Geoderma 325 (2018) 208–217

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Zinc fertilizers influence greenhouse gas emissions and nitrifying and denitrifying communities in a non-irrigated arable cropland

Mónica Montoya[a,*], Antonio Castellano-Hinojosa[b], Antonio Vallejo[a], José Manuel Álvarez[a], Eulogio J. Bedmar[b], Jaime Recio[a], Guillermo Guardia[a]

[a] ETSI Agronómica, Alimentaria y de Biosistemas, Centro de Estudios e Investigación para la Gestión de Riesgos, Agrarios y Medioambientales (CEIGRAM), Universidad Politécnica de Madrid, Ciudad Universitaria s/n, 28040 Madrid, Spain
[b] Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín, CSIC, Granada, Spain

ARTICLE INFO

Handling editor: Jan Willem Van Groenigen

Keywords:
Global warming potential
Nitrification inhibitors
Nitrogen cycling
Calcareous soil
Fertilizer rate
Micronutrient chelate

ABSTRACT

Fertilization with micronutrients (e.g., zinc, Zn) is essential in order to overcome the global nutritional problems associated with human micronutrient deficiencies. However, little is known about the effect of micronutrient fertilizers and their interaction with nitrogen (N) on greenhouse gas (GHG) emissions and soil microbial processes involved in nitrous oxide (N_2O) fluxes. In this context, a one-year field experiment was carried out using a winter wheat (*Triticum aestivum* L.) crop in Central Spain. Winter wheat was treated with different Zn sources (Zn-sulphate, Zn-lignosulphonate, Zn with a mixture of synthetic chelating compounds DTPA-HEDTA-EDTA and Zn-humic/fulvic acids) and N rates (0, 120 and 180 kg N ha^{-1}). Zn sources were applied at 10 kg Zn ha^{-1} for Zn-sulphate and 0.36 kg Zn ha^{-1} for the rest of treatments. Nitrous oxide, methane (CH_4) and respiration fluxes were measured (two-three times per week during the first month after each fertilization and thereafter with decreasing frequency), as were the total abundances of soil Bacteria and Archaea, ammonia-oxidizing Bacteria and Archaea, and denitrifying bacteria. The DTPA-HEDTA-EDTA reduced cumulative N_2O losses by 21.4% and respiration fluxes by 24.4% from those of the no Zn application. The chelating of metal co-factors (mainly copper, Cu) of the enzymes involved in the nitrification and denitrification steps was the probable mechanism for the reduction of N_2O emissions as bacterial *amoA*, *nirK*, *nirS* and *norB* gene abundances, as well as the extractable Cu content, decreased in this treatment. Unexpectedly, the DTPA-HEDTA-EDTA increased the copy number of *nosZ* by 31.2% over that of the no Zn application. The Zn applied together with the humic/fulvic acids mixture caused significant increases of total bacterial abundance and nitrifier and denitrifier communities, particularly the *norB* gene, thereby leading to the highest N_2O emissions. The optimum N rate was 120 kg N ha^{-1} since it resulted in the lowest yield-scaled N_2O losses and N surplus. The application of synthetic Zn chelates can be recommended as a win-win mitigation and adaptation strategy aimed at reducing yield-scaled GHG emissions and at the enhancement of Zn biofortification.

1. Introduction

Agriculture is a substantial contributor to climate change via the emissions of three greenhouse gases (GHGs) to the atmosphere: carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (IPCC, 2014). Reducing GHG emissions from agriculture is essential in order to avoid increases in the mitigation cost in other sectors and to guarantee the achievement of the objective to limit warming to 2 °C above pre-industrial levels (Wollenberg et al., 2016). Major efforts in direct GHG mitigation from agriculture are focused on N_2O, due to its calculated high cumulative forcing of 265 times that of CO_2 over a 100 year time horizon (IPCC, 2014) and because of its role in the depletion of stratospheric ozone (O_3) (Ravishankara et al., 2009). Regarding CH_4 fluxes, non-flooded agricultural soils are generally sinks of this GHG by its oxidation (Snyder et al., 2009).

It is critical to reduce environmental pollution without compromising food security in the context of an increasing worldwide population (Braun, 2007; Frank et al., 2017). Mitigation practices should therefore achieve the reduction of yield-scaled emissions, the maintenance or increase of crop yields and the improvement of net gross margins for farmers via increasing N use efficiency or crop quality (e.g., increased protein or micronutrient concentrations), while also addressing climate change adaptation (Billen et al., 2015). Several mitigation strategies based on N fertilization, (e.g., management of N source

* Corresponding author.
E-mail address: monica.montoya@upm.es (M. Montoya).

https://doi.org/10.1016/j.geoderma.2018.03.035
Received 22 January 2018; Received in revised form 23 March 2018; Accepted 25 March 2018
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including inhibitors or controlled-release fertilizers, placement, time of application or N rate) can reduce area-scaled emissions (Xia et al., 2017). However, the influence of micronutrients and the interaction with N on N_2O emissions still remains poorly understood.

Nitrous oxide is released from agricultural soils mainly through autotrophic nitrification and heterotrophic denitrification (Ussiri and Lal, 2013). The critical step of nitrification, the oxidation of ammonia (NH_3) to hydroxylamine (NH_2OH), is carried out by ammonia-oxidizing bacteria (AOB) or archaea (AOA) which produce the enzyme ammonia monooxygenase encoded by the *amoA* gene. Denitrification, the dominant process in wet soils, is initiated with the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) by nitrate reductase enzymes (encoded by the *narG/napA* genes), followed by NO_2^- reduction by nitrite reductase enzymes (encoded by the *nirS* and *nirK* genes), nitric oxide (NO) reduction by nitric oxide reductase (encoded by *norB/norC* gene) and finally, the conversion of N_2O by nitrous oxide reductase enzymes (encoded by *nosZ* genes), leading to the generation of N_2 as an end-product (Bueno et al., 2012). Enzymes that catalyse these stepwise biochemical processes contain trace metals as co-factors such as copper (Cu), zinc (Zn) or iron (Fe) (Glass and Orphan, 2012). The same accounts for CH_4 emission and consumption. As a consequence, previous studies have found an effect of Zn on N_2O and CH_4 fluxes (Chen et al., 2014; Pramanik and Kim, 2017). However, so far it has not been clarified whether if this effect is micronutrient-dependent (Ruyters et al., 2010; De Brouwere et al., 2007) or source-dependent. Widespread products which inhibit nitrification, such as dicyandiamide (DCD) or 3,4-dimethylpyrazole phosphate (DMPP) act as metal chelators (Ruser and Schulz, 2015). Specifically, Duncan et al., 2017 showed that the chelation of Cu as the co-factor of the ammonia monooxygenase was the inhibition mechanism for DMPP and nitrapyrin in low-Cu content soils. The influence of Cu availability on ammonium (NH_4^+) oxidation has been demonstrated in several studies (e.g. Bédard and Knowles, 1989 or Singh and Verma, 2007). Therefore, chelated agents as ethylenediaminetetraacetic acid (EDTA), which are used to apply micronutrients with enhanced efficacy (Alvarez, 2010), have been shown to inhibit nitrification (Hu et al., 2003). However, studying the specific inhibition mechanism, as well as the effect on other metal-dependent biochemical processes such as denitrification, is still needed.

Both the management of macronutrients fertilization (right time, right source, right place and right rate) (Venterea et al., 2016) and the study of its interactions with micronutrients (e.g., Zn, Cu, Fe) need to be considered to achieve an adequate nutrient balance. Micronutrients supply is essential to avoid severe penalties in growth, yield and crop quality associated to their deficiencies (Singh, 1996), and also for sustaining human and animal health (Kumar et al., 2016). Zinc has been recognized as one of the main target micronutrients, since its supplementation is associated with reductions of the incidence of infectious diseases such as pneumonia, particularly among children in areas with insufficient Zn supply (Gibson, 2012). A high incidence of Zn deficiency in humans has been associated with regions with soils with low Zn phytoavailability (Cakmak et al., 2016). Usually, calcareous and/or alkaline soils are associated with deficiencies of P or micronutrients (Singh, 1996; Deb et al., 2009). Besides, it is necessary to find synergistic effects between nutrients (Fageria and Baligar, 2005; Hamnér et al., 2017), with the aim to improve nutrient use efficiencies (e.g. N, thus reducing potential pollution impacts) and also crop quality through biofortification in deficient soils (Cakmak et al., 2016).

So far, the effect of Zn fertilizers on N_2O and CH_4 emissions has not been studied in non-flooded crops, and an improved understanding of how these molecules modulate soil nitrifying and denitrifying microbiota is still needed. To that end, the main objective of this experiment was to study the effect of several Zn sources (conventional, synthetic chelates and natural chelates) and N fertilization rates on GHG emissions and yield-scaled emissions, quantifying also the microbial populations involved in N_2O fluxes. We explored the hypothesis that N_2O emissions (from both nitrification and denitrification processes) could be directly affected by Zn sources (particularly chelates).

2. Materials and methods

2.1. Site description

The field experiment was located in the National Center of Irrigation Technology, "CENTER" (latitude 40°25′1.31″N, longitude 3°29′45.07″W) in the Madrid region (Spain). According to the Soil Taxonomy of the USDA, the soil is a *Typic xerofluvent* (Soil Survey Staff, 2014) with a silt loam texture (10% clay, 59.5% silt, and 30.5% sand) on the upper horizon (0–20 cm). The major physicochemical properties of the topsoil, measured by conventional methods, were: organic matter (Walkley-Balck), 20.7 g kg^{-1}; total Nitrogen, 1.64 g kg^{-1}; bulk density, 1.27 Mg m^{-3}; water pH, 8.2; $CaCO_3$, 8.16 g kg^{-1}; extractable P (Olsen), 28.4 mg kg^{-1}; total K, 3.14 g kg^{-1}; exchangeable K, 0.51 g kg^{-1}; DTPA-extractable Cu, 1.56 mg kg^{-1}, DTPA-extractable Zn 0.81 mg kg^{-1} (values below 1 mg kg^{-1} indicate Zn deficiency, Brennan et al., 1993); and DTPA-extractable Fe, 4.41 mg kg^{-1}. According to data from the meteorological station placed in the field, the mean annual average temperature and rainfall during the last 10 years were 14.1 °C and 393 mm, respectively. Data of rainfall and air and soil temperatures (at 10 cm depth) were obtained daily from the meteorological station located at the field site.

2.2. Experimental design and management

A field experiment was carried out from October 2015 to July 2016. A total of 45 plots (4 m × 5 m) were arranged in a three-replicate randomized block design. Each plot was a result of a factorial combination of three N rates (0, 120 and 120 + 60 kg N ha^{-1}) with five Zn sources: control without Zn application (Zn0); Zn sulphate (S, $ZnSO_4$ 35% Zn; w/w); Zn-lignosulphonate (LS, 7.5% Zn; w/w); Zn applied with a mixture of chelating compounds (Ch, Zn-DTPA-HEDTA-EDTA 7% Zn; w/w); and Zn-humic/fulvic acids (HuFu, 7% Zn; w/w). Zn sources were applied in two dressing applications. At each time, the application was 0 kg Zn ha^{-1} for Zn0; 0.18 kg Zn ha^{-1} for LS, Ch and HuFu; and 5 kg Zn ha^{-1} for S (Martens and Westermann, 1991; Mortvedt and Gilkes, 1993). Therefore, total Zn rate was 10 kg Zn ha^{-1} for S and 0.36 kg Zn ha^{-1} for the rest of treatments.

The field was seeded on October 27th with winter wheat (*Triticum aestivum* L. 'Ingenio') at 200 kg ha^{-1}. No fertilizers (N, P, or K) were applied at seeding according to the previous soil analysis. Nitrogen and Zn-based fertilizers were split into two dressing applications, at the beginning and the end of the stem elongation stage (Z30 and Z39, Zadoks et al., 1974). In the case of N, the second dressing fertilization (60 kg N ha^{-1}) was only carried out in the N120 + 60 treatment, while the plots corresponding to both N-fertilized treatments (N120 and N120 + 60) received 120 kg N ha^{-1} at the first dressing application. All of the N was applied through urea (46% N; EuroChem Agro). Both N and Zn sources were applied through liquid solutions which were sprayed homogeneously through a knapsack sprayer (foliar-soil application). The wheat was harvested on June 21st with a research plot combine (Wintersteiger Inc.). The field was kept free of weeds, pests and diseases following local practices.

2.3. GHG sampling and analyses

During the first month after fertilization, gas and soil samples were collected 2–3 times per week, considering it to be the most critical period of high gas emissions. Afterwards, the frequency of sampling was decreased progressively, increasing after rainfall events. The GHG (N_2O, CH_4 and CO_2) fluxes were measured using the closed chamber technique. Opaque cylindrical static chambers of 19.3 L were placed in each plot and fitted into stainless steel rings which had been inserted into the soil to a depth of 10 cm at the beginning of the experiment in

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