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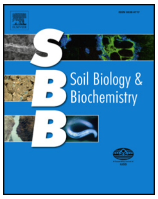

Dynamic biochar effects on soil nitrous oxide emissions and underlying microbial processes during the maize growing season

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ABSTRACT

Biochar application is a promising approach to decrease nitrous oxide (N_2O) emissions from agricultural soils, yet little is known about how biochar affects N_2O-producing and consuming microbial processes under changing field conditions. We conducted a field study to assess if growing season patterns in soil N_2O emissions were correlated with the underlying microbial processes of nitrification and denitrification. We measured soil N_2O emissions, potential nitrification and denitrification rates, and the abundance of key soil nitrogen (N)-cycling functional genes in an intensive maize production field trial in Illinois, USA that included the following four treatments: Control (unamended), Biochar (100 Mg ha^{-1}), Nitrogen (269 kg N ha^{-1} as Urea Ammonium Nitrate fertilizer), and Biochar + Nitrogen (100 Mg ha^{-1} and 269 kg N ha^{-1}, respectively). Biochar increased potential nitrification rates when soil ammonium concentrations were high following fertilizer application, thus enhancing N_2O emissions in the Biochar + Nitrogen treatment early in the season which were likely nitrification-associated. However, over the full growing season, biochar application reduced cumulative N_2O emissions in Biochar + Nitrogen plots to levels similar to the unamended Control. This could be attributed to biochar suppression of potential denitrification throughout the growing season. The treatments amended with biochar avoided large pulses of N_2O emissions following intense rain events in the mid-season, while also sustaining lower N_2O emissions in the late-season. Our study demonstrates that biochar can have dynamic effects on soil N_2O emissions and the underlying microbial processes that depend on changing edaphic conditions, such as soil inorganic nitrogen availability and moisture, over the growing season.

1. Introduction

Nitrogen fertilizer application is necessary for sustaining global crop yields (Mueller et al., 2014), but excessive inputs of reactive N to agroecosystems results in negative environmental consequences associated with nitrate (NO_3^-) leaching and soil emissions of N gases, such as the potent greenhouse gas (GHG) nitrous oxide (N_2O) (Galloway et al., 2008). The application of biochar, a carbon-rich material produced by pyrolyzing biomass at low-oxygen conditions, has recently gained attention as a strategy for mitigating these unintended consequences, particularly soil N_2O emissions (Atkinson et al., 2010; Lehmann and Kleber, 2015; He et al., 2017; Kammann et al., 2017; Verhoeven et al., 2017). Meta-analyses of studies conducted across a range of cropping systems around the world and using a variety of biochar types have shown that biochar application reduces soil N_2O emissions by 44–60% on average (Cayuela et al., 2014; Van Zwieten et al., 2015; Zhao et al., 2017). Despite the large body of biochar literature, there is little consensus on how biochar and various N-cycling processes interact under field conditions to decrease N_2O emissions (Clough and Condron, 2010; Wang et al., 2013; Cayuela et al., 2014; DeLuca et al., 2015). Moreover, controlled laboratory or greenhouse experiments used to investigate potential mechanisms often show higher biochar efficacy than field experiments, which suggests that there may be other factors inhibiting the efficacy of biochar in reducing N_2O emissions in the field (Cayuela et al., 2015; He et al., 2017; Verhoeven et al., 2017). The challenge of advancing a process-level understanding of the N_2O mitigation potential of biochar is complex because N_2O is produced in soil primarily by the distinct microbially-mediated processes of nitrification and denitrification, among others, and it is also consumed in soil by denitrification. Characterization of

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biochar effects on these pathways of N_2O production and consumption over the growing season in a field setting is needed to guide mechanistic studies that will enable the development of recommended practices that maximize biochar N_2O mitigation potential.

The direction of biochar effects on nitrification and its associated N_2O production is still uncertain, making it difficult to delve into the mechanisms controlling these effects. Some studies have suggested that biochar inhibits nitrification-derived N_2O emissions based on observations of reduced N_2O emissions at relatively low water-filled pore space assumed to be conducive only for the aerobic process of nitrification (Scheer et al., 2011; Taghizadeh-Toosi et al., 2011; Angst et al., 2014). However, ^{15}N tracer studies that directly measured gross nitrification rates or nitrification-derived N_2O emissions showed a stimulatory rather than inhibitory effect of biochar (Prommer et al., 2014; Sánchez-García et al., 2014; Wells and Baggs, 2014). These direct measurements of process rates are consistent with observations that biochar enhances the size and diversity of ammonia-oxidizing communities responsible for nitrification (Prommer et al., 2014; Song et al., 2014). It is unclear if the inconsistent findings in the literature are due to differences in methodology or to the potential for biochar to have contrasting effects on nitrification-derived N_2O emissions depending on context (e.g., edaphic conditions, biochar type, or biochar application rate). Additional studies linking measurements of nitrification activity with soil N_2O emissions are needed.

Although there is consensus that biochar reduces N_2O emissions derived from the anaerobic process of denitrification, there are two possible pathways leading to this effect. Denitrification is an anaerobic process in which nitrate (NO_3^-) is reduced to dinitrogen (N_2) through a sequence of intermediates, including N_2O. Denitrifying microbes can release N_2O rather than reducing it to N_2, through a pathway called incomplete denitrification. Assessing biochar effects on complete versus incomplete denitrification is difficult because N_2O reduction to N_2 is notoriously difficult to measure (Groffman et al., 2006). Some studies utilizing ^{15}N stable isotope approaches have documented decreased total denitrification with biochar application, but these challenging measurements are less common (Cayuela et al., 2013). Instead, biochar suppression of denitrification-derived N_2O emissions is typically attributed to increased rates of complete denitrification to N_2 (Cayuela et al., 2013). This assumes that, because biochar often increases soil pH, it ameliorates the known detrimental impact of acidic pH on N_2O reduction to N_2 by denitrifiers (Šimek and Cooper, 2002; Obia et al., 2015). Although studies have shown biochar-induced increases in gene or transcript abundance of *nosZ*, the gene encoding for the N_2O reductase enzyme responsible for the last step of denitrification (Scala and Kerkhof, 1998; Henry et al., 2006), there is mixed support for the pH mechanism driving these responses to biochar (Kammann et al., 2017). While there are other possible mechanisms for biochar to decrease denitrification-derived N_2O emissions, such as biochar acting as an electron shuttle to facilitate N_2O reduction (Kappler et al., 2014), biochar toxicity to denitrifiers (Wang et al., 2013), physical sorption of N_2O by biochar (Spokas and Reicosky, 2009), or significant increases in pH beyond the capacity of the denitrifiers (Šimek et al., 2002). These discrepancies suggests that biochar effects on the pathways of complete versus incomplete denitrification are not yet settled.

Controlled laboratory or greenhouse experiments are necessary to isolate N_2O pathways and test mechanisms driving biochar effects on these pathways, however application of these results should be supplemented with field studies to develop a more complete understanding of biochar's effect on soil N_2O emissions. Biochar application rates in field trials are often significantly lower than those used in incubation or pot studies due to the high cost of applying biochar at large scales (Shackley et al., 2011). Soil N_2O emissions mitigation increases with biochar application rates (Ducey et al., 2013), such that this difference has been suggested to account for the lower field mitigation potential of biochar. However, Felber et al. (2014) observed lower reductions of N_2O emissions in a field trial compared to laboratory incubations using the same biochar applied at the same rate. They concluded that the more heterogeneous incorporation of biochar in the field compared to the laboratory led to less interaction of biochar with soil nitrogen, thereby decreasing the N_2O mitigation potential in the field. Biochar also impacts N-cycling processes differently in the presence versus absence of plants (Xu et al., 2014), with the latter condition more typical of laboratory incubation studies. Interestingly, seldom addressed in laboratory incubations is the role of changing conditions, including soil temperature, moisture, and nitrogen availability, in the field setting. This could not only change the relative contribution of nitrification and denitrification to soil N_2O dynamics but also change how biochar effects these microbial processes. Furthermore, exposure to field conditions can age biochar, causing changes to its characteristics that could limit its potential for mitigating N_2O emissions. While aged biochar has been shown to be less effective at reducing N_2O emissions than fresh biochar (Spokas, 2013), aged biochar can still reduce N_2O emissions relative to untreated soils (Hagemann et al., 2017). The resulting variable biochar effect on soil N_2O emissions over the course of a growing season can lead to cumulatively lower N_2O mitigation compared to incubation and pot studies performed under tightly controlled conditions. To date, the majority of studies investigating biochar effects on soil N_2O emissions have been conducted in the laboratory or greenhouse (Cayuela et al., 2014; He et al., 2017). Field studies that attempt to link soil N_2O dynamics to their underlying microbial processes can provide insight into how the results from these studies can be used to predict the N_2O mitigation potential of biochar in the field.

Here, we conducted a study to improve understanding of the relationship between microbial processes and changes in N_2O dynamics following biochar addition under changing field conditions. The objectives of this study were to i) evaluate *in situ* soil N_2O fluxes in a representative, high-yielding maize production field over an entire growing season, ii) characterize patterns in potential nitrification and denitrification rates over the same time period to assess their relative contribution to soil N_2O emissions, and iii) quantify the abundance of key N-cycling functional genes to further link soil N_2O emissions to the underlying microbial processes.

2. Materials and methods

2.1. Study site

The field study was conducted at the University of Illinois Crop Sciences Research and Education Center in Urbana, IL (40.06 N, 88.23 W) from April to September 2016. The experimental site was planted with maize (*Zea mays* L.) in 2016 as part of a two-year maize-soybean (*Glycine max* L.) crop rotation that is typical of the US Midwest. Soils at this site are classified as a Drummer silty clay loam-Flanagan silt loam association (Fine-silty, mixed, superactive, mesic Typic Endoaquolls and Fine, smectitic, mesic Aquic Argiudolls, respectively) (Soil Survey Staff, NRCS, USDA). The following soil characteristics were determined for the 0–18 cm depth by A&L Great Lakes Laboratories (Fort Wayne, Indiana, USA): 6.4 pH, 10% sand, 59% silt, 31% clay, 4.0% organic matter, 0.19% total N, 12.4 $\mathrm{cmol_c\ kg^{-1}}$ CEC, 40.5 $\mathrm{mg\ kg^{-1}}$ Bray-1 P, and 237 $\mathrm{mg\ kg^{-1}}$ extractable K. The long-term average growing season temperature and cumulative precipitation during May through September at this site is 21.0° C and 533 mm, respectively, while in 2016 it was 22.0° C and 653 mm, respectively (NOAA Applied Climate Information System, http://www.rcc-acis.org/).

2.2. Experimental design

The field trial was arranged as a randomized complete block design with four treatments: an unamended control (Control), biochar applied at 100 $\mathrm{Mg\ ha^{-1}}$ (Biochar), N fertilizer addition at 269 $\mathrm{kg\ N\ ha^{-1}}$ (Nitrogen), and biochar + N fertilizer addition at 100 $\mathrm{Mg\ ha^{-1}}$ and 269 $\mathrm{kg\ N\ ha^{-1}}$, respectively (Biochar + Nitrogen). Each block was