High Application Rates of Biochar to Mitigate N₂O Emissions From a N-Fertilized Tropical Soil Under Warming Conditions

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Biochar application has been suggested as a strategy to decrease nitrous oxide emissions from agricultural soils while increasing soil C stocks, especially in tropical regions. Climate change, specifically increasing temperatures, will affect soil environmental conditions and thereby directly influence soil N₂O fluxes. Here, we show that Miscanthus giganteus biochar applied at high rates suppresses the typical warming-induced stimulation of N₂O emissions. Specifically, in experiments with high biochar addition (25 Mg ha⁻¹), N₂O emissions under 40°C were equal to or even lower compared to those observed at 20°C. In this sense, the mitigation potential of biochar for N₂O emissions might increase under the auspices of climate change.

Keywords: greenhouse gases, black C, fertilizers, Miscanthus giganteus, climate change

INTRODUCTION

Biochar is the product of biomass pyrolysis and has been applied to the soil with the purpose of improving soil quality and increasing soil carbon (C) stocks, especially in tropical regions. Furthermore, biochar may also have the potential to decrease greenhouse gas (GHG) emissions, especially nitrous oxide (N₂O) (Matuštík et al., 2020; Zhang et al., 2020). Among the GHGs, N₂O has received special attention because it remains in the atmosphere for more than 114 years and has a warming potential 298 times >CO₂, with N fertilization in agricultural lands as one of its main sources (Reay et al., 2012). N₂O is formed by microbial N turnover processes, not only by the reductive process of denitrification but also by the oxidative process of nitrification (Venterea and Rolston, 2000), and thus, its production is dependent not only on substrate availability and oxidative status of soils, which may be affected by biochar, but also by temperature. Biochar addition may affect N₂O emissions by changing soil ammonium and nitrate concentrations (Liang et al., 2006; Cheng et al., 2008), decreasing soil bulk density (Karhu et al., 2011), facilitating N₂O consumption in the terminal step of denitrification (Aamer et al., 2020), and adding labile carbon and nitrogen compounds to the soil (Spokas and Reicosky, 2009).

In a global meta-analysis, it was observed that biochar application significantly decreased soil N₂O emissions by on average 38%. The application rate was identified as the most influential variable affecting the mitigation potential of biochar applications to soils (Zhang et al., 2020).
Recent review studies show that N$_2$O production is significantly reduced with biochar application rates of 1–2% by weight (Cayuela et al., 2014; Kammann et al., 2017). A decrease in N$_2$O emissions ranging between 21 and 92% was reported in four contrasting soils compared to untreated controls, with the mitigation potential strongly increasing with increasing biochar additions (1–20% by weight) (Stewart et al., 2013). An 80–88% reduction in N$_2$O efflux was also found when 5, 10, and 20 g kg$^{-1}$ biochar was applied to soil with and without added manure (Rogovska et al., 2011). Such variable responses of N$_2$O reduction might be due to differences in characteristics of the biochar, soils, or prevailing environmental conditions (Kammann et al., 2017).

Climate change, specifically increasing temperatures, will affect soil environmental conditions and thereby directly influence soil N$_2$O fluxes. Under temperate conditions, a 2-year field study found that biochar-warming interactions led to higher total N$_2$O emissions than the control (Bamminger et al., 2017). According to the authors, the observed stimulation of soil N$_2$O emissions in warmed biochar plots may be due to the (i) reduction of the nitrate sorption capacity of biochar by soil warming; (ii) stimulation of soil organic matter mineralization under warming, which increases the amount of available C and N in the soil and at the same time results in lowered soil oxygen concentration due to the stimulation of soil respiration, thereby creating anaerobic zones for denitrification; (iii) increases in soil moisture by biochar application due to the increased water-holding capacity of soils, especially under dry conditions; and (iv) changes in the microbial community because of soil warming and biochar application.

Considering the potential of biochar application for mitigating GHG emissions in tropical regions (Rittl et al., 2015), the influence of expected increases in temperature on the N$_2$O emissions of biochar-amended soils requires investigation, mainly in a scenario of climate change. Specifically, little information is available on the interactive response of tropical soil N$_2$O emissions to temperature changes and biochar addition rates (Bamminger et al., 2017). Consequently, we deployed a laboratory experiment targeted to investigate the changes in the N$_2$O emissions from N-fertilized biochar-amended soils, as affected by elevated temperature and biochar addition rates.

**MATERIALS AND METHODS**

**Biochar and Soil Characteristics**

Biochar was produced from Miscanthus giganteus grass. The biomass was dried at $\sim$125°C in a reactor and then carbonized at 450°C for 15 min (Mimmo et al., 2014). The chemical characteristics of biochar used in this study are presented in Supplementary Table S1. Soil was collected from the top layer (0–20 cm) under a native Atlantic Rain Forest patch in Piracicaba, São Paulo, Brazil (22°90'74" S; 48°24'01" W). The soil was classified as an Entisol Quartzipsamment (USDA, 2014), sandy (7.8% clay, 2.2% silt, and 90% sand), with pH in H$_2$O of 3.9, total C of 0.86%, and 0.06% of total N at the 0–20-cm layer.

**Experimental Design**

To evaluate the effect of biochar amendment rates on N$_2$O emissions from N-fertilized soil, an 88-day incubation experiment was performed using an existent experiment (Rittl et al., 2020). Shortly, the previous experiment was carried on with half-liter incubation jars filled with 100 g of soil and amended with biochar at various application rates (0, 0.24, 0.48, and 0.96 g of biochar, respectively, and 6.25, 12.5, and 25 Mg ha$^{-1}$) and subsequently preincubated at 20°C or 40°C in a full factorial design ($n = 3$ each). After 144 days, the experiment was finished, and our study started by adding in each jar 0.132 g of NH$_4$NO$_3$, which corresponded to a surface application of 90 kg N ha$^{-1}$, and N$_2$O measurements started for a period of 88 days. Soil moisture was adjusted to 60% field capacity and maintained at that level throughout the experiment.

**Measurements of N$_2$O Efflux**

Emissions of N$_2$O were determined daily for the first 15 days, two to three times per week for the next 25 days, and weekly for the next 48 days (Supplementary Figure S1). Gas emissions were determined by taking gas samples at time 0 and 32 min after jars were gas-tight closed with a plastic syringe of 20 ml. Gas emissions in the 24 jars were measured sequentially. Gas samples were injected in a gas chromatograph (SRI GC8610, Torrance, CA, USA) equipped with an electron capture detector (ECD) for quantification of N$_2$O, with helium carrier gas. The oven temperatures and the temperatures of the ECD were set to 72 and 325°C, respectively. The N$_2$O emissions were calculated on a per gram basis considering the concentration difference between time 0 and 32 min and the headspace volume (Abbruzzini et al., 2017).

**Data Analyses**

Statistical analyses were performed using Assisstat 7.7 and Origin 8.5 (OriginLab, Co.). The data were checked for normality and homogeneity of variances to meet the assumptions of ANOVA. Two-way repeated measures ANOVA was performed on JASP 0.8.6.0 to test the effects of biochar rate and temperature on mean N$_2$O daily emissions. A paired-samples t-test was conducted to compare daily mean N$_2$O emissions from the treatments.

**RESULTS**

We observed that Miscanthus biochar addition significantly suppressed N$_2$O emissions (Figure 1; Supplementary Table S2; $P < 0.001$). The mean N$_2$O daily emissions from untreated soil were higher (0.071–0.216 µg N$_2$O g$^{-1}$ dry soil) than those from biochar-treated soils (0.09–0.164 µg N$_2$O g$^{-1}$ dry soil). In our study, incubation at warmer temperatures significantly increased mean N$_2$O daily emissions not only in untreated soils but also in soils with low biochar application rates (6.25 and 12.5 Mg ha$^{-1}$) by 332 and 150%, respectively (Figure 1). However, in experiments with high biochar addition (25 Mg ha$^{-1}$), N$_2$O emissions under 40°C were equal to or even lower compared to those observed at 20°C (Figure 1).
**DISCUSSION**

*Miscanthus* biochar application decreased the N$_2$O emissions from a N-fertilized tropical soil (Figure 1; Supplementary Table S2). This finding is in agreement with other studies and the general understanding of biochar effects on soil N$_2$O emission and has been explained by an “electron shuttle” effect that biochar might play during the denitrification process, facilitating N$_2$O reduction to dinitrogen (N$_2$) (Cayuela et al., 2014). The application of biochar from hardwood trees in sandy loam soil at a rate of 28 Mg ha$^{-1}$ suppressed 91% of the total N$_2$O emissions (Case et al., 2012). Under controlled conditions, the combined application of 30 Mg ha$^{-1}$ of *Miscanthus* biochar pyrolyzed at 600°C and N-rich litter resulted in a reduction of 42% in N$_2$O emissions (Bamminger et al., 2014).

Soil warming has been shown to increase N$_2$O emissions through stimulation of or shifts in the microbial community responsible for N cycling (Cantarel et al., 2012). Furthermore, warming might increase soil anaerobiosis and thus N$_2$O emissions derived from denitrification as a consequence of increased soil respiration (Butterbach-Bahl et al., 2013). However, N$_2$O emissions from *Miscanthus* biochar-treated tropical sandy soils were always lower than those from untreated soils at both incubation temperatures (Figure 1). With increasing biochar addition rate, biochar addition may have counteracted these temperature-mediated shifts, possibly by reducing nitrogen availability (Liang et al., 2006; Cheng et al., 2008) and/or promoting N$_2$O reduction to the terminal denitrification product N$_2$ (Aamer et al., 2020). Interestingly, in our study with a higher biochar addition rate, the temperature-induced stimulation of N$_2$O emissions completely diminished, as also confirmed by the significant interaction between temperature and biochar effects (Supplementary Table S2). This shows that biochar at high application rates (25 Mg ha$^{-1}$) fully hampers the typical increase in soil N$_2$O emissions with rising temperatures. We hypothesize that this effect is due to the observed biochar-promoted reduction of N$_2$O to N$_2$ in soils, i.e., before its emission to the atmosphere (Butterbach-Bahl et al., 2013).

**FIGURE 1** | Mean daily N$_2$O emissions (±SE, n = 3) observed for an 88-day laboratory incubation of tropical soil with different *Miscanthus* biochar application rates (0, 6.25, 12.5, and 25 Mg ha$^{-1}$). Yellow and red colors show the results for 20 and 40°C incubation temperatures. Uppercase letters indicate significant differences between biochar rates at 40°C. Lowercase letters indicate significant differences between biochar rates at 20°C. The percentage (%) indicates an increase or decrease in N$_2$O emissions at 40°C compared to 20°C (ANOVA, paired-samples t-test, p < 0.001).
Our findings provide empirical evidence about the role of biochar in mitigating N\textsubscript{2}O emissions from N fertilization under warming conditions, a contentious issue in agricultural lands of tropical regions, mainly in a scenario of climate change. In a tropical sandy soil, doubled incubation temperature resulted in several-fold increased N\textsubscript{2}O emissions, an effect that diminished the highest Miscanthus biochar addition rates. This indicates that biochar effects on N\textsubscript{2}O reduction to N\textsubscript{2} in soils increase with increasing temperatures and that at biochar application rates of 25 Mg ha\textsuperscript{-1}, the typical temperature-induced stimulation of N\textsubscript{2}O emission can be avoided. In summary, Miscanthus biochar offset warming effects on N\textsubscript{2}O emissions in a tropical sandy soil, and the mitigating results of biochar are strongest at high biochar rates and high temperatures. This might explain the high mitigation potential of N\textsubscript{2}O in tropical soils reported here and could indicate that the potential of biochar to mitigate N\textsubscript{2}O emissions will increase with global warming.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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**AUTHOR CONTRIBUTIONS**

TR: conceptualization, experimentation, sampling and lab analysis, data analysis, writing—original draft, and writing—review and editing. DO: writing—review and editing. LC: experimentation, sampling and lab analysis. ES, KB-B, and MD: conceptualization, writing—review and editing. CC: conceptualization, supervision, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2020.611873/full#supplementary-material
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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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