A. P., Bibar, M. P. S., Coscione, A. R., Packer, A. P., & de Andrade, C. A. (2018). Carbon stability and mitigation of fertilizer induced N2O emissions in soil amended with biochar. *Science of the Total Environment*, *625*, 1459–1466. https://doi.org/10.1016/j.scitotenv.2017.12.196

Guenet, B., Gabrielle, B., Chenu, C., Arrouays, D., Balesdent, J., Bernoux, M., Bruni, E., Caliman, J. P., Cardinael, R., Chen, S., Ciais, P., Desbois, D., Fouche, J., Frank, S., Henault, C., Lugato, E., Naipal, V., Nesme, T., Obersteiner, M., … Zhou, F. (2021). Can N2O emissions offset the benefits from soil organic carbon storage? *Global Change Biology*, *27*(2), 237–256. https://doi.org/10.1111/gcb.15342

Haider, G., Joseph, S., Steffens, D., Müller, C., Taherymoosavi, S., Mitchell, D., & Kammann, C. I. (2020). Mineral nitrogen captured in field-aged biochar is plant-available. *Scientific Reports*, *10*(1), 1–12. https://doi.org/10.1038/s41598-020-70586-x

Harter, J., Krause, H. M., Schuettler, S., Ruser, R., Fromme, M., Scholten, T., Kappler, A., & Behrens, S. (2014). Linking N2O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. In *ISME Journal* (Vol. 8, Issue 3, pp. 660–674). https://doi.org/10.1038/ismej.2013.160

Huang, H., Reddy, N. G., Huang, X., Chen, P., Wang, P., Zhang, Y., Huang, Y., Lin, P., & Garg, A. (2021). Effects of pyrolysis temperature, feedstock type and compaction on water retention of biochar amended soil. *Scientific Reports*, *11*(1), 1–19. https://doi.org/10.1038/s41598-021-86701-5

Huang, Y., Tao, B., Lal, R., Lorenz, K., Jacinthe, P. A., Shrestha, R. K., Bai, X., Singh, M. P., Lindsey, L. E., & Ren, W. (2023). A global synthesis of biochar's sustainability in climate-smart agriculture - Evidence from field and laboratory experiments. *Renewable and Sustainable Energy Reviews*, *172*(November 2022), 113042. https://doi.org/10.1016/j.rser.2022.113042

IUSS Working Group WRB. 2022. World Reference Base for Soil Resources. International soil classification system for naming soils and creating legends for soil maps. 4th edition. International Union of Soil Sciences (IUSS), Vienna, Austria.

Jantalia, C. P., dos Santos, H. P., Urquiaga, S., Boddey, R. M., & Alves, B. J. R. (2008). Fluxes of nitrous oxide from soil under different crop rotations and tillage systems in the South of Brazil. *Nutrient Cycling in Agroecosystems*, *82*(2), 161–173. https://doi.org/10.1007/s10705-008-9178-y

Jesus, S. De, Carlos, D., Pellegrino, E., Tatiana, C., Rittl, F., Fernanda, T., Blanca, A., & Prado, L. (2023). Biochar Physical and Hydrological Characterization to Improve Soil Attributes for Plant Production. *Journal of Soil Science and Plant Nutrition*. https://doi.org/10.1007/s42729-023-01273-9

Jiang, X., Haddix, M. L., & Cotrufo, M. F. (2016). Interactions between biochar and soil organic carbon decomposition: Effects of nitrogen and low molecular weight carbon compound addition. *Soil Biology and Biochemistry*, *100*, 92–101. https://doi.org/10.1016/j.soilbio.2016.05.020

Jindo, K., Audette, Y., Higashikawa, F. S., Silva, C. A., Akashi, K., Mastrolonardo, G., Sánchez-Monedero, M. A., & Mondini, C. (2020). Role of biochar in promoting circular economy in the agriculture sector. Part 1: A review of the biochar roles in soil N, P and K cycles. *Chemical and Biological Technologies in Agriculture*, *7*(1), 1–12. https://doi.org/10.1186/s40538-020-00182-8

Joseph, S., Cowie, A. L., Van Zwieten, L., Bolan, N., Budai, A., Buss, W., Cayuela, M. L., Graber, E. R., Ippolito, J. A., Kuzyakov, Y., Luo, Y., Ok, Y. S., Palansooriya, K. N., Shepherd, J., Stephens, S., Weng, Z. (Han), & Lehmann, J. (2021). How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar. *GCB Bioenergy*, *January*, 1–34. https://doi.org/10.1111/gcbb.12885

Kaur, N., Kieffer, C., Ren, W., & Hui, D. (2023). How much is soil nitrous oxide emission reduced with biochar application? An evaluation of meta-analyses. *GCB Bioenergy*, *15*(1), 24–37. https://doi.org/10.1111/gcbb.13003

Keeney, D. R., & Nelson, D. W. (2015). *Nitrogen-Inorganic Forms* (pp. 643–698). https://doi.org/10.2134/agronmonogr9.2.2ed.c33

Kern, J., Giani, L., Teixeira, W., Lanza, G., & Glaser, B. (2019). What can we learn from ancient fertile anthropic soil (Amazonian Dark Earths, shell mounds, Plaggen soil) for soil carbon sequestration? *Catena*, *172*, 104–112. https://doi.org/10.1016/J.CATENA.2018.08.008

Kravchenko, A. N., Toosi, E. R., Guber, A. K., Ostrom, N. E., Yu, J., Azeem, K., Rivers, M. L., & Robertson, G. P. (2017). Hotspots of soil N 2 O emission enhanced through water absorption by plant residue. *Nature Geoscience*, *10*(7), 496–500. https://doi.org/10.1038/ngeo2963

Kuypers, M. M. M., Marchant, H. K., & Kartal, B. (2018). The microbial nitrogen-cycling network. *Nature Reviews Microbiology*, *16*(5), 263–276. https://doi.org/10.1038/nrmicro.2018.9

Lefebvre, D., Williams, A., Kirk, G. J. D., Meersmans, J., Sohi, S., Goglio, P., & Smith, P. (2021). An anticipatory life cycle assessment of the use of biochar from sugarcane residues as a greenhouse gas removal technology. *Journal of Cleaner Production*, *312*(January), 127764. https://doi.org/10.1016/j.jclepro.2021.127764

Lefebvre, D., Williams, A., Meersmans, J., Kirk, G. J. D., Sohi, S., Goglio, P., & Smith, P. (2020). Modelling the potential for soil carbon sequestration using biochar from sugarcane residues in Brazil. *Scientific Reports*, *10*(1), 1–11. https://doi.org/10.1038/s41598-020-76470-y

Lehmann, J., Cowie, A., Masiello, C., Kammann, C., Woolf, D., Amonette, J., Cayuela, M., Camps-Arbestain, M., & Whitman, T. (2021). Biochar in climate change mitigation. *Nat Geosci (in Press)*, *14*(December). https://doi.org/10.1038/s41561-021-00852-8

Lenth, R. (2022). *emmeans: Estimated Marginal Means, aka Least-Squares Means_* (R package version 1.8.0). https://CRAN.R-project.org/package=emmeans..

Liang, D., & Robertson, G. P. (2021). Nitrification is a minor source of nitrous oxide (N2O) in an agricultural landscape and declines with increasing management intensity. *Global Change Biology*, *27*(21), 5599–5613. https://doi.org/10.1111/gcb.15833

Liu, X., Mao, P., Li, L., & Ma, J. (2019). Impact of biochar application on yield-scaled greenhouse gas intensity: A meta-analysis. *Science of the Total Environment*, *656*, 969–976. https://doi.org/10.1016/j.scitotenv.2018.11.396

Liu, X., Shi, Y., Zhang, Q., & Li, G. (2021). Effects of biochar on nitrification and denitrification-mediated N2O emissions and the associated microbial community in an agricultural soil. *Environmental Science and Pollution Research*, *28*(6), 6649–6663. https://doi.org/10.1007/s11356-020-10928-4

Lourenço, K. S., Dimitrov, M. R., Pijl, A., Soares, J. R., do Carmo, J. B., van Veen, J. A., Cantarella, H., & Kuramae, E. E. (2018). Dominance of bacterial ammonium oxidizers and fungal denitrifiers in the complex nitrogen cycle pathways related to nitrous oxide emission. *GCB Bioenergy*, *10*(9), 645–660. https://doi.org/10.1111/gcbb.12519

Meinshausen, M., Lewis, J., McGlade, C., Gütschow, J., Nicholls, Z., Burdon, R., Cozzi, L., & Hackmann, B. (2022). Realization of Paris Agreement pledges may limit warming just below 2 °C. *Nature*, *604*(7905), 304–309. https://doi.org/10.1038/s41586-022-04553-z

Menandro, L. M. S., Cantarella, H., Franco, H. C. J., Kölln, O. T., Pimenta, M. T. B., Sanches, G. M., Rabelo, S. C., & Carvalho, J. L. N. (2017). Comprehensive assessment of sugarcane straw: implications for biomass and bioenergy production. In *Biofuels, Bioproducts and Biorefining* (Vol. 11, Issue 3, pp. 488–504). https://doi.org/10.1002/bbb.1760

Menandro, L. M. S., de Moraes, L. O., Borges, C. D., Cherubin, M. R., Castioni, G. A., & Carvalho, J. L. N. (2019). Soil Macrofauna Responses to Sugarcane Straw Removal for Bioenergy Production. *Bioenergy Research*, *12*(4), 944–957. https://doi.org/10.1007/s12155-019-10053-2

Morales, S. E., Cosart, T., & Holben, W. E. (2010). Bacterial gene abundances as indicators of greenhouse gas emission in soils. *ISME Journal*, *4*(6), 799–808. https://doi.org/10.1038/ismej.2010.8

Novais, S. V., Oliveira Zenero, M. D., Frade Junior, E. F., Paiva de Lima, R., & Pelegrino Cerri, C. E. (2017). Mitigation of Greenhouse Gas Emissions from Tropical Soils Amended with Poultry Manure and Sugar Cane Straw Biochars. *Agricultural Sciences*, *08*(09), 887–903. https://doi.org/10.4236/as.2017.89065

Okuno, F. M., Cardoso, T. de F., Duft, D. G., Luciano, A. C. dos S., Neves, J. L. M., Soares, C. C. dos S. P., & Leal, M. R. L. V. (2019). Technical and Economic Parameters of Sugarcane Straw Recovery: Baling and Integral Harvesting. *Bioenergy Research*, *12*(4), 930–943. https://doi.org/10.1007/s12155-019-10039-0

Otto, R., Castro, S. A. Q., Mariano, E., Castro, S. G. Q., Franco, H. C. J., & Trivelin, P. C. O. (2016). Nitrogen Use Efficiency for Sugarcane-Biofuel Production: What Is Next? *Bioenergy Research*, *9*(4), 1272–1289. https://doi.org/10.1007/s12155-016-9763-x

Pereira, L. G., Cavalett, O., Bonomi, A., Zhang, Y., Warner, E., & Chum, H. L. (2019). Comparison of biofuel life-cycle GHG emissions assessment tools: The case studies of ethanol produced from sugarcane, corn, and wheat. *Renewable and Sustainable Energy Reviews*, *110*(October 2017), 1–12. https://doi.org/10.1016/j.rser.2019.04.043

Piepho, H.-P., Büchse, A., & Emrich, K. (2003). A hitchhiker's guide to mixed models for randomized experiments. *Journal of Agronomy and Crop Science* , 310–322.

Pinheiro, J., Bates, D., & R Core Team. (2022). *nlme: Linear and Nonlinear Mixed Effects Models* (R package version. 3.1-157). https://CRAN.R-project.org/package=nlme.

Pinheiro, J. C., & Bates, D. M. (2000). Mixed-Effects Models in S and S-PLUS. Springer-Verlag. https://doi.org/10.1007/b98882

Raij, B. van, Andrade, J. C. de, Cantarella, H., & Quaggio, J. A. (2001). Análise química para avaliação da fertilidade de solos tropicais. (1st ed.). Instituto Agronômico.

Quassi de Castro, S. A., Otto, R., Bohórquez Sánchez, C. E., Tenelli, S., Sermarini, R. A., & Trivelin, P. C. O. (2021). Sugarcane straw preservation results in limited immobilization and improves crop N-fertilizer recovery. *Biomass and Bioenergy*, *144*(December 2020). https://doi.org/10.1016/j.biombioe.2020.105889

R Core Team, (2020). (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna,Austria. https://www.r-project.org/

Rasche, L., & Diego, R. S. (2020). Pros and Cons of Sugarcane Straw Recovery in São Paulo. *Bioenergy Research*, *13*(1), 147–156. https://doi.org/10.1007/s12155-019-10078-7

Rittl, T. F., Oliveira, D. M. S., Canisares, L. P., Sagrilo, E., Butterbach-Bahl, K., Dannenmann, M., & Cerri, C. E. P. (2021). High Application Rates of Biochar to Mitigate N2O Emissions From a N-Fertilized Tropical Soil Under Warming Conditions. *Frontiers in Environmental Science*, *8*(January), 1–5. https://doi.org/10.3389/fenvs.2020.611873

Roozen, A. (2015). *Availability of sustainable lignocellulosic biomass residues in Brazil for export to the EU* [Master Thesis]. Utrecht University.

Ruiz Corrêa, S. T., Barbosa, L. C., Menandro, L. M. S., Scarpare, F. V., Reichardt, K., de Moraes, L. O., Hernandes, T. A. D., Franco, H. C. J., & Carvalho, J. L. N. (2019). Straw Removal Effects on Soil Water Dynamics, Soil Temperature, and Sugarcane Yield in South-Central Brazil. *BioEnergy Research*, *12*(4), 749–763. https://doi.org/10.1007/s12155-019-09981-w

Sainju, U. M. (2016). A global meta-analysis on the impact of management practices on net global warming potential and greenhouse gas intensity from cropland soils. *PLoS ONE*, *11*(2), 1–26. https://doi.org/10.1371/journal.pone.0148527

Soares, J. R., Cassman, N. A., Kielak, A. M., Pijl, A., Carmo, J. B., Lourenço, K. S., Laanbroek, H. J., Cantarella, H., & Kuramae, E. E. (2016). Nitrous oxide emission related to ammonia-oxidizing bacteria and mitigation options from N fertilization in a tropical soil. *Scientific Reports*, *6*(July), 1–11. https://doi.org/10.1038/srep30349

Song, Q., Zhu, J., Gong, Z., Feng, Y., Wang, Q., Sun, Y., Zeng, X., & Lai, Y. (2021). Effect of straw retention on carbon footprint under different cropping sequences in Northeast China. *Environmental Science and Pollution Research*, *28*(39), 54792–54801. https://doi.org/10.1007/s11356-021-14316-4

Stein, L. Y. (2019). Insights into the physiology of ammonia-oxidizing microorganisms. *Current Opinion in Chemical Biology*, *49*, 9–15. https://doi.org/10.1016/j.cbpa.2018.09.003

Stein, L. Y. (2020). The Long-Term Relationship between Microbial Metabolism and Greenhouse Gases. *Trends in Microbiology*, *28*(6), 500–511. https://doi.org/10.1016/j.tim.2020.01.006

Stein, L. Y., & Klotz, M. G. (2016). The nitrogen cycle. *Current Biology*, *26*(3), R94–R98. https://doi.org/10.1016/j.cub.2015.12.021

Tenelli, S., Bordonal, R. O., Cherubin, M. R., Cerri, C. E. P., & Carvalho, J. L. N. (2021). Multilocation changes in soil carbon stocks from sugarcane straw removal for bioenergy production in Brazil. *GCB Bioenergy*, *13*(7), 1099–1111. https://doi.org/10.1111/gcbb.12832

Thompson, W., & Meyer, S. (2013). Second generation biofuels and food crops: Co-products or competitors? *Global Food Security*, *2*(2), 89–96. https://doi.org/10.1016/j.gfs.2013.03.001

Tian, H., Xu, R., Canadell, J. G., Thompson, R. L., Winiwarter, W., Suntharalingam, P., Davidson, E. A., Ciais, P., Jackson, R. B., Janssens-Maenhout, G., Prather, M. J., Regnier, P., Pan, N., Pan, S., Peters, G. P., Shi, H., Tubiello, F. N., Zaehle, S., Zhou, F., … Yao, Y. (2020). A comprehensive quantification of global nitrous oxide sources and sinks. *Nature*, *586*(7828), 248–256. https://doi.org/10.1038/s41586-020-2780-0

Tozzi, F. V. D. N., Coscione, A. R., Puga, A. P., Carvalho, C. S., Pellegrino Cerri, C. E., & de Andrade, C. A. (2019). Carbon stability and biochar aging process after soil application. *Horticulture International Journal*, *3*(6), 320–329. https://doi.org/10.15406/hij.2019.03.00148

United Nations Department of Economic and Social Affairs, P. D. (2022). *World Population Prospects 2022: Summary of Results*.

Vargas, V. P., Soares, J. R., Oliveira, B. G., Lourenço, K. S., Martins, A. A., Del Grosso, S. J., do Carmo, J. B., & Cantarella, H. (2019). Sugarcane Straw, Soil Temperature, and Nitrification Inhibitor Impact N2O Emissions from N Fertilizer. *Bioenergy Research*, *12*(4), 801–812. https://doi.org/10.1007/s12155-019-10015-8

Vasconcelos, A. L. S., Cherubin, M. R., Cerri, C. E. P., Feigl, B. J., Borja Reis, A. F., & Siqueira-Neto, M. (2022). Sugarcane residue and N-fertilization effects on soil GHG emissions in south-central, Brazil. *Biomass and Bioenergy*, *158*(January 2021). https://doi.org/10.1016/j.biombioe.2022.106342

Vasconcelos, A. L. S., Cherubin, M. R., Feigl, B. J., Cerri, C. E. P., Gmach, M. R., & Siqueira-Neto, M. (2018). Greenhouse gas emission responses to sugarcane straw removal. *Biomass and Bioenergy*, *113*(September 2017), 15–21. https://doi.org/10.1016/j.biombioe.2018.03.002

Wan, S., Lin, Y., Ye, G., Fan, J., Hu, H. W., Zheng, Y., Jin, S. S., Duan, C., & He, J. Z. (2023). Long-term manure amendment reduces nitrous oxide emissions through decreasing the abundance ratio of amoA and nosZ genes in an Ultisol. *Applied Soil Ecology*, *184*(August 2022), 104771. https://doi.org/10.1016/j.apsoil.2022.104771

Wang, L., Quiceno, R., Price, C., Malpas, R., & Woods, J. (2014). Economic and GHG emissions analyses for sugarcane ethanol in Brazil: Looking forward. *Renewable and Sustainable Energy Reviews*, *40*, 571–582. https://doi.org/10.1016/j.rser.2014.07.212

Wolfinger, R. D. (1996). Heterogeneous Variance: Covariance Structures for Repeated Measures. *Journal of Agricultural, Biological, and Environmental Statistics*, *1*(2), 205. https://doi.org/10.2307/1400366

Woolf, D., Lehmann, J., Ogle, S., Kishimoto-Mo, A. W., McConkey, B., & Baldock, J. (2021). Greenhouse Gas Inventory Model for Biochar Additions to Soil. *Environmental Science & Technology*, *55*(21), 14795–14805. https://doi.org/10.1021/acs.est.1c02425

Zhang, L., Jing, Y., Chen, C., Xiang, Y., Rezaei Rashti, M., Li, Y., Deng, Q., & Zhang, R. (2021). Effects of biochar application on soil nitrogen transformation, microbial functional genes, enzyme activity, and plant nitrogen uptake: A meta-analysis of field studies. *GCB Bioenergy*, *13*(12), 1859–1873. https://doi.org/10.1111/gcbb.12898

Supplementary information

Table S1. Real-time qPCR conditions and primer utilized for the quantification of funcional marker genes.

Gene name	Primers	Primer sequence (5' -> 3')	Thermal cycling conditions	Amplicon size (pb)	References
16S rRNA Bacteria	Eub338 Eub518	5´-CCTACGGGAGGCAGCAG-3' 5´-ATTACCGCGGCTGCTGG-3'	95°C - 10 min.; 40 cycles: 95°C - 30 s.; 53°C - 40 s.; 72°C - 40 s.*	180	Muyzer, *et al.*, 1993
nosZ	nosZ2F nosZ2R	5'-CGCRACGGCAASAAGGTSMSSGT-3' 5'-CAKRTGCAKSGCRTGGCAGAA-3'	95°C - 10 min; 40 cycles: 95°C - 40 s.; 63°C - 30 s.; 72°C - 40 s.*	267	Henry *et al.* 2006
AOA-*amoA*	Arch-amoAF Arch-amoAR	5'-STAATGGTCTGGCTTAGACG-3' 5'-GCGGCCATCCATCTGTATGT-3'	95°C - 5 min.; 40 cycles: 95°C - 40 s.; 56°C - 30 s.; 72°C - 1min.*	635	Francis *et al.* 2005
AOB-*amoA*	amoA1F amoA2R	5'-GGGGTTTCTACTGGTGGT-3' 5'-CCCCTCKGSAAAGCCTTCTTC-3'	95°C - 10 min; 40 cycles: 95°C - 40 s.; 56°C - 30 s.; 72°C - 1 min.*	491	Rotthauwe *et al.* 1997
nirK	NirK876 NirK1040	5'-ATYGGCGGVAYGGCGA-3' 5'-GCCTCGATCAGRTTRTGGTT-3'	95°C - 10 min; 40 cycles: 95°C - 15 s.; 63°C - 30 s.; 72°C - 30 s.*	165	Henry *et al.* 2004
nirS	nirScd3aF nirSR3cd	5'- GTSAACGTSAAGGARACSGG -3' 5'- GASTTCGGRTGSGTCTTGA -3'	95°C-10 min.; 40 cycles 95°C-20 s.; 63°C-30 s.; 72°C-30 s.*	425	Throbäck *et al.* 2004

Table S2. Analysis of variance (ANOVA) results for cumulative N_2O, biomass production, N_2O emissions intensity, soil C, N and C/N ratio as a response to treatments; and repeated-measures analysis of variance (MANOVA) for soil mineral N ($N\text{-}NHO_3^-$ and $N\text{-}NH_4^+$) concentrations as a response to treatments and time, measured in days after fertilizer application (DAF).

ANOVA

Source of variation	**DF**	**Residual DF**	**Cumulative N_2O**		**N_2O Emission Intensity**		**Aboveground Biomass**		**Belowground Biomass**		**Total C**		**Total N**		**C/N Ratio**	
			F-value	*P-value*	*F-value*	*P-value*	*F-value*	*P-value*	*F-value*	*P-value*	*F-value*	*P-value*	*F-value*	*P-value*	*F-value*	*P-value*
Treatment	4	20	119.79	<.0001	88.60	<.0001	11.94	<.0001	11.94	<.0001	3.56	<.05	2.47	0.07	2.47	0.07

MANOVA

Source of variation	**DF**	**Residual DF**	**$N\text{-}NO_3^-$**		**$N\text{-}NH_4^+$**	
			F-value	*P-value*	*F-value*	*P-value*
Treatment	4	15	22.5	<.0001	2.2	0.124
DAF	4	60	152.4	<.0001	170.2	<.0001
Treatment x DAF	16	60	18.6	<.0001	16.2	<.0001

DF: Degrees of freedom; DAF: Days after fertilizer application.

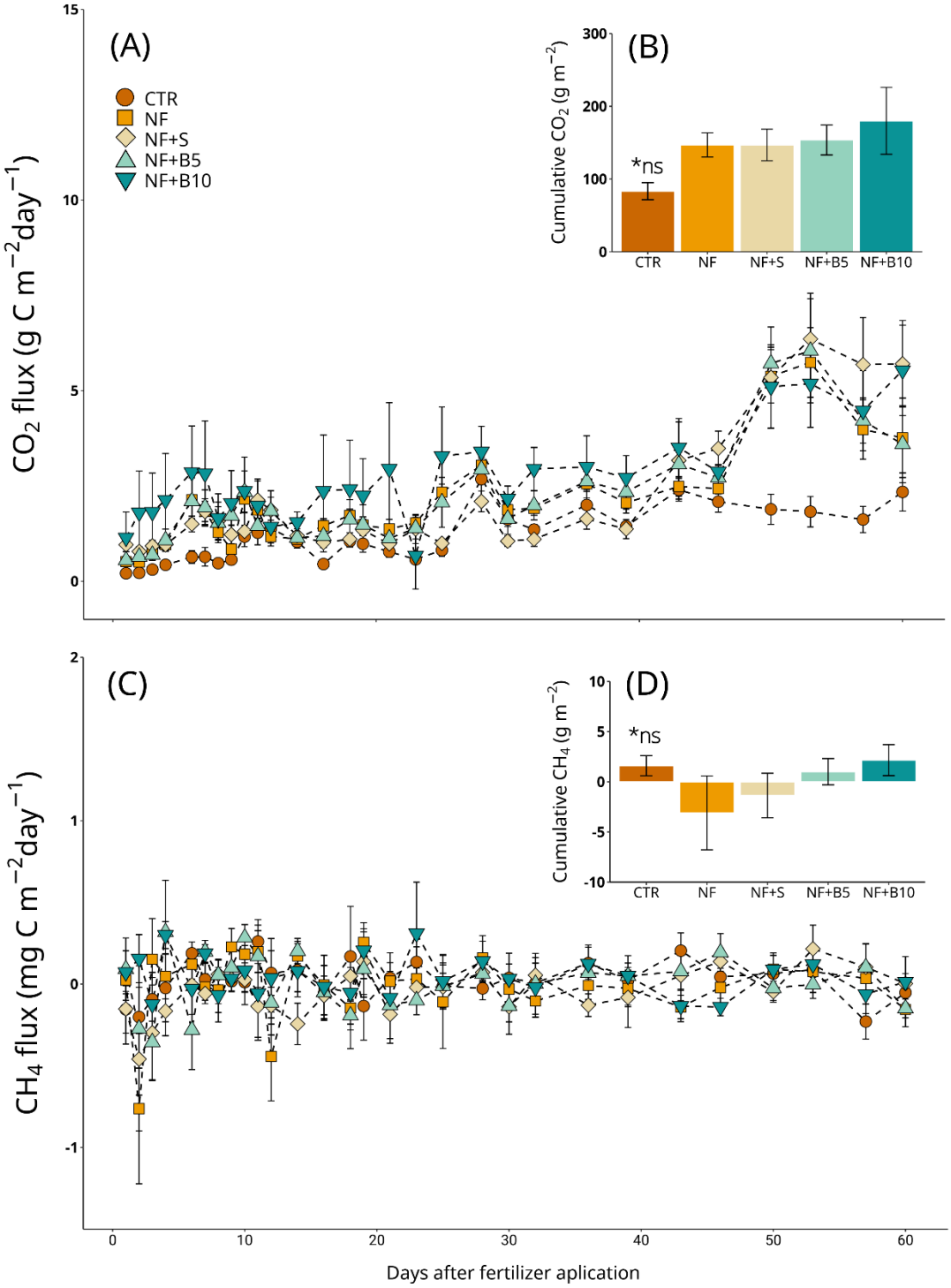

Figure S1. CO_2 flux (a), cumulative CO_2 emissions (b), CH_4 flux (c), and cumulative CH_4 emissions (d); in response to N Fertilizer application. alone (NF) or combined with straw (NF+S) or variable biochar doses – 5 (NF+B5) or 10 Mg ha^{-1} (NF+B10). over a 60-day greenhouse pot experiment. Data (mean ± SE. n=5) followed by (*ns) are not different by ANOVA(p <0.05).

Table S3. Repeated-measures analysis of variance (MANOVA) for the relative abundance of the *amoA*, *nirK*, *nirS*, and *nosZ* functional genes, and 16S total abundance as a response to treatments and time, measured in days after fertilizer application (DAF).

MANOVA

Source of variation	**DF**	**Residual DF**	**16 S**		**AOA**		**AOB**		**nirK**		**nirS**		**nosZ**	
			F-value	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*
Treatment	4	15	3.4	0.03	1.9	0.14	4.1	0.01	4.0	0.02	22.5	<.0001	5.0	<.05
DAF	3	44	44.8	<.0001	2.1	0.10	14.1	<.0001	19.6	<.0001	152.4	<.0001	8.0	<.05
Treatment x DAF	12	44	2.4	<.05	1.9	0.06	2.8	0.005	3.4	0.001	18.6	<.0001	2.5	<.05

DF: Degrees of freedom; DAF: Days after fertilizer application. AOA: *amoA*-containing archaeas. AOB: *amoA containing bacterias.*

Table S4. Soil total N, and C/N ratio in response to N fertilizer application, alone (NF) or combined with straw (NF+S) or variable biochar doses – 5 (NF+B5) or 10 Mg ha^{-1} (NF+B10), over a 60-day greenhouse pot experiment. Data (mean ± SE. n=5) followed by the same letters do not differ significantly by Tukey's test (p <0.05).

Treatment	N g kg^{-1}	C/N ratio
CTR	1.83 ± 0.06 a	13.2 ± 0.11 a
NF	1.77 ± 0.04 a	13.2 ± 0.05 a
NF+S	1.81 ± 0.03 a	13.1 ± 0.16 a
NF+B5	1.88 ± 0.03 a	13.3 ±0.24 a
NF+B10	1.95 ± 0.02 a	13.5 ±0.24 a

3. THE N_2O EMISSION RESPONSE TO THE APPLICATION OF DIFFERENT TYPES OF BIOCHARS

Abstract

Biochar is the solid by-product of thermal conversion from organic biomasses, and its use by agriculture can represent a cost-effect solution to decrease soil nitrous oxide (N_2O) emissions. Currently, in Brazil, agricultural and forestry residues represent the main available biomasses to be utilized as feedstock for biochar production. Thus, the present study focused on evaluating if the use of different biomasses would impact the biochar capacity to influence N_2O emissions. To assess these effects, we conducted a 60-day greenhouse pot experiment with sugarcane plants, with 6 treatments: soil only (CTR); soil + N fertilizer (NF); NF + sugarcane (*Saccharum* spp.) straw biochar (NF+SB); NF + sugarcane bagasse biochar (NF+BB); NF + residue biochar of *Pinus* spp. (NF+PB); and NF with residue biochar of *Eucalyptus* spp. (NF+EB). Our results showed that regardless of the feedstock material utilized, all evaluated biochars reduced the cumulative N_2O emissions by 25-50%. The highest mitigation capacity was observed under NF+SB, whereas NF+EB had a lower ability to suppress N_2O emissions than sugarcane-derived biochar. Moreover, the feedstock material also influenced the different chemical properties found in the biochar, where forestry residues biochar showed a higher C content and aromatic C functional groups on its surface. The higher C presence in those materials resulted in an increase in soil C levels above the ones registered for sugarcane-based biochars. Our results showed that N fertilizer and biochar application, regardless of the feedstock material, did not influence biomass production by sugarcane plants. Biochar application also did not affect $N\text{-}NH_4^+$ availability. Conversely, overall soils under NF+BB, NF+PB, and NF+EB registered lower $N\text{-}NO_3^-$ concentration than NF, indicating that these materials could retain the anion. Our findings suggest that biochar produced from eucalyptus residue is the least recommended option if the final objective of the practice is N_2O suppression. At the same time, pine biomass residue was the best option to decrease N_2O emissions and increase soil C storage. Based on our findings, the relationship between feedstock material and N_2O emissions suggests that this parameter could be used as a possible GHG response predictor under tropical environments. Hence, biochar application in tropical soils is a recommended strategy to decrease N_2O emissions while increasing soil C storage, confirming that it should be considered in future projects as a nature-based solution for reducing GHG emissions by agriculture.

Keywords**:** scanning electron microscopy; SEM, x-ray photoelectron spectroscopy; XPS; greenhouse gas; GHG.

3.1. Introduction

Biochar, also known as "black-carbon (C)", is a carbonaceous material obtained from the thermal conversion of organic materials under high temperatures and limited oxygen conditions (Kambo & Dutta, 2015; Kan et al., 2016; Wang & Wang, 2019). Research over the past years have pointed out several unique characteristics of biochar that make its use as soil amendment be considered a nature-based solutions (Lehmann et al., 2006, 2021; Woolf et al., 2010). Nature-based solution is an umbrella term recently adopted by policymakers that includes initiatives to protect biodiversity and ensure the functioning of ecosystem services while tackling social challenges such as climate change (Seddon et al., 2019). The use of biochar in agriculture as a nature-based solution relies on two important mechanisms: the promotion of carbon (C) sequestration and the decrease of greenhouse gas (GHG) emissions (Blanco-Canqui, 2021). The impact of biochar on soil C sequestration can persist for years, as seen in Terra preta soils, where the material is still present after being added by human activity centuries ago (Kern et al., 2019; Spokas, 2010). Regarding soil GHG emissions, the use of biochar tends to decrease nitrous oxide (N_2O) emissions from croplands (Cayuela et al., 2013; Huang et al., 2023).

The N_2O is a GHG with a global warming potential 273 times greater than CO_2 (Foster et al., 2021). An increase in the N_2O fluxes derived from agricultural activities has been observed in the past years, mainly caused by the N input through organic and inorganic fertilizers (Montzka et al., 2011; Tian et al., 2020). Despite several observations about the biochar capacity to mitigate N_2O emissions (Kaur et al., 2023), some studies have also registered neutral effects from this practice (Munera-Echeverri et al., 2022; Ramlow et al., 2019). The variability in the N_2O-biochar interaction is due to the influence of several factors, some of them linked to soil management practices, N fertilizer rate and type, presence of organic residues, soil pH, and biochar application rate (Boateng et al., 2020; Feng et al., 2018; Wang et al., 2018; Wu et al., 2018). Moreover, the physicochemical properties of biochar (e.g., feedstock material, pyrolisis temperature, C: N ratio, N content, surface area, and pH) may also affect the response of N_2O emissions (Kollah et al., 2022; Liao et al., 2021; Lyu et al., 2022). Because of the relationship among these variables, the sum between them will modulate the response of soil-plant-atmosphere system to its use as a soil amendment.

Recent studies have sought to investigate the mechanisms of biochar that may influence soil N_2O emissions dynamics, but no conclusive answer has been achieved yet (Cayuela et al., 2014; Joseph et al., 2021). In this context, previous reports have considered that the chemical composition of different feedstock biomasses could influence the response of N_2O in biochar-

amended soils. Moreover, the feedstock material has a relevant influence on physicochemical properties of the biochar (Ippolito et al., 2020), as wood-based biochar can express distinct characteristics from biochar produced from herbaceous biomasses. Overall, lignocellulosic biomass-biochar can be categorized into two groups; wood-biochars are considered to be made from forestry and wood residues, while herbaceous-biochars include crops and their plant residues (Tomczyk et al., 2020).

Among the available residual biomasses in Brazil, residues from forestry and sugarcane production are possible sources for biochar production, as they represent a cost-viable option (Cervi et al., 2021; Roozen, 2015). Although both herbaceous and wood-derived biochar has been reported to be able to suppress N_2O emissions (Li et al., 2019; Lyu et al., 2022; Zhang et al., 2023), some studies have pointed out differences in the magnitude of this response (Lan et al., 2019; Ramlow et al., 2019). Previous reports have mentioned that the feedstock material can influence electrochemical properties of biochar (Chacón et al., 2017), which can be altered by the concentration and type of the functional groups expressed in the surface of biochar (Yuan et al., 2021; Yuan et al., 2019). The electrochemical character has been pointed out as one of the studied hypotheses for the mitigation of N_2O emissions registered under biochar-amended soils, as its "electron transfer" capacity can enhance the N_2O reduction to dinitrogen gas (Cayuela et al., 2013; Yuan et al., 2019). Moreover, a recent study observed that the feedstock characteristics could influence N_2O reduction, where materials with poor lignin content can express a higher capacity to mitigate N_2O emissions (Pascual et al., 2020). Therefore, understanding the effect of the soil-plant-atmosphere system response to biochar application produced from different feedstocks can help to predict the environmental and agronomic benefits of this management strategy.

Hence, we hypothesized that wood-derived biochar would have a lower capacity to mitigate N_2O emissions driven by N fertilizer than crop residue biochar due to their specific physicochemical characteristics. For that, the present study was made with the objective of a) characterizing the chemical properties of biochar from different feedstocks and correlate it with soil N_2O emissions, b) assessing the influence of feedstock materials utilized for biochar production on soil N_2O emissions

3.2. Material and Methods

3.2.1. Biochar production and physicochemical properties

A greenhouse experiment was conducted with sugarcane plants (*Saccharum* spp.), where four types of biochar produced from sugarcane crop (straw and bagasse) and forestry (*Pinus* spp. and *Eucalyptus grandis*) residues were evaluated over 60 days. Forestry biomass comprised post-harvest residues (i.e., pruning wastes and tree bark). The biochars were obtained using a fluidized bed fast-pyrolysis reactor, operating at temperatures ranging from 450 to 500°C. The range in the temperature was due to the characteristics of the auto-thermal pyrolysis utilized, where the differences in the lignocellulosic biomasses resulted in distinct environmental conditions necessary to keep the pyrolysis temperature stable. The feedstock material used for biochar production together with the pyrolysis temperature and code can be found on Table 4.

Table 4: Feedstock materials and pyrolysis temperature for biochar obtention.

Feedstock material	**Biochar code**	**Pyrolisis temperature** (°C)
Sugarcane straw	SB	450
Sugarcane bagasse	BB	450
Pine residue	PB	500
Eucalyptus residue	EB	450

The carbon (C) and nitrogen (N) total content of the biochars were performed before its application on the soil. The samples were prepared by grinding biochar particles with a pestle and mortar and passed through a 0.5 mm sieve. Total C and N (%) quantification was performed by the dry combustion method using a TruSpec CHN elemental analyzer (LECO - St. Joseph, MI, USA). The results of biochar chemical characterization, as well as the C/N ratio results, can be found in Table 5.

Table 5. Chemical characteristics of biochar obtained from different feedstocks (n=3).

Feedstock material	**pH**	**C** (%)	**N** (%)	**C/N Ratio**
Sugarcane straw	7.5	45.5	0.7	64.1
Sugarcane bagasse	8.8	43.8	0.6	73.1
Pine residue	7.8	60.8	0.5	114.4
Eucalyptus residue	8.9	62.9	0.4	138.3

The biochar samples were examined through scanning electron microscopy (SEM) to assess the microstructural and morphological characteristics of the materials produced from different feedstocks. Biochar samples were prepared by passing the material through a 0.5 mm sieve. Chemical composition and distribution analysis of the biochars were obtained using dispersive X-ray spectroscopy (EDS), which quantifies the elemental particle composition from the outermost layer (~100 nm) to approximately 2 μm inside the sample (Hagemann et al., 2017). Both SEM and EDS images were obtained in an Inspect F50® (ThermoFisher Scientific - Waltham, MA, USA) microscope equipped with an EDS sensor. The x-ray photoelectron spectroscopy (XPS) technique was utilized for chemical quantification and characterization of the elements and functional carbon (C) groups present in the surface layer of the materials. This technique consists of the emission of x-rays on the sample to detect the photoelectrons emitted by each atom type, thus quantifying the elemental composition and chemical bonds found at 10 nm from the surface (Joy et al., 1986). The XPS analysis was performed in triplicates, in different sample areas with a spot size of 400 μm, on a K-Alpha XPS (ThermoFisher Scientific) using an A1 K α (1486 eV). A pass energy of 200 eV was utilized for survey scans and 50 eV for a specific region (C1s). Spectra were fitted through the Thermo Avantage software (version 5.921), and core level binding energies were adjusted to the C1s region of 285.0 eV. The atomic percentage of the elements was calculated using the relative peak area in the spectrum by the total peak area of each element. The elemental composition was used to compare the different biochar samples and not to imply the absolute composition of the samples.

3.2.2. Experimental design

A pot experiment was conducted in a greenhouse environment over 60 days. One pre-sprouted sugarcane seedling was planted (var. CTC 9001) per pot (27,5 x 27 x 21 cm), where each pot consisted of an experimental unit. Six treatments were arranged in a completely randomized design with four repetitions. The treatments consisted of soil without the addition of nitrogen fertilizer and biochar as the control (CTR); soil with N fertilizer addition (NF); NF with sugarcane straw biochar (NF+SB); NF with sugarcane bagasse biochar (NF+BB); NF with pine biochar (NF+PB); and NF with eucalyptus biochar (NF+EB). Each pot was filled with 7 kg of sieved dry soil (< 2 mm mesh). The soil (0-20 cm) was collected from a sugarcane field in São Paulo – Brazil and classified as an Ferrasol (IUSS, 2022) with 40% clay, 11% silt, 47% sand, pH in $CaCl_2$ of 6.5, Al of <0.1 $mmol_c$ dm^{-1}, available-P of 239 mg dm^{-3}, K of 0.9 mmol dm^{-3}, and

organic matter of 35.7 g dm^{-3}. All soil physicochemical characterization analyses were performed before the establishment of the experiment, following the methods proposed by Raij et al., (2001).

For the treatments with biochar application, 70 g of biochar was mixed and homogenized to the soil at a rate of 1 % (w/w) (equivalent to 20 Mg ha^{-1}) before the pots were filled. Soil moisture was adjusted to 60% of water-filled pore space (WFPS) as it better represent the conditions found on brazilian soils, with this moisture level was maintained throughout the experiment period. The pots were pre-incubated for 10 days to stabilize microbial activity; after that, the N fertilizer was applied, which represents the beginning of the experiment. Nitrogen fertilizer was applied at a rate of 3 g N kg^{-1} rate using ammonium sulfate in all treatments except for the control. To attend to the nutritional demands of the plant, phosphorus (P) and potassium (K) were applied in all treatments at the following rates: 2 g $soil^{-1}$ of P_2O_5 via single superphosphate; and 3 g $soil^{-1}$ of K_2O via potassium chloride. Based on the volumetric water content obtained using a soil humidity sensor (TEROS 10, Meter – São José dos Campos, SP, Brazil), water field pore space (WFPS) was maintained at 60%.

3.2.3. GHG sampling and measurements

During the experimental period, gas samples for N_2O analysis were collected from a mini PVC static chamber with 5 cm in diameter and 15 cm in height. One chamber was installed per experimental unit and remained open except during the gas sampling events. The chamber was closed with a lid containing a hole sealed with a 1.5 mm silicon septa. Gas samples were collected from the headspace of the chamber at 0, 15, and 30 minutes after the lid was closed of each experimental unit, using a syringe equipped with a stainless needle (0.13 mm diameter). After that, gas samples were placed in 12 ml pre-evacuated vials (Exetainer; Labco Inc., UK). The N_2O concentration of the gas samples was quantified by Shimadzu gas chromatography (GC 2014, Japan) through an electron capture detector (ECD). Based on these results, the daily fluxes were estimated from the linear increase of the N_2O concentration values obtained from the 0, 15, and 30-minute samples (Jantalia et al., 2008). Daily fluxes were adjusted based on chamber headspace, soil temperature, and atmospheric pressure. The cumulative N_2O emission was calculated by linear interpolation between two daily fluxes over the 60-day experimental period.

3.2.4. Total C, total N, and mineral N soil analysis

Soil samples (20 g) were collected in four representative sampling days (on four representative sampling days (4, 11, 39, and 60 after N fertilizer application) to determine the dynamics of mineral N ($N\text{-}NH_4^+$ and $N\text{-}NO_3^-$) concentrations. Based on the N_2O flux, four sampling points (4, 11, 39, and 60 days after fertilizer application) were selected for the mineral N analysis procedure. For the mineral N extraction, 5 g soil subsamples was added to 50 ml of a 2 M KCl solution (Buresh et al., 1982), agitated for 1 h, and gravity filtered. In order to convert the results to a dry-weight basis (mg kg^{-1}), 12 g subsamples were weighed before and after oven-drying at 105ºC until reaching a constant weight to determine the moisture content. From the extracted solution, soil $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ concentrations were quantified through the steam distillation method (Keeney & Nelson, 2015).

Soil samples for total C and N analysis were collected in the surface layer of the pot (0-10cm) at the end of the experiment. These samples were oven-dried at 40°C for 48 hours, and then ground in pestle and mortar until all the soil passed through a 150 mm sieve. The samples were then, analyzed for total C and N determination by the dry combustion method, using a TruSpec CHN elemental analyzer (LECO - St. Joseph, MI, USA).

3.2.5. Biomass production and N_2O emission intensity

Sugarcane yield was measured by quantifying the above and belowground biomass. To separate both parts, the sugarcane plants were cut close to the soil surface after 60 days from the beginning of the experiment. To prepare the collected belowground biomass for measurement, it was thoroughly washed to remove soil particles. For dry weight determination of both parts, the materials were air dried at 35°C until maintaining a constant weight. The total biomass produced was considered the sum between sugarcane roots and leaves. This result was later utilized to calculate the N_2O emissions intensity parameter, obtained by the ratio between CO_2 eq emissions (g pot^{-1}) and total biomass (kg pot^{-1}). The CO_2eq emissions were obtained by converting the cumulative N_2O emissions to a CO_2 equivalent basis, considering the global warming potential of 273 (Foster et al., 2021).

3.2.6. Statistical analysis

All data analysis was performed using the R software (R Core Team, 2022). A one-way analysis of variance (ANOVA) was performed to assess the influence of N fertilizer application and biochar type on N_2O cumulative emissions, soil parameters (total C, total N, total C/N), and biomass production. The chemical composition and functional groups identified at the biochar surface were also compared through ANOVA. In the cases where the difference among treatments was significant at a 5% probability level, mean comparisons were performed according to Tukey's post hoc test using the *emmeans* package (Lenth, 2022). To evaluate the $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ concentration dynamics over time, a repeated-measures analysis of variance (MANOVA) test was performed (p <0.05) using the *"nmle"* (Pinheiro et al., 2022). Treatment was set as a fixed effect, whereas the interaction among replicates, treatments, and time dynamics was set as random effects.

As different sources of N affect mineralization over time, the inclusion of treatments or DAF was tested as the *a priori* known within-group heteroscedasticity structure to account for unequal variances (*varIdent, nlme*) in the MANOVA (Pinheiro & Bates, 2000). The weighted least squares model with treatments established as the heteroscedastic group was the one that best fitted the experimental data, and the inclusion of covariance structures did not improve the overall fit of the model. Normality and heterogeneity of the residual improvement was utilized to evaluate the addition of heteroscedasticity group and covariance structures. The goodness of fit between the simple and more complex models was assessed according to the AIC fit statistic (Burnham & Anderson, 2002). Additionally, principal component analysis (PCA) was used to assess the relationships between the cumulative soil N_2O emission, mineral N concentration, and the biochar surface characterization data (chemical composition and functional groups) by x-ray photoelectron spectroscopy (XPS), using the base R prcomp() function (n=4).

3.3. Results

3.3.1. Physicochemical characteristics of biochars

In order to obtain the chemical distribution of elements found in the biochar particles, captured by energy-dispersive x-ray spectroscopy (EDS), high-resolution images were obtained (Figure 8). The EDS spectrum showed that sugarcane straw biochar (SB) presented a higher proportion of O in its "bulk" elemental composition. Overall, the SB and BB showed higher

silica (Si) presence in their constitution than those obtained from forestry residues (Figure 8 a, b). Likewise, the EDS images obtained for PB and EB showed more significant calcium (Ca) concentration (Figure 8 c, d) than sugarcane-derived biochar.

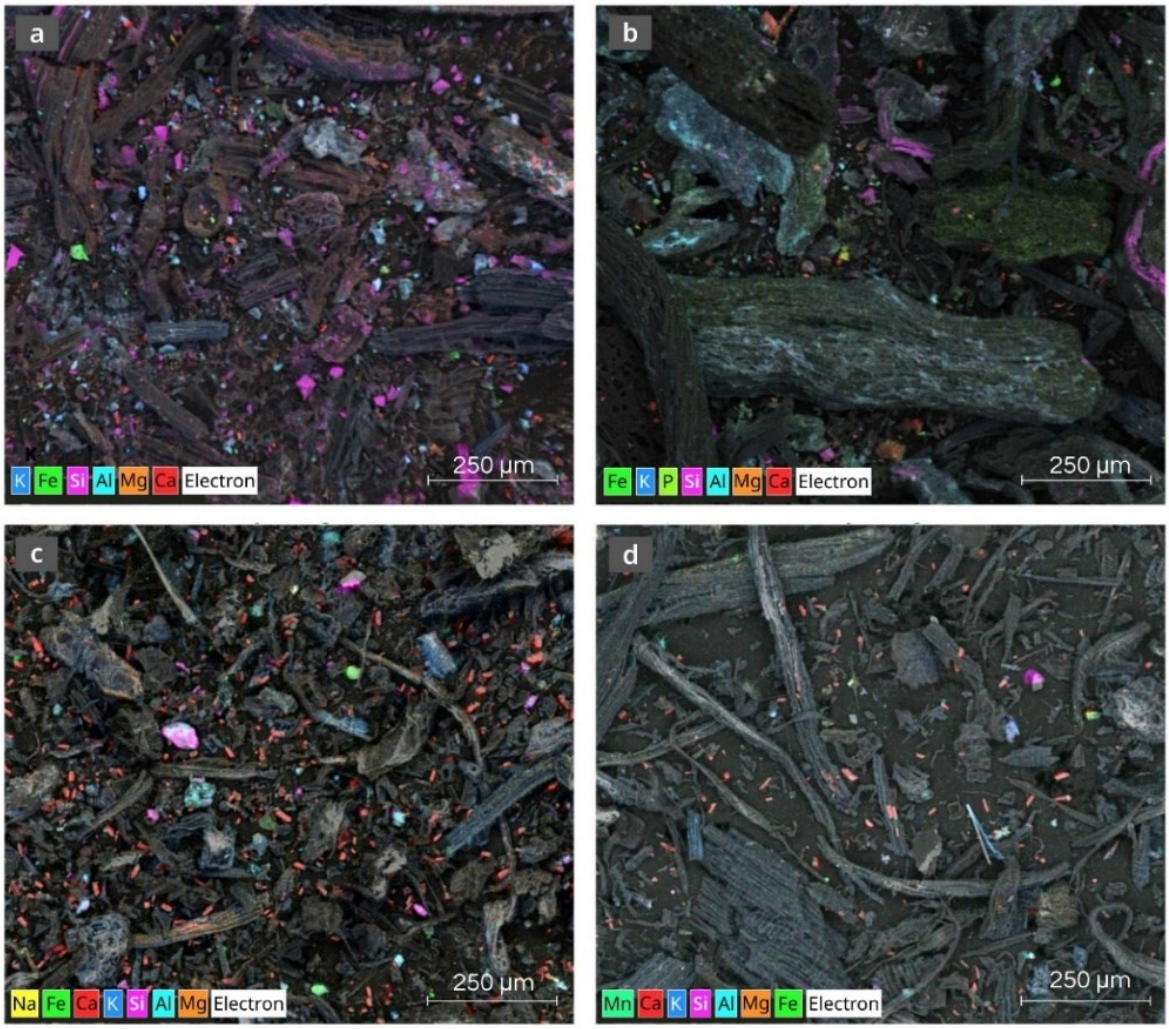

Figure 8. Chemical mapping by dispersive X-ray spectroscopy (EDS) of fresh biochar obtained from sugarcane straw (a) and bagasse (b), pine (c), and eucalyptus biomass (d).

The survey x-ray photoelectron spectroscopy (XPS) analysis showed that the chemical composition of the surface layer of the biochar was generally similar among sugarcane-derived biochars (SB and BB) (Figure 9). Likewise, PB and EB exhibited close similarity in the elemental characterization. The surface layer of wood-derived biochar particles had more C than SB and BB, which conversely presented 35-37% more O than PB and EB. The increase in the amount of O observed for SB and BB was seen mainly in the forms of hydroxyl/ether (C−O) and carbonyl/carboxyl (C=O) (Figure 10). Minor amounts of Si, Al, and N were also quantified for

the sugarcane residues biochar on a surface, which was not seen for those produced from pine and eucalyptus biomasses (Figure 9).

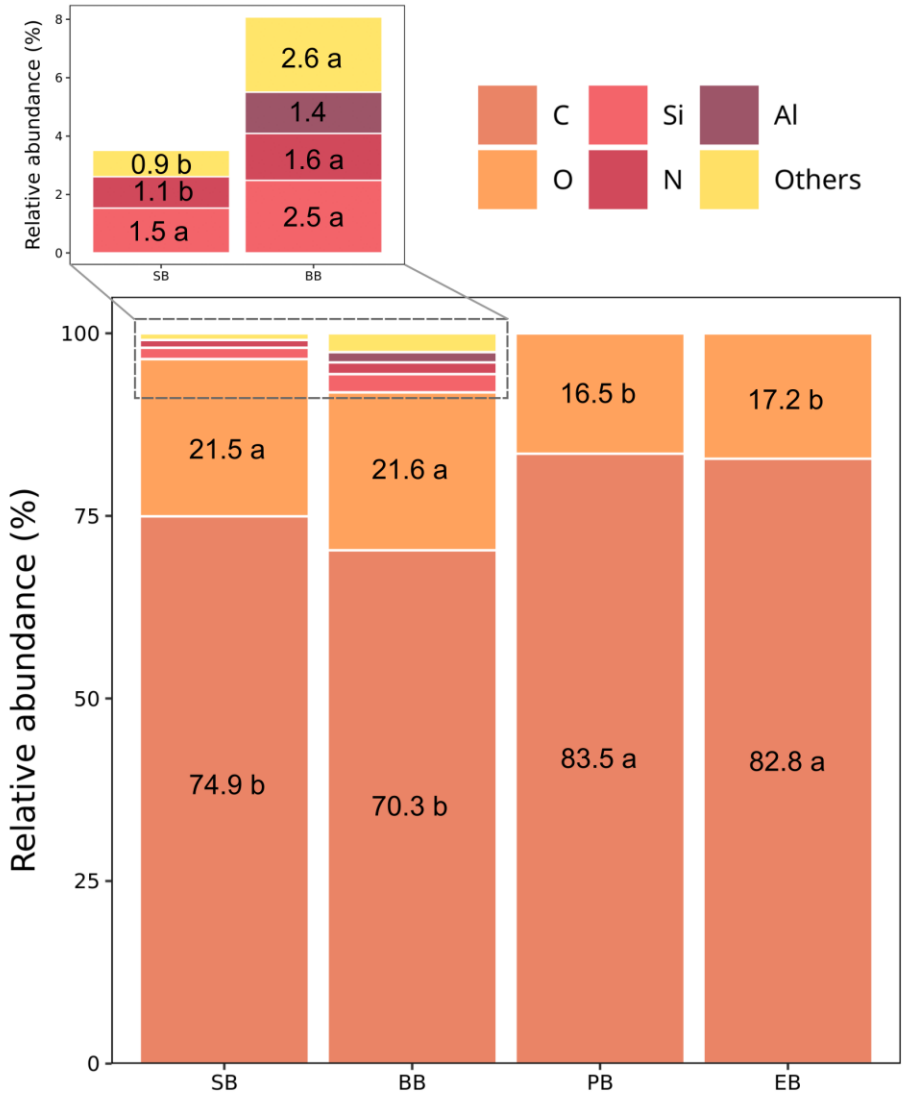

Figure 9. Survey x-ray photoelectron spectroscopy (XPS) analysis derived element composition of fresh biochar surface obtained from straw (SB), bagasse (BB), pine (PB), and eucalyptus (EB) biomass. The quantification elements of less than 1% were included in the "Others" category, including calcium, phosphorus, magnesium, and potassium Data (mean, n=3) followed by different letters denote significant differences among treatments by Tukey's test ($p < 0.05$).

The different types of biochar had a significant interaction with the chemical bonds identified at the surface of the biochar particles ($p < 0.05$; Table S5). All the biochars presented different C functional groups, albeit the dominant component from all samples was the aromatic C (C−C/C=C) (Figure 10). The analysis revealed that PB had the lowest share of oxidized C species among the evaluated biochars (C−O; C=O; O−C=O), while SB exhibited the highest

value. As for the presence of carbonyl/carboxyl (C=O) groups, SB had higher values than EB, being the only difference among the evaluated types of biochar. Moreover, EB exhibited the highest share of hydroxyl/ether (C−O) among the evaluated biochars (p<0.05). The analysis also revealed more carboxylic acid (O-C=O) molecules in SB and BB.

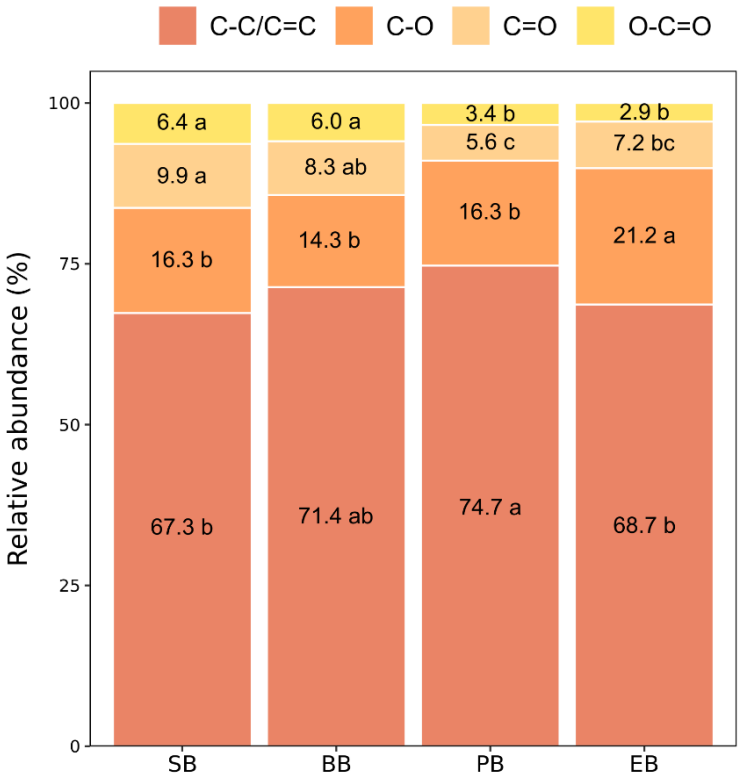

Figure 10. High-resolution carbon (HRC) x-ray photoelectron spectroscopy (XPS) results of the C-1s peak regional scan of different types of biochar obtained from straw (SB), bagasse (BB), pine (PB), and eucalyptus (EB) biomass. Data (mean, n=3) followed by different letters denote significant differences among treatments by Tukey's test (p < 0.05).

High-resolution images obtained using scanning electron microscopy (SEM) revealed highly porous and heterogenous surfaces of biochar particles, where biochar obtained from different feedstock biomasses presented distinct microstructure (Figure 11). Figure 11 b and d shows the similarity between the structures of parenchyma and xylem fibers in SB and BB, respectively, that could still be identified after pyrolysis. EB and PB amplification images reveal a structure with many pores and cracks on the material's surface (Figure 11 f, h). The wood-derived biochar also exhibited more heterogenicity in the images, as its feedstock material comprises different plant parts (leaves, twigs pieces, and bark).

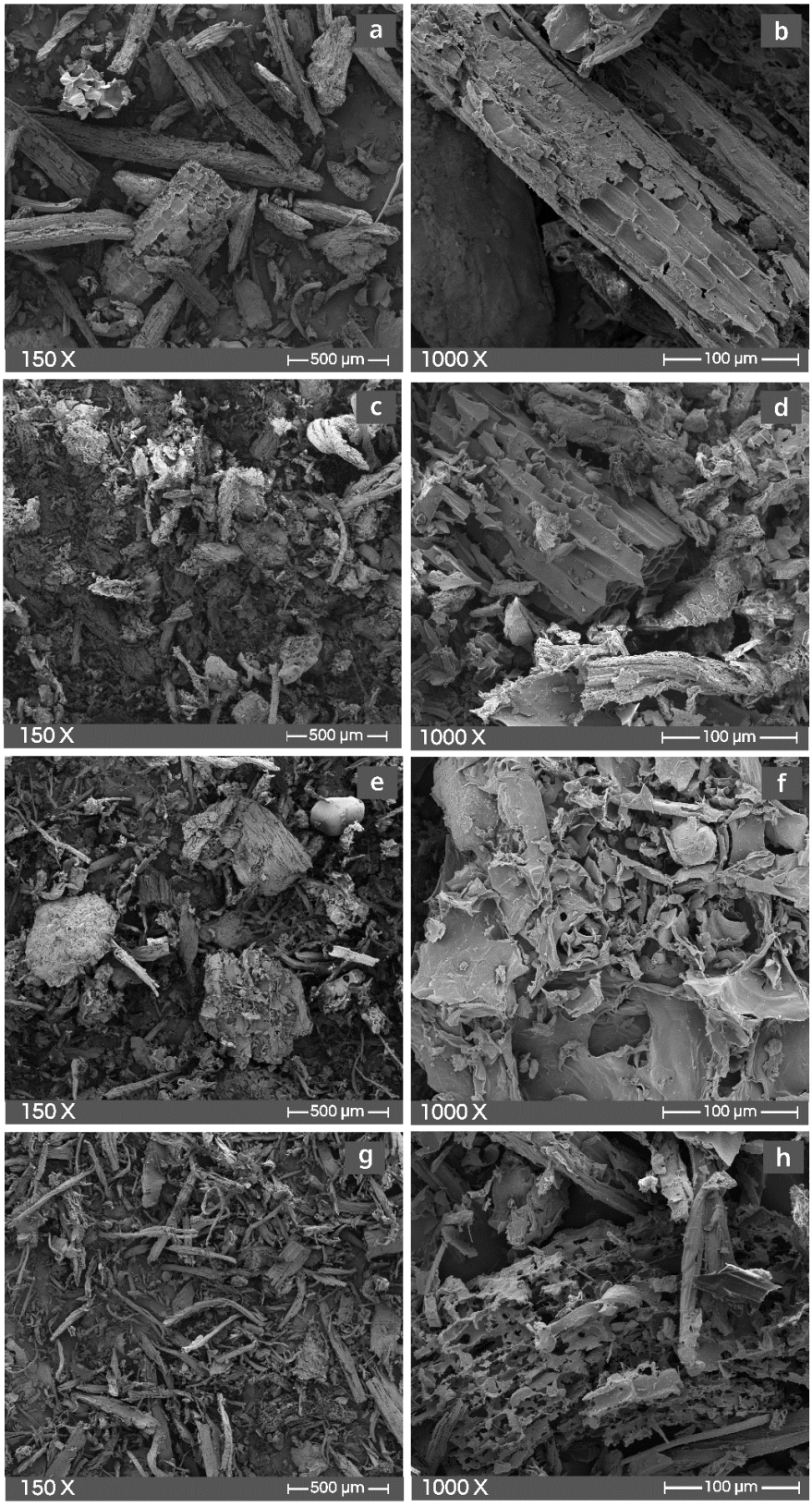

Figure 11. Particle surface microstructure images captured by scanning electron microscopy (SEM) of fresh biochar obtained from straw (a, b), bagasse (c, d), pine (e, f), and eucalyptus (g, h) biomass, at 150x (left column; scale bar 500 µm), and 1000x magnifications (right column; scale bar 100 µm).

3.3.2. N_2O fluxes and cumulative emissions

Increases in soil N_2O emissions appeared in the first 10 days after fertilizer application (DAF) (Figure 12 A). Among the treatments with N fertilizer addition, the N_2O fluxes were generally lower in soils under biochar application. Furthermore, the dynamics of N_2O fluxes showed similar patterns among biochar treatments throughout the experiment, where NF+EB registered the highest peak emission of 74 mg N m^{-2} day^{-1} at day 7. Soils under NF treatment emitted >80% of their total N_2O emissions only on the 43rd day, whereas this occurred between 17 to 25 DAF for biochar treatments. The peak emission under no N fertilizer application (CTR) was 29 mg N m^{-2} day^{-1} at day 3, stabilizing near zero from day 15. The ANOVA results showed that the cumulative N_2O emissions from biochar-containing treatments were lower than NF, regardless of the biochar type (p<0.05; Table S6). The most significant reduction was observed for NF+S, which had a cumulative N_2O emission 50% lower than NF (Figure 12 B). Reductions of 35, 35, and 25% were also seen for NF+BB, NF+PB, and NF+EB, respectively, compared to NF. The only difference between biochar-amended soils was seen for NF+EB and NF+SB, where the sugarcane-straw biochar led to a lower cumulative N_2O emission than NF+EB. Cumulative N_2O emission under NF was 1066 mg m^{-2}, representing an increase of 4.9 folds compared to CTR (219 mg m^{-2}).

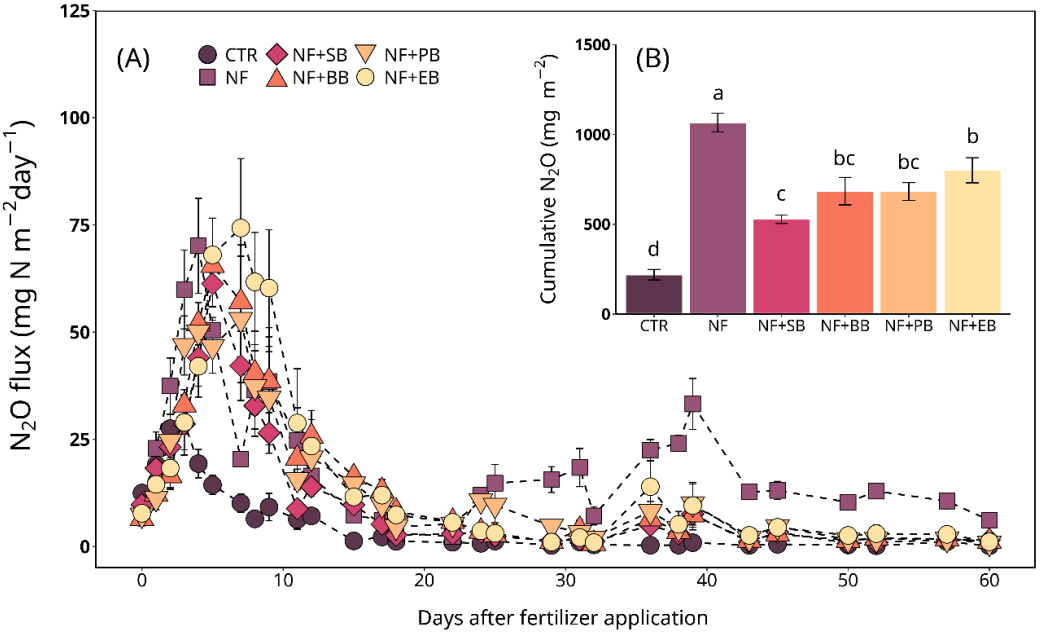

Figure 12. N_2O temporal fluxes (A) and cumulative emissions (B) in response to soil only (CTR), N fertilizer addition alone (NF), or combined with biochar produced from sugarcane straw (NF+SB), sugarcane bagasse (NF+BB), pine (NF+PB) and eucalyptus (NF+EB). Data (mean ± SE, n=4) followed by different letters denote significant differences among treatments by Tukey's test ($p < 0.05$).

3.3.3. Soil mineral N concentration dynamic

The availability of soil mineral N (N-NH_4^+ and N-NO_3^-) was significantly influenced by the addition of N fertilizer and biochar throughout the experiment ($p < 0.05$; Table S6). Soil N-NH_4^+ ranged from below detection levels under CTR, to 1202 mg N kg^{-1} under NF+BB at 4 DAF (Figure 13 A). By the 11 DAF, NF+SB exhibited higher soil N-NH_4^+ availability than NF. For N-NO_3^- concentration, only NF+BB had more N-NO_3^- available than CTR at 11 DAF (Figure 13 B). All treatments with biochar addition presented N-NO_3^- concentration levels bellow NF values at 39 DAF. At this period, NF+SB soils also exhibited more N-NO_3^- available than NF+PB soils. By the end of the experiment, NF+BB, NF+PB, and NF+EB continued to present soil N-NO_3^- levels below NF values, albeit all biochar-amended soils presented similar results. Moreover, NF+SB soils presented N-NO_3^- levels similar to NF.

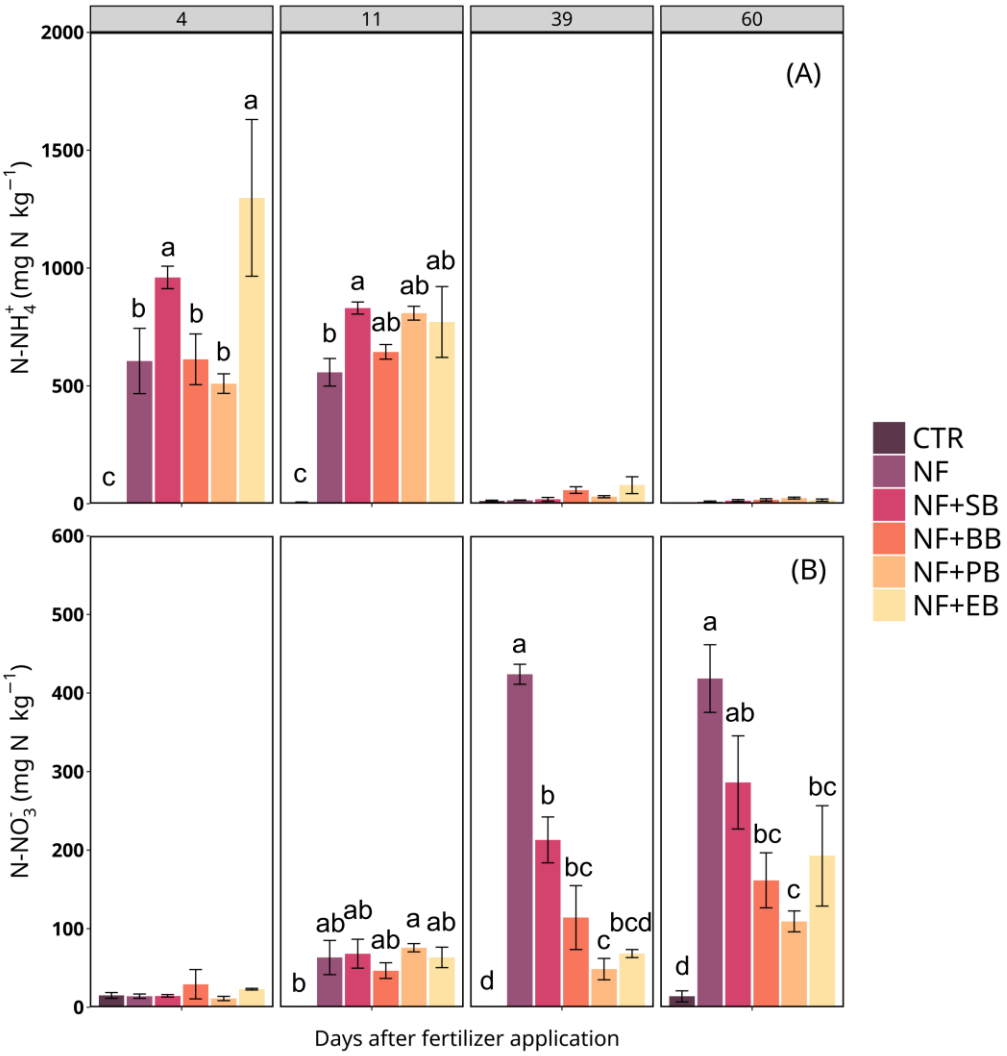

Figure 13: Ammonium N-NH_4^+ (A) and nitrate N-NO_3^- (B) soil concentration in response to soil only (CTR), N fertilizer addition alone (NF), or combined with biochar produced from sugarcane straw (NF+SB), sugarcane bagasse (NF+BB), pine (NF+PB) and eucalyptus (NF+EB). Data (mean ± SE, n=4) followed by different letters denote significant differences among treatments by Tukey's test ($p < 0.05$).

3.3.4. Soil total C, N, and C/N ratio

The ANOVA results showed a significant interaction between the evaluated treatments and soil C concentrations (Table S6). The soil C content ranged from 11 to 18 g kg- under NF and NF+PB, respectively (Figure 14). Soil C concentrations were highest under soils amended with wood-derived biochar, where NF+PB and NF+EB exhibited soil C levels 39% to 40% higher than NF. Soils amended with NF+BB presented C levels similar to CTR and NF. Whereas, NF+SB had soil C levels higher than NF, albeit being not different from CTR. Regarding total N concentration, NF+EB presented superior values than NF and CTR (p<0.05;

Table S7). The soil C/N ratio obtained under treatments with N fertilizer addition was similar, except for NF+PB which exhibited a superior C/N ratio (Table S7).

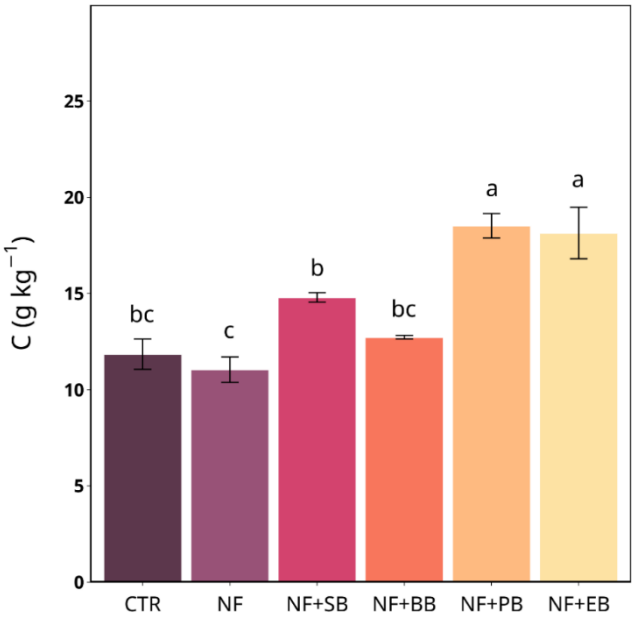

Figure 14. Soil total C concentration in response to soil only (CTR), N fertilizer addition alone (NF), or combined with biochar produced from sugarcane straw (NF+SB), sugarcane bagasse (NF+BB), pine (NF+PB) and eucalyptus (NF+EB). Data (mean ± SE, n=4) followed by different letters denote significant differences among treatments at Tukey's test ($p < 0.05$).

3.3.5. Biomass production and N_2O emission intensity

The aboveground biomass production varied from 13 - 16 g pot^{-1} under CTR and NF+B, respectively, and no significant differences among the treatments were observed (Table 6). Nevertheless, the one-way ANOVA analysis showed that belowground biomass and N_2O emission intensity had a significant interaction with treatments (p<0.05; Table S6). All treatments with NF addition had a similar effect on the belowground biomass parameter (Table 6). However, for belowground biomass, a higher value was observed NF+BB compared with CTR. The highest N_2O-emission intensity results were observed under treatments with N fertilizer addition, which presented EI values from 2 to 4.5-folds higher than CTR (Table 6). Soils under the NF presented higher N_2O emission intensity than biochar-amended soils, except for the NF+EB treatment. The NF+SB, NF+BB, and NF+PB treatments exhibited lower N_2O emission intensity by 40-55% compared to NF.

Table 6. N_2O Emission intensity, sugarcane aboveground and belowground sugarcane biomass (dry basis) in response to soil only (CTR), N fertilizer addition alone (NF), or combined with biochar produced from sugarcane straw (NF+SB), sugarcane bagasse (NF+BB), pine (NF+PB) and eucalyptus (NF+EB). Data (mean ± SE, n=4) followed by different letters denote significant differences among treatments by Tukey's test ($p < 0.05$).

Treatment	Aboveground Biomass (g pot $^{-1}$)	Belowground Biomass (g pot $^{-1}$)	N_2O Emission Intensity (g CO_2 eq. kg biomass $^{-1}$)
CTR	13.3 ± 0.4 a	13.7 ± 0.6 b	270 ± 39.7 d
NF	14.0 ± 0.5 a	14.6 ± 1.0 ab	1235 ± 61.9 a
NF+SB	14.5 ± 0.8 a	17.4 ± 1.5 ab	592 ± 8.7 c
NF+BB	16.7 ± 1.7 a	19.4 ± 1.8 a	680 ± 85.8 bc
NF+PB	15.1 ± 0.7 a	15.6 ± 0.4 ab	745 ± 79.0 bc
NF+EB	14.3 ± 1.0 a	15.2 ± 1.6 ab	924 ± 103.0 ab

3.3.6. Correlation between N_2O emissions, mineral N availability, and biochar functional groups

The PCA was used to explore the relationships between response variables (i.e. soil mineral N and cumulative N_2O emissions and biochar chemical properties (Figure 15). Taken together the cumulative proportion between the first (PC1) and second (PC2) principal components, it can explain 73.3% of the total variance observed in the data. The PC1 separated the different biochars into two groups: ones with high O content, carbonyl/carboxyl (C=O), carboxylic acid (O-C=O) on its surface and a high soil mineral N content; and biochars with higher C content, aromatic C (C−C/C=C), hydroxyl/ether (C−O), and N_2O emissions. The first group with more O-containing functional groups was associated with the sugarcane-derived biochar (SB and BB), whereas the group with more aromatic C and hydroxyl/ether (C−O) forms were linked with the wood-derived biochar (PB and EB). Overall, the PC2 splits wood-derived biochars with high aromatic C at the top (PB), and those with high hydroxyl/ether and N_2O values (EB) at the bottom. Increases in the hydroxyl/ether (C−O)form influenced a higher soil N_2O emission, as indicated by the strong positive relationship seen on both axes between the two variables. The content of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ correlated positively with the presence of O on the biochar surface, together with the carbonyl/carboxyl (C=O), and carboxylic acid (O-C=O) forms. Furthermore, carboxylic acid (O-C=O) forms showed a positive interaction with $N\text{-}NO_3^-$ concentrations.

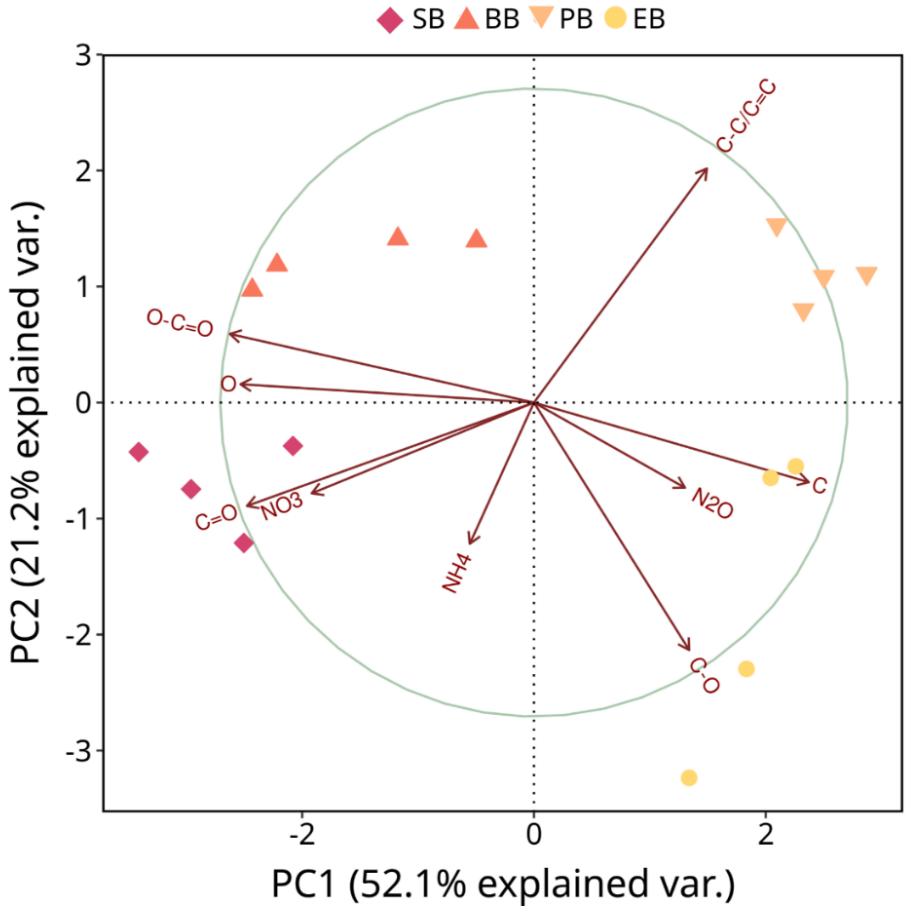

Figure 15: Principal component analysis (PCA) for soil cumulative N_2O emissions and N mineral content by the end of the experiment, and surface chemical composition (C and O) and C functional groups of biochars. Variables are represented by arrows, being better represented when the arrow points are close to the correlation circle. SB: sugarcane straw biochar; BB: sugarcane bagasse biochar; PB: pine biochar, EB: eucalyptus biochar. PC1: first principal component; PC2: second principal component.

3.4. Discussion

Overall, the biochar application in soil reduced N_2O emissions driven by N fertilizer regardless of the feedstock material used for biochar production. The magnitude of these reductions is consistent with previous meta-analyses that reported a range of 10 - 54% mitigation in N_2O emissions due to biochar application (Cayuela et al., 2014; Kaur et al., 2023; Shakoor et al., 2021). This effect has been observed in several climatic regions; however, few observations have been reported under tropical environments (Huang et al., 2023). Though, the data variability regarding this response can be high, with some reports mentioning no effect caused by biochar application on GHG consumption/emission patterns (Munera-Echeverri et al., 2022; Toczydlowski et al., 2023), or even stimulation of N_2O emission (Deng et al., 2019; Lin et al.,

2017; Mahmud et al., 2018). The lack of response is attributed to many factors, one of them being the biochar application at lower rates low rates (<10 Mg ha^{-1}), as greater N_2O suppression is reportedly associated with higher rates. (Borchard et al., 2019; Lyu et al., 2022; Zhang et al., 2023). Previous reports also mention that feedstock biomass can influence the final physicochemical properties of the biochar, thus influencing its capacity to suppress N_2O emissions (Cayuela et al., 2014; Lyu et al., 2022).

In our study, the feedstock used to produce biochar significantly influenced the magnitude of the reduction of N_2O emissions. This result is supported by previous works pointing out that the type of feedstock utilized for biochar production interacts primarily with cumulative N_2O emissions (Deng et al., 2021; Tarin et al., 2021). The most significant reduction was seen in soils amended with sugarcane straw biochar, where N_2O emissions were 50% lower than in NF (Figure 12 B). This finding corroborates with studies that have observed suppression of N_2O emissions caused by straw-based biochars (Abbruzzini et al., 2019; Hamad et al., 2023; Zhang et al., 2022). Conversely to previous studies that reported no reductions in soil N_2O emissions in response to woody biochar application (Lan et al., 2019; Ramlow et al., 2019), we observed that both wood-based biochars reduced N_2O emissions. Moreover, BB and PB biochar were similarly effective in reducing N_2O emissions by 35% compared to straw-based biochar. Overall, we observed that straw-biochar was more efficient in reducing N_2O emissions than the eucalyptus derived-biochar. In line with our observations, Grutzmacher et al. (2018) found that biochar from sugarcane filter-cake presented a greater capacity to reduce N_2O emissions induced by N fertilizer application than the biochar from eucalyptus-sawdust. This effect might be related to the lignin content of the eucalyptus biomass since feedstocks biomasses with higher lignin content can result in a biochar with a lower N_2O mitigation capacity (Li et al., 2023; Pascual et al., 2020).

Recent studies have observed that specific O-containing functional groups in the biomass can reduce the biochar capacity to transfer electrons to N_2O-consuming microorganisms, further impairing N_2O reduction to N_2 (Yuan et al., 2021; Yuan et al., 2019). A hypothesis for the lowest mitigation effect registered for EB is the higher share of hydroxyl/ether (C−O) groups seen in the material (Figure 10), which exhibited a strong positive relationship with N_2O emissions). One of the possible reasons for this effect is the link between O-containing functional groups and the biochar capacity to reduce N_2O emissions by acting as an "electron shuttle" (Cayuela et al., 2013; Yuan et al., 2019). The biochar capacity to mediate extracellular electron transfer reactions is due to reactive surface O functional groups, which can influence the

metabolism of microorganisms involved in the N cycle (Joseph et al., 2021; Lovley & Holmes, 2022). Conversely to our findings, these previous studies concluded that carboxyl and carbonyl (C=O) are the main functional groups most likely to inhibit N_2O reduction. Nevertheless, these mechanisms are not completely elucidated, and more specific studies are necessary to unravel the impact of functional groups of feedstock biochar on N-related microorganisms in tropical soils.

Along with the feedstock material influence on N_2O emissions, we found that it also affected the different amounts of functional groups and the chemical composition of the biochar surface. One hypothesis for this interaction could be the effect of the feedstock properties on functional groups expressed by biochar, such as the relative proportions of cellulose, hemicellulose, and lignin (Chacón et al., 2017). Overall, the biochars were separated into two groups based on their similarity between chemical properties and impacts on N-related parameters: sugarcane-based biochars (SB and BB) and wood-derived biochars (PB and EB). Our results showed that biochars derived from sugarcane had a higher oxygen content on its surface than wood-derived biochars (Figure 9). In line with our findings, previous studies highlighted that biochar produced from crop residues, such as SB and BB, exhibits higher cation exchange capacity (CEC) than wood-based biochar (Ippolito et al., 2020).

Furthermore, we observed a positive relationship between $N\text{-}NH_4^+$ soil concentration, O surface content, carbonyl/carboxyl (C=O), and carboxylic acid (O-C=O) groups, which were higher in SB and BB (Figure 15). An explanation for this correlation is the link between cation exchange capacity (CEC) and O-containing functional groups in biochar, which can attract electrostatically available cations in the soil solution (Dai et al., 2021; Gai et al., 2014; Hansen et al., 2016). Thus, biochar with more acidic functional groups, such as carboxylic acid and carboxyl, will express more negative charges in a standard pH range found in soils. However, no relevant influences of biochar types on the soil $N\text{-}NH_4^+$ dynamics were observed during the experimental period under soils amended with biochar (Figure 13 A). Similar results have been reported, highlighting that CEC of biochar particles was ineffective for $N\text{-}NH_4^+$ retention (Abbruzzini et al., 2019; Castejón-del Pino et al., 2023). A possible explanation for this lack of response may be that biochar CEC increases with the residence time of the material in soil due to the oxidation of aliphatic and aromatic C forms (Joseph et al., 2021; Liang et al., 2006) and, the short period of 10 days between biochar application, and subsequent N fertilizer application and soil $N\text{-}NH_4^+$ evaluation (< 1 month) may not have been enough to show major changes on $N\text{-}NH_4^+$ dynamics.

The most considerable retention effect caused by biochar in soil mineral N was registered for $N\text{-}NO_3^-$ concentrations (Figure 13 B). Despite the biochar having a low anionic

exchange capacity (Gai et al., 2014), we observed while evaluating the $N\text{-}NO_3^-$ soil dynamics a significant reduction in the anion availability under soils amended with biochar. This reduction only appeared after an increase in the anion availability was registered, which occurred as the nitrification process of the $N\text{-}NH_4^+$ added through N fertilizer. Our findings corroborate previous works highlighting a decrease in soil $N\text{-}NO_3^-$ values following biochar application (Jia et al., 2023) due to $N\text{-}NO_3^-$ retention inside the biochar particles (Hagemann et al., 2017; Haider et al., 2020). Among the evaluated treatments, only NF+SB presented values similar to NF in the sampling periods with significative differences, indicating a lower retention capacity of $N\text{-}NO_3^-$. However, the soil $N\text{-}NO_3^-$ response to biochar application can be highly variable and even unrelated to the biochar feedstock material (Borchard et al., 2019).

The interaction between biochar and soil N also influenced the N_2O emission intensity (Table 6). Although all biochar-amended soils had similar emission intensity, only the eucalyptus-derived did not differ from the N fertilizer treatment. Previous studies highlighted the biochar capacity to decrease N_2O emission intensity (Huang et al., 2023; Liu et al., 2019). However, Huang et al. (2023) observed that reductions in soil emission intensity were higher for soils after the application of wood-based biochar than herbaceous biochar, which was inconsistent with our findings. As the emission intensity infers on the yield-scaled N_2O emissions (Campanha et al., 2019; Sainju, 2016), the low differences among biomass production can result in an N_2O emission intensity more similar to the cumulative GHG values. Thus, a hypothesis for our contrasting results regarding wood-based biochar impact on N_2O emission intensity might be due to slight changes in the sugarcane aboveground biomass production (Table 6). This response differs from previous works that have highlighted the biochar capacity to increase crop yield under tropical conditions (Huang et al., 2023; Liu et al., 2019). Regarding belowground biomass, the only difference was seen for NF+BB, which presented higher production than CTR. However, the increase between the two treatments was negligible since the values of biomass production were low. One of the results of biochar soil application is the increase in water hold capacity (Jesus et al., 2023), which can benefit plant growth due to higher water availability. As soil moisture was kept at the same level for all treatments, this could have hampered a key mechanism by which biochar could increase plant productivity in field conditions.

Previous studies have registered increases in soil C content following biochar application, where the magnitude of this response can be influenced by the biochar feedstock material, with higher soil C accumulation associated primarily with wood sources (Huang et al., 2023; Liu et al., 2016). Consistent with these findings, we observed a higher increase in the C

storage under soils amended with wood-based biochar than the ones derived from sugarcane residues, albeit C concentration in biochar-amended soil from sugarcane straw was higher than in the NF treatment (Figure 14). Reportedly, wood-derived biochar has a higher C share in its chemical composition (Ippolito et al., 2020; Li et al., 2023). Thus, applying biochar derived from wood sources would increase the C input to agricultural soils. Regarding the C content on the biochar surface, we also observed that PB and EB presented higher values than straw and bagasse-based biochars (Figure 9). Thus, the feedstock material derived from wood proved to be the most appropriate type of biochar to be adopted for an immediate increase in soil C levels. Analyzing the C forms expressed in each biochar, we found that the PB exhibited a slightly higher proportion of aromatic C structures (Figure 10), which may contribute to increasing its mean residence time in soil (Leng et al., 2019; McBeath et al., 2015; Yang et al., 2022). This effect may be more linked with the higher temperature utilized in the pyrolysis of PB, as it is reported to be the main factor influencing the degree of C condensation into aromatic forms (Paiva et al., 2022; Singh et al., 2014; Tomczyk et al., 2020).

Two critical aspects related to biochar usage as a soil amendment are the increase of soil C storage while simultaneously reducing GHG emissions (Paustian et al., 2016; Woolf et al., 2021), and both were observed in our study, confirming the "win-win" status of some biochar feedstocks. The present research has advanced our understanding of the effects that feedstock biomass could have on the biochar capacity to mitigate N_2O emissions. Overall, the biochar application to soil of all treatments was able to reduce N_2O emissions caused by N fertilizer applied, while the wood-derived biochars showed greater C storage in the soil. Hence, the significant interaction between the feedstock material and N_2O emissions indicates that this parameter could be considered as a possible predictor of GHG response under tropical environments in future studies. However, it is important to highlight that the production cost associated with biochar production can limit its adoption on a larger scale (Maroušek et al., 2019), so the need to develop strategies for more efficient biochar production is crucial to enhance the chances of adoption. Thus, our results regarding the different feedstock materials available on a larger scale in Brazil and their effects on the soil-plant-atmosphere system can help predict its use and production benefits, encouraging future sustainable strategies that aim to incorporate this practice as a nature-based solution.

3.5. Conclusions

Our findings suggest that biochar usage as a soil amendment reduced N_2O emissions, and some feedstock materials increased soil C sequestration. Both sugarcane residues biomasses (straw and bagasse) are considered feasible feedstocks, resulting in biochar capable of decreasing N_2O fluxes. Our hypothesis was partially confirmed, as eucalyptus-biochar expressed a lower capacity to reduce the amount of N_2O emitted from the soil than sugarcane straw biochar. However, pine-derived biochar exhibited a similar interaction with N_2O as the straw and bagasse biochar. Thus, among the available biomasses evaluated, using eucalyptus biochar is the least recommended option if the final objective of the practice is the suppression of N_2O fluxes. In a scenario where forest residues are explored for biochar production, pine residue is a viable option to decrease N_2O emissions to levels similar to herbaceous sources while promoting a high C input and soil C storage compared to other feedstocks. Furthermore, the feedstock material influenced the final chemical composition and C forms found on the biochar surface, where forestry residue biochar had a higher C content than sugarcane residues. Hence, the significant interaction between the feedstock material and N_2O emissions indicates that this parameter could be considered a possible predictor of GHG response under tropical environments in future studies.

References

Abbruzzini, T. F., Davies, C. A., Toledo, F. H., & Cerri, C. E. P. (2019). Dynamic biochar effects on nitrogen use efficiency, crop yield and soil nitrous oxide emissions during a tropical wheat-growing season. *Journal of Environmental Management*, *252*(September), 109638. https://doi.org/10.1016/j.jenvman.2019.109638

Blanco-Canqui, H. (2021). Does biochar improve all soil ecosystem services? *GCB Bioenergy*, *13*(2), 291–304. https://doi.org/10.1111/gcbb.12783

Boateng, K. K., Obeng, G. Y., & Mensah, E. (2020). Eco-Friendly Yield and Greenhouse Gas Emissions as Affected by Fertilization Type in a Tropical Smallholder Rice System, Ghana. *Sustainability*, *12*(24), 10239. https://doi.org/10.3390/su122410239

Borchard, N., Schirrmann, M., Cayuela, M. L., Kammann, C., Wrage-Mönnig, N., Estavillo, J. M., Fuertes-Mendizábal, T., Sigua, G., Spokas, K., Ippolito, J. A., & Novak, J. (2019). Biochar, soil and land-use interactions that reduce nitrate leaching and N 2 O emissions: A meta-analysis. *Science of the Total Environment*, *651*, 2354–2364. https://doi.org/10.1016/j.scitotenv.2018.10.060

Buresh, R., Austin, E., & Craswell, E. (1982). Analytical methods in15N research. *Fertilizer Research*, *3*(1), 37–62. https://doi.org/10.1007/BF01063408

Burnham, K. P., & Anderson, D. R. (2002). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach* (2nd ed.). Springer.

Campanha, M. M., de Oliveira, A. D., Marriel, I. E., Gontijo Neto, M. M., Malaquias, J. V., Landau, E. C., de Albuquerque Filho, M. R., Ribeiro, F. P., & de Carvalho, A. M. (2019). Effect of soil tillage and N fertilization on N2O mitigation in maize in the Brazilian Cerrado. *Science of the Total Environment*, *692*, 1165–1174. https://doi.org/10.1016/j.scitotenv.2019.07.315

Castejón-del Pino, R., Cayuela, M. L., Sánchez-García, M., & Sánchez-Monedero, M. A. (2023). Nitrogen availability in biochar-based fertilizers depending on activation treatment and nitrogen source. *Waste Management*, *158*(December 2022), 76–83. https://doi.org/10.1016/j.wasman.2023.01.007

Cayuela, M. L., Sánchez-Monedero, M. A., Roig, A., Hanley, K., Enders, A., & Lehmann, J. (2013). Biochar and denitrification in soils: When, how much and why does biochar reduce N2O emissions? *Scientific Reports*, *3*(Experiment 2), 1–7. https://doi.org/10.1038/srep01732

Cayuela, M. L., Van Zwieten, L., Singh, B. P., Jeffery, S., Roig, A., & Sánchez-Monedero, M. A. (2014). Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. *Ecosystems and Environment*, *191*, 5–16. https://doi.org/10.1016/j.agee.2013.10.009

Cervi, W. R., Lamparelli, R. A. C., Gallo, B. C., de Oliveira Bordonal, R., Seabra, J. E. A., Junginger, M., & van der Hilst, F. (2021). Mapping the environmental and techno-economic potential of biojet fuel production from biomass residues in Brazil. *Biofuels, Bioproducts and Biorefining*, *15*(1), 282–304. https://doi.org/10.1002/bbb.2161

Chacón, F. J., Cayuela, M. L., Roig, A., & Sánchez-Monedero, M. A. (2017). Understanding, measuring and tuning the electrochemical properties of biochar for environmental applications. *Reviews in Environmental Science and Biotechnology*, *16*(4), 695–715. https://doi.org/10.1007/s11157-017-9450-1

Dai, W., Xu, M., Zhao, Z., Zheng, J., Huang, F., Wang, H., Liu, C., & Xiao, R. (2021). Characteristics and quantification of mechanisms of Cd2+ adsorption by biochars derived from three different plant-based biomass. *Arabian Journal of Chemistry*, *14*(5), 103119. https://doi.org/10.1016/j.arabjc.2021.103119

Dawar, K., Khan, H., Zaman, M., Muller, C., Alam, S. S., Fahad, S., Alwahibi, M. S., Alkahtani, J., Saeed, B., Saud, S., Hammad, H. M., & Nasim, W. (2021). The Effect of Biochar and Nitrogen Inhibitor on Ammonia and Nitrous Oxide Emissions and Wheat Productivity. *Journal of Plant Growth Regulation*, *0123456789*. https://doi.org/10.1007/s00344-020-10283-1

Deng, B. L., Wang, S. L., Xu, X. T., Wang, H., Hu, D. N., Guo, X. M., Shi, Q. H., Siemann, E., & Zhang, L. (2019). Effects of biochar and dicyandiamide combination on nitrous oxide emissions from Camellia oleifera field soil. *Environmental Science and Pollution Research*, *26*(4), 4070–4077. https://doi.org/10.1007/S11356-018-3900-3

Deng, B., Yuan, X., Siemann, E., Wang, S., Fang, H., Wang, B., Gao, Y., Shad, N., Liu, X., Zhang, W., Guo, X., & Zhang, L. (2021). Feedstock particle size and pyrolysis temperature regulate effects of biochar on soil nitrous oxide and carbon dioxide emissions. *Waste Management*, *120*, 33–40. https://doi.org/10.1016/j.wasman.2020.11.015

Feng, Z., Sheng, Y., Cai, F., Wang, W., & Zhu, L. (2018). Separated pathways for biochar to affect soil N2O emission under different moisture contents. *Science of the Total Environment*, *645*, 887–894. https://doi.org/10.1016/j.scitotenv.2018.07.224

Foster, P., Storelvmo, T., Armour, K., Collins, W., Dufresne, J. L., Frame, D., Lunt, D. J., Mauritsen, T., Palmer, M. D., Watanabe, M., Wild, M., & Zhang, H. (2021). The Earth's Energy Budget, Climate Feedbacks, and Climate Sensitivity. In 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, & B. Zhou (Eds.), *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 923–1054). Cambridge University Press.

Gai, X., Wang, H., Liu, J., Zhai, L., Liu, S., Ren, T., & Liu, H. (2014). Effects of feedstock and pyrolysis temperature on biochar adsorption of ammonium and nitrate. *PLoS ONE*, *9*(12), 1–19. https://doi.org/10.1371/journal.pone.0113888

Grutzmacher, P., Puga, A. P., Bibar, M. P. S., Coscione, A. R., Packer, A. P., & de Andrade, C. A. (2018). Carbon stability and mitigation of fertilizer induced N2O emissions in soil amended with biochar. *Science of the Total Environment*, *625*, 1459–1466. https://doi.org/10.1016/j.scitotenv.2017.12.196

Hagemann, N., Joseph, S., Schmidt, H. P., Kammann, C. I., Harter, J., Borch, T., Young, R. B., Varga, K., Taherymoosavi, S., Elliott, K. W., McKenna, A., Albu, M., Mayrhofer, C., Obst, M., Conte, P., Dieguez-Alonso, A., Orsetti, S., Subdiaga, E., Behrens, S., & Kappler, A. (2017). Organic coating on biochar explains its nutrient retention and stimulation of soil fertility. *Nature Communications*, *8*(1), 1–11. https://doi.org/10.1038/s41467-017-01123-0

Haider, G., Joseph, S., Steffens, D., Müller, C., Taherymoosavi, S., Mitchell, D., & Kammann, C. I. (2020). Mineral nitrogen captured in field-aged biochar is plant-available. *Scientific Reports*, *10*(1), 1–12. https://doi.org/10.1038/s41598-020-70586-x

Hamad, A. A. A., Ni, L., Shaghaleh, H., Elsadek, E., & Hamoud, Y. A. (2023). Effect of Carbon Content in Wheat Straw Biochar on N2O and CO2 Emissions and Pakchoi Productivity Under Different Soil Moisture Conditions. *Sustainability*, *15*(6), 5100. https://doi.org/10.3390/su15065100

Hansen, V., Müller-Stöver, D., Munkholm, L. J., Peltre, C., Hauggaard-Nielsen, H., & Jensen, L. S. (2016). The effect of straw and wood gasification biochar on carbon sequestration, selected soil fertility indicators and functional groups in soil: An incubation study. *Geoderma*, *269*, 99–107. https://doi.org/10.1016/j.geoderma.2016.01.033

Huang, Y., Tao, B., Lal, R., Lorenz, K., Jacinthe, P. A., Shrestha, R. K., Bai, X., Singh, M. P., Lindsey, L. E., & Ren, W. (2023). A global synthesis of biochar's sustainability in climate-smart agriculture - Evidence from field and laboratory experiments. *Renewable and Sustainable Energy Reviews*, *172*(November 2022), 113042. https://doi.org/10.1016/j.rser.2022.113042

Ippolito, J. A., Cui, L., Kammann, C., Wrage-Mönnig, N., Estavillo, J. M., Fuertes-Mendizabal, T., Cayuela, M. L., Sigua, G., Novak, J., Spokas, K., & Borchard, N. (2020). Feedstock choice, pyrolysis temperature and type influence biochar characteristics: a comprehensive meta-data analysis review. *Biochar*, *2*(4), 421–438. https://doi.org/10.1007/s42773-020-00067-x

IUSS Working Group WRB. 2022. World Reference Base for Soil Resources. International soil classification system for naming soils and creating legends for soil maps. 4th edition. International Union of Soil Sciences (IUSS), Vienna, Austria.

Jantalia, C. P., dos Santos, H. P., Urquiaga, S., Boddey, R. M., & Alves, B. J. R. (2008). Fluxes of nitrous oxide from soil under different crop rotations and tillage systems in the South of Brazil. *Nutrient Cycling in Agroecosystems*, *82*(2), 161–173. https://doi.org/10.1007/s10705-008-9178-y

Jesus, S. De, Carlos, D., Pellegrino, E., Tatiana, C., Rittl, F., Fernanda, T., Blanca, A., & Prado, L. (2023). Biochar Physical and Hydrological Characterization to Improve Soil Attributes for Plant Production. *Journal of Soil Science and Plant Nutrition*. https://doi.org/10.1007/s42729-023-01273-9

Jia, X., Yan, W., Ma, H., & Shangguan, Z. (2023). Antagonistic and synergistic interactions dominate GHGs fluxes, soil properties and yield responses to biochar and N addition. *Frontiers in Environmental Science*, *11*(March), 1–12. https://doi.org/10.3389/fenvs.2023.1123897

Joseph, S., Cowie, A. L., Van Zwieten, L., Bolan, N., Budai, A., Buss, W., Cayuela, M. L., Graber, E. R., Ippolito, J. A., Kuzyakov, Y., Luo, Y., Ok, Y. S., Palansooriya, K. N., Shepherd, J., Stephens, S., Weng, Z. (Han), & Lehmann, J. (2021). How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar. *GCB Bioenergy*, *January*, 1–34. https://doi.org/10.1111/gcbb.12885

Joy, D. C., Romig, A. D., & Goldstein, J. I. (Eds.). (1986). *Principles of Analytical Electron Microscopy*. Springer US. https://doi.org/10.1007/978-1-4899-2037-9

Kambo, H. S., & Dutta, A. (2015). A comparative review of biochar and hydrochar in terms of production, physico-chemical properties and applications. *Renewable and Sustainable Energy Reviews*, *45*, 359–378. https://doi.org/10.1016/j.rser.2015.01.050

Kan, T., Strezov, V., & Evans, T. J. (2016). Lignocellulosic biomass pyrolysis: A review of product properties and effects of pyrolysis parameters. *Renewable and Sustainable Energy Reviews*, *57*, 1126–1140. https://doi.org/10.1016/j.rser.2015.12.185

Kaur, N., Kieffer, C., Ren, W., & Hui, D. (2023). How much is soil nitrous oxide emission reduced with biochar application? An evaluation of meta-analyses. *GCB Bioenergy*, *15*(1), 24–37. https://doi.org/10.1111/gcbb.13003

Keeney, D. R., & Nelson, D. W. (2015). *Nitrogen-Inorganic Forms* (pp. 643–698). https://doi.org/10.2134/agronmonogr9.2.2ed.c33

Kern, J., Giani, L., Teixeira, W., Lanza, G., & Glaser, B. (2019). What can we learn from ancient fertile anthropic soil (Amazonian Dark Earths, shell mounds, Plaggen soil) for soil carbon sequestration? *Catena*, *172*, 104–112. https://doi.org/10.1016/J.CATENA.2018.08.008

Kollah, B., Ahirwar, U., Parmar, R., Devi, M. H., Atoliya, N., Shinoji, K. C., Patra, A., Singh, A. B., Dubey, G., & Mohanty, S. R. (2022). How do biochar size fractions and organic fertilizers interactively influence nitrous oxide emission from a tropical vertisol? *Journal of Plant Nutrition and Soil Science*, *185*(6), 888–900. https://doi.org/10.1002/jpln.202100150

Lan, Z. M., Chen, C. R., Rezaei Rashti, M., Yang, H., & Zhang, D. K. (2019). Linking feedstock and application rate of biochars to N2O emission in a sandy loam soil: Potential mechanisms. *Geoderma*, *337*(August 2018), 880–892. https://doi.org/10.1016/j.geoderma.2018.11.007

Lehmann, J., Cowie, A., Masiello, C., Kammann, C., Woolf, D., Amonette, J., Cayuela, M., Camps-Arbestain, M., & Whitman, T. (2021). Biochar in climate change mitigation. *Nat Geosci (in Press)*, *14*(December). https://doi.org/10.1038/s41561-021-00852-8

Lehmann, J., Gaunt, J., & Rondon, M. (2006). Bio-char sequestration in terrestrial ecosystems - A review. *Mitigation and Adaptation Strategies for Global Change*, *11*(2), 403–427. https://doi.org/10.1007/s11027-005-9006-5

Leng, L., Huang, H., Li, H., Li, J., & Zhou, W. (2019). Biochar stability assessment methods: A review. *Science of the Total Environment*, *647*, 210–222. https://doi.org/10.1016/j.scitotenv.2018.07.402

Lenth, R. (2022). *emmeans: Estimated Marginal Means, aka Least-Squares Means_* (R package version 1.8.0). https://CRAN.R-project.org/package=emmeans..

Li, L., Long, A., Fossum, B., & Kaiser, M. (2023). Effects of pyrolysis temperature and feedstock type on biochar characteristics pertinent to soil carbon and soil health: A meta-analysis. *Soil Use and Management*, *39*(1), 43–52. https://doi.org/10.1111/sum.12848

Li, S., Harris, S., Anandhi, A., & Chen, G. (2019). Predicting biochar properties and functions based on feedstock and pyrolysis temperature: A review and data syntheses. *Journal of Cleaner Production*, *215*, 890–902. https://doi.org/10.1016/j.jclepro.2019.01.106

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad, J. O., Thies, J., Luizão, F. J., Petersen, J., & Neves, E. G. (2006). Black Carbon Increases Cation Exchange Capacity in Soils. *Soil Science Society of America Journal*, *70*(5), 1719–1730. https://doi.org/10.2136/sssaj2005.0383

Liao, J., Hu, A., Zhao, Z., Liu, X., Jiang, C., & Zhang, Z. (2021). Biochar with large specific surface area recruits N2O-reducing microbes and mitigate N2O emission. *Soil Biology and Biochemistry*, *156*(May 2020), 108212. https://doi.org/10.1016/j.soilbio.2021.108212

Lin, Y., Ding, W., Liu, D., He, T., Yoo, G., Yuan, J., Chen, Z., & Fan, J. (2017). *Wheat straw-derived biochar amendment stimulated N 2 O emissions from rice paddy soils by regulating the amoA genes of ammonia-oxidizing bacteria*. https://doi.org/10.1016/j.soilbio.2017.06.001

Liu, S., Zhang, Y., Zong, Y., Hu, Z., Wu, S., Zhou, J., Jin, Y., & Zou, J. (2016). Response of soil carbon dioxide fluxes, soil organic carbon and microbial biomass carbon to biochar amendment: A meta-analysis. *GCB Bioenergy*, *8*(2), 392–406. https://doi.org/10.1111/gcbb.12265

Liu, X., Mao, P., Li, L., & Ma, J. (2019). Impact of biochar application on yield-scaled greenhouse gas intensity: A meta-analysis. *Science of the Total Environment*, *656*, 969–976. https://doi.org/10.1016/j.scitotenv.2018.11.396

Lovley, D. R., & Holmes, D. E. (2022). Electromicrobiology: the ecophysiology of phylogenetically diverse electroactive microorganisms. *Nature Reviews Microbiology*, *20*(1), 5–19. https://doi.org/10.1038/S41579-021-00597-6

Lyu, H., Zhang, H., Chu, M., Zhang, C., Tang, J., Chang, S. X., Mašek, O., & Ok, Y. S. (2022). Biochar affects greenhouse gas emissions in various environments: A critical review. *Land Degradation & Development*, *January 2021*, 1–16. https://doi.org/10.1002/ldr.4405

Mahmud, A. F., Camps-Arbestain, M., & Hedley, M. (2018). Investigating the influence of biochar particle size and depth of placement on nitrous oxide (N2 o) emissions from simulated urine patches. *Agriculture (Switzerland)*, *8*(11). https://doi.org/10.3390/agriculture8110175

Maroušek, J., Strunecký, O., & Stehel, V. (2019). Biochar farming: defining economically perspective applications. *Clean Technologies and Environmental Policy*, *21*(7), 1389–1395. https://doi.org/10.1007/s10098-019-01728-7

McBeath, A. V., Wurster, C. M., & Bird, M. I. (2015). Influence of feedstock properties and pyrolysis conditions on biochar carbon stability as determined by hydrogen pyrolysis. *Biomass and Bioenergy*, *73*, 155–173. https://doi.org/10.1016/j.biombioe.2014.12.022

Montzka, S. A., Dlugokencky, E. J., & Butler, J. H. (2011). Non-CO 2 greenhouse gases and climate change. *Nature*, *476*(7358), 43–50. https://doi.org/10.1038/nature10322

Munera-Echeverri, J. L., Martinsen, V., Dörsch, P., Obia, A., & Mulder, J. (2022). Pigeon pea biochar addition in tropical Arenosol under maize increases gross nitrification rate without an effect on nitrous oxide emission. *Plant and Soil*, *474*(1–2), 195–212. https://doi.org/10.1007/s11104-022-05325-4

Paiva, M. V. de, Bettini, J., Colombari, F. M., Fazzio, A., & Strauss, M. (2022). Unveiling electrical anisotropy of hierarchical pyrolytic biocarbons from wood cellulose. *Journal of Materials Science*, *57*(48), 21980–21995. https://doi.org/10.1007/s10853-022-08033-7

Pascual, M. B., Sanchez-Monedero, M. A., Cayuela, M. L., Li, S., Haderlein, S. B., Ruser, R., & Kappler, A. (2020). Biochar as electron donor for reduction of N2O by Paracoccus denitrificans. *FEMS Microbiology Ecology*, *96*(8), 1–11. https://doi.org/10.1093/femsec/fiaa133

Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P., & Smith, P. (2016). Climate-smart soils. *Nature*, *532*(7597), 49–57. https://doi.org/10.1038/nature17174

Pinheiro, J., Bates, D., & R Core Team. (2022). *nlme: Linear and Nonlinear Mixed Effects Models* (R package version. 3.1-157). https://CRAN.R-project.org/package=nlme.

Pinheiro, J. C., & Bates, D. M. (2000). *Mixed-Effects Models in S and S-PLUS*. Springer-Verlag. https://doi.org/10.1007/b98882

R Core Team, (2020). (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna,Austria. https://www.r-project.org/

Raij, B. van, Andrade, J. C. de, Cantarella, H., & Quaggio, J. A. (2001). *Análise química para avaliação da fertilidade de solos tropicais*. (1st ed.). Instituto Agronômico.

Ramlow, M., Foster, E. J., Del Grosso, S. J., & Cotrufo, M. F. (2019). Broadcast woody biochar provides limited benefits to deficit irrigation maize in Colorado. *Agriculture, Ecosystems & Environment*, *269*(November 2017), 71–81. https://doi.org/10.1016/j.agee.2018.09.017

Roozen, A. (2015). *Availability of sustainable lignocellulosic biomass residues in Brazil for export to the EU* [Master Thesis]. Utrecht University.

Sainju, U. M. (2016). A global meta-analysis on the impact of management practices on net global warming potential and greenhouse gas intensity from cropland soils. *PLoS ONE*, *11*(2), 1–26. https://doi.org/10.1371/journal.pone.0148527

Seddon, N., Sengupta, S., García-Espinosa, M., Hauler, I., Herr, D., & Rizvi, A. R. (2019). *Nature-based Solutions in Nationally Determined Contributions: Synthesis and recommendations for enhancing climate ambition and action by 2020*.

Shakoor, A., Shahzad, S. M., Chatterjee, N., Arif, M. S., Farooq, T. H., Altaf, M. M., Tufail, M. A., Dar, A. A., & Mehmood, T. (2021). Nitrous oxide emission from agricultural soils: Application of animal manure or biochar? A global meta-analysis. *Journal of Environmental Management*, *285*(January), 112170. https://doi.org/10.1016/j.jenvman.2021.112170

Singh, B., Fang, Y., Cowie, B. C. C., & Thomsen, L. (2014). NEXAFS and XPS characterisation of carbon functional groups of fresh and aged biochars. *Organic Geochemistry*, *77*, 1–10. https://doi.org/10.1016/j.orggeochem.2014.09.006

Soil Survey Staff - USDA-NRCS. (2014). *Keys to soil taxonomy* (Resources Conservation Service, Ed.; 12th edn).

Spokas, K. A. (2010). Review of the stability of biochar in soils: Predictability of O:C molar ratios. *Carbon Management*, *1*(2), 289–303. https://doi.org/10.4155/cmt.10.32

Tarin, M. W. K., Khaliq, M. A., Fan, L., Xie, D., Tayyab, M., Chen, L., He, T., Rong, J., & Zheng, Y. (2021). Divergent consequences of different biochar amendments on carbon dioxide (CO2) and nitrous oxide (N2O) emissions from the red soil. *Science of the Total Environment*, *754*, 141935. https://doi.org/10.1016/j.scitotenv.2020.141935

Tian, H., Xu, R., Canadell, J. G., Thompson, R. L., Winiwarter, W., Suntharalingam, P., Davidson, E. A., Ciais, P., Jackson, R. B., Janssens-Maenhout, G., Prather, M. J., Regnier, P., Pan, N., Pan, S., Peters, G. P., Shi, H., Tubiello, F. N., Zaehle, S., Zhou, F., … Yao, Y. (2020). A comprehensive quantification of global nitrous oxide sources and sinks. *Nature*, *586*(7828), 248–256. https://doi.org/10.1038/s41586-020-2780-0

Toczydlowski, A. J. Z., Slesak, R. A., Venterea, R. T., & Spokas, K. A. (2023). Pyrolysis temperature has greater effects on carbon and nitrogen biogeochemistry than biochar feedstock when applied to a sandy forest soil. *Forest Ecology and Management*, *534*(November 2022), 120881. https://doi.org/10.1016/j.foreco.2023.120881

Tomczyk, A., Sokołowska, Z., & Boguta, P. (2020). Biochar physicochemical properties: pyrolysis temperature and feedstock kind effects. *Reviews in Environmental Science and Biotechnology*, *19*(1), 191–215. https://doi.org/10.1007/s11157-020-09523-3

Wang, J., & Wang, S. (2019). Preparation, modification and environmental application of biochar: A review. *Journal of Cleaner Production*, *227*, 1002–1022. https://doi.org/10.1016/j.jclepro.2019.04.282

Wang, Y. Q., Bai, R., Di, H. J., Mo, L. Y., Han, B., Zhang, L. M., & He, J. Z. (2018). Differentiated mechanisms of biochar mitigating straw-induced greenhouse gas emissions in two contrasting paddy soils. *Frontiers in Microbiology*, *9*(NOV), 1–19. https://doi.org/10.3389/fmicb.2018.02566

Woolf, D., Amonette, J. E., Street-Perrott, F. A., Lehmann, J., & Joseph, S. (2010). Sustainable biochar to mitigate global climate change. *Nature Communications*, *1*(5). https://doi.org/10.1038/ncomms1053

Woolf, D., Lehmann, J., Ogle, S., Kishimoto-Mo, A. W., McConkey, B., & Baldock, J. (2021). Greenhouse Gas Inventory Model for Biochar Additions to Soil. *Environmental Science & Technology*, *55*(21), 14795–14805. https://doi.org/10.1021/acs.est.1c02425

Wu, D., Senbayram, M., Zang, H., Ugurlar, F., Aydemir, S., Brüggemann, N., Kuzyakov, Y., Bol, R., & Blagodatskaya, E. (2018). Effect of biochar origin and soil pH on greenhouse gas emissions from sandy and clay soils. *Applied Soil Ecology*, *129*(May), 121–127. https://doi.org/10.1016/j.apsoil.2018.05.009

Yang, Y., Sun, K., Han, L., Chen, Y., Liu, J., & Xing, B. (2022). Biochar stability and impact on soil organic carbon mineralization depend on biochar processing, aging and soil clay content. *Soil Biology and Biochemistry*, *169*(December 2021), 108657. https://doi.org/10.1016/j.soilbio.2022.108657

Yuan, D., Yuan, H., He, X., Hu, H., Qin, S., Clough, T., Wrage-Mönnig, N., Luo, J., He, X., Chen, M., & Zhou, S. (2021). Identification and verification of key functional groups of biochar influencing soil N2O emission. *Biology and Fertility of Soils*, *57*(4), 447–456. https://doi.org/10.1007/s00374-021-01541-9

Yuan, H., Zhang, Z., Li, M., Clough, T., Wrage-Mönnig, N., Qin, S., Ge, T., Liao, H., & Zhou, S. (2019). Biochar's role as an electron shuttle for mediating soil N 2 O emissions. *Soil Biology and Biochemistry*, *133*(September 2018), 94–96. https://doi.org/10.1016/j.soilbio.2019.03.002

Zhang, J., Sun, H., Ma, J., Zhang, X., Wang, C., & Zhou, S. (2022). Effect of straw biochar application on soil carbon, greenhouse gas emissions and nitrogen leaching: A vegetable crop rotation field experiment. *Soil Use and Management*, *December 2022*, 729–741. https://doi.org/10.1111/sum.12877

Zhang, X., Lv, J., Zhang, Y., Li, S., Chen, X., & Sha, Z. (2023). A Meta-Analysis Study on the Use of Biochar to Simultaneously Mitigate Emissions of Reactive Nitrogen Gases (N2O and NO) from Soils. *Sustainability (Switzerland)*, *15*(3). https://doi.org/10.3390/su15032384

Supplementary information

Table S5: Analysis of variance (ANOVA) from the data of high resolution carbon (HRC) and high resolution oxygen (HRO) images, obtained by x-ray photoelectron spectroscopy (XPS).

ANOVA									
Survey Relative Abundance (%)									
Source of variation	**DF**	**Residual DF**	**C**				**O**		
			F-value		*p-value*		*F-value*		*p-value*
Treatment	3	8	32.8		<0.001		9.1		<0.01
Source of variation	**DF**	**Residual DF**	**Si**		**N**		**Others**		
			F-value	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*	
Treatment	1	4	4.37	0.1	188.6	<0.001	30.2	<0.01	
High Resolution Carbon (HRC)									
Source of variation	**DF**	**Residual DF**	**C−C/C=C**		**C−O**		**C=O**		**O-C=O**	
			F-value	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*
Treatment	3	8	9.17	<0.05	7.4	<0.01	11.7	<0.01	10.2	<0.01

DF: Degrees of freedom.

Table S6. Repeated-measures analysis of variance (MANOVA) and analysis of variance (ANOVA), as a response to treatments and time, measured in days after fertilizer application (DAF) (*n*=4).

ANOVA

Source of variation	DF	Residual DF	Cumulative N_2O		N_2O Emission Intensity		Aboveground Biomass		Belowground Biomass		Total C		Total N		C/N Ratio	
			F-value	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*
Treatment	4	20	27.71	<.0001	21.19	<.0001	1.44	0.257	2.80	<.05	5.29	<0.005	8.53	<.0005	6.37	<.005

MANOVA

Source of variation	DF	Residual DF	$N\text{-}NO_3^-$		$N\text{-}NH_4^+$	
			F-value	*p-value*	*F-value*	*p-value*
Treatment	4	15	22,5	<.0001	2,2	0,124
DAF	4	60	152,4	<.0001	170,2	<.0001
Treatment x DAF	4	60	18,6	<.0001	16,2	<.0001

DF: Degrees of freedom; DAF: Days after fertilizer application.

Table S7. Soil total N. and C/N ratio in response to soil only (CTR). N fertilizer addition alone (NF). or combined with biochar produced from sugarcane straw (NF+SB). sugarcane bagasse (NF+BB). pine (NF+PB) and eucalyptus (NF+EB). Data (mean ± SE. n=4) followed by different letters denote significant differences between treatments at Tukey's test ($p < 0.05$).

Treatment	**N** g kg^{-1}	**C/N ratio**
CTR	0.83 ± 0.05 c	14.2 ± 0.51 a
NF	1.24 ± 0.06 bc	8.96 ± 0.50 b
NF+SB	1.70 ± 0.19 ab	10.0 ± 0.66 b
NF+BB	1.70 ± 0.11 ab	8.64 ±0.87 b
NF+PB	1.58 ± 0.20 ab	12.2 ±1.35 a
NF+EB	1.82 ± 0.02 a	9.99 ±0.82 b