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Effect of the earthworm gut-stimulated denitrifiers on soil nitrous oxide emissions

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ABSTRACT

Earthworms may influence soil N_2O emissions by stimulating free-living denitrifiers due to the restructuring of the soil environment, and by inoculating gut-stimulated denitrifying bacteria during the excretion process. However, the relative importance of each effect on soil N_2O emissions is still not clear. To determine the differences in earthworm (*Eisenia fetida*)-induced N_2O emissions, sterilized (from which the impacts of indigenous soil microbes are limited) and unsterilized soils were used in the 30-days microcosm experiment. Earthworm inoculation improved soil ammonium (NH_4^+–N), nitrate (NO_3^-–N) and dissolved organic carbon (DOC) concentrations in both soils. Soil N_2O emission also increased with the addition of earthworms, but the significant difference on cumulative N_2O emissions were only detected in the sterilized soil (362.22 and 645.86 μg N_2O–N kg^{-1} dry soil with and without earthworm inoculation, respectively). Redundancy analysis indicated that the earthworm inoculation changed the soil microbial community structure in both soils. The relative density of *nirS* genes was significantly increased due to earthworm inoculation in sterilized soil, but no obvious difference was detected in the unsterilized soil. In conclusion, by changing the soil bacterial community via gut-stimulated denitrifiers, earthworms may increase N_2O emissions in the sterilized soil, while this effect may be diminished by competition with indigenous soil microorganisms in unsterilized soil.

1. Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas with a global warming potential (GWP) of 298-fold greater than that of carbon dioxide (CO_2) on a 100-year horizon. More than 60% of global N_2O emissions are derived from biological activities in soils [1]. Earthworms represent the major soil animal biomass in most terrestrial temperate ecosystems, and their interactions with soil microbes are thought to be an important factor on influencing the N_2O balance of soils [2], although earthworms hardly produce any N_2O themselves [3].

Through decomposing [4], mineralizing [5], and burrowing activities [6], earthworms can improve soil nutrition, change soil structure, and thereby stimulate indigenous soil microbial activity and/or change microbial community composition, which may in turn affect N_2O emissions [7,8]. In addition, the earthworm gut is a near optimal environment for N_2O production in terms of microflora, anaerobicity and concentrations of mineral N and available C. As a result, N_2O emissions from the ingested soil are elevated as compared to the surrounding soil matrix, and gut-stimulated denitrifying bacteria may also be responsible for the *in vivo* emission of N_2O [9–11].

Generally, earthworms may influence soil N_2O emissions by stimulating free-living denitrifiers (living in the soil and not passing through the earthworm gut) due to the restructuring of the soil environment, and by inoculating gut-stimulated denitrifying bacteria during the excretion process. However, the relative importance of each effect on soil N_2O emissions is still not clear due to the difficulty of distinguishing these effects, which usually occur simultaneously in time and space. Thus, sterilized soils, from which the impacts of indigenous soil microbes are considered to be eliminated within a certain time period, were used in this microcosm experiment and compared with unsterilized soils to determine whether there were differences in earthworm induced-N_2O emissions during the 30-day incubation. The aim of this study was to assess the effect of gut-stimulated denitrifiers on soil N_2O emission with the interactions between earthworm and soil microorganisms.

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2. Materials and methods

2.1. Soil and earthworm preparation

Soil with a clay loamy texture was collected (0–30 cm depth) from a vegetable field in Wuhan, Hubei province, China (30°28.7 N and 114°21.6 E). This field was fertilized with vermicompost and cattle dung for several years, and the dominant earthworm species in this field was *Eisenia fetida* (~80 individuals m^{-2}). The soil was sieved through a 5 mm sieve to remove debris, and then sterile distilled water was added to achieve a moisture content of 63% water-filled pore space (WFPS), which was corresponded to the optimal moisture level for earthworm activity. The soil was pre-incubated at 25 °C for seven days to stabilize the microbial activity, and then half of soil was placed on the dishes and sterilized twice at 103.4 kPa, 121 °C with the autoclaving method for 1 h each time to prevent indigenous soil organisms from affecting the characteristics of the soil [12]. The time interval for the twice sterilization was 24 h. The moisture was adjusted again by adding sterile distilled water after sterilization. The properties of the sterilized soil at the beginning of incubation were presented in Fig. 1.

Rice straw was collected from the cultivated rice filed after harvesting, oven-dried at a temperature of 60 °C for seven days and then chopped to pass through a 2 mm stainless steel sieve. Total C and N contents in rice straw were 376.8 and 7.9 g kg^{-1}, respectively, with a corresponding C/N ratio of 48.

Adult earthworms, *E. fetida*, were collected from the fields and transferred to a chamber containing rice straw and the soils used in this study, and were maintained at 25 °C for seven days prior to the commencement of experiment.

2.2. Experimental design

The experiment with three replicates was carried out using microcosm system incubation and consisted of four treatments: sterilized soil with (TE) and without earthworm (TO), and unsterilized soil with (UE) and without earthworm (UO).

One and a half kilograms (fresh weight, which was moistened to 63% WFPS with sterile distilled water) of either sterilized or unsterilized soil were placed in a 2 L glass bottle. Subsequently, 15 g of rice straw was manually incorporated into soil using a sterilized spoon as carefully as possible to provide adequate food for *E. fetida*. Depending on the treatments, five adult individual earthworms were added to the microcosm after voiding their guts for 24 h following the filter paper method and flushing with sterile distilled water twice [7]. We used a higher earthworm density (~200 individuals m^{-2}) in this experiment because (1) earthworm densities of up to 277 individuals m^{-2} have been reported in the red soil of China [13], and (2) high earthworm density can enhance the influence of earthworms on soil to compensate for the short-duration incubation.

Each microcosm was covered with a black polyethylene cloth and a piece of plastic mesh (1.5 mm) held tightly with a rubber band to prevent earthworms from escaping and to allow aeration. All microcosms were incubated in the dark at temperatures of 25 ± 2 °C for 30-days. After a flux measurement, soil moisture content was manually adjusted every 2 days by adding sterile distilled water.

2.3. Sampling and measurements

2.3.1. N_2O emissions

The measurements of N_2O flux were taken daily for the first 10 days of the experiment and then with an interval of two days for the remaining 20 days. According to the published literature [14,15], the bottles were flushed with ambient air for 30 min and then closed for 2 h. Gas samples (30 ml) were collected from the headspace of the bottles using a gastight syringe at 0 and 2 h after bottle closure. The incubation bottles were kept open after sampling, and the gas samples were analyzed immediately using the gas chromatograph (GC-7890A, Agilent Technologies, USA) equipped with an electron-capture detector (ECD) for N_2O analysis. Fluxes were calculated assuming a linear increase in the concentration of gas over time while the lid was closed. Cumulative gas emissions were calculated assuming linear changes between subsequent measurements [16].

2.3.2. Soil chemical properties and microbial biomass

Soil subsamples were collected from the bottles on days 3, 6, 9, 14 and 20 after gas sampling as carefully as possible via the soil drill method to reach the bottom of the bottle, in order to minimize the

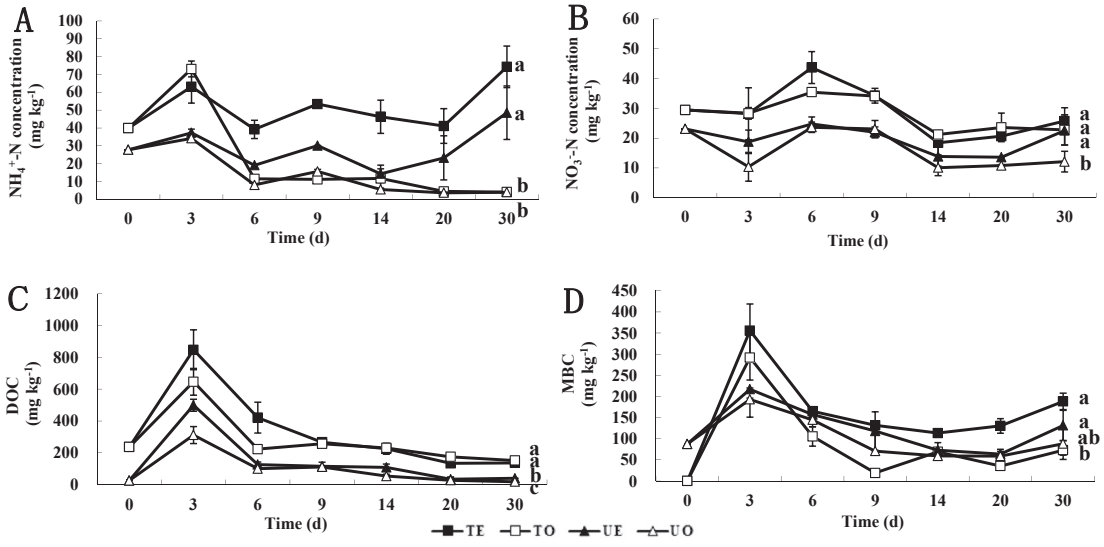

Fig. 1. Variations in NH_4^+–N (A), NO_3^-–N (B), DOC (C) concentration and MBC (D) among the different treatments during the 30-day incubation. Error bars denote standard errors (n = 3). Values on day 30 followed by the same letter(s) are not significant difference ($P < 0.05$) for different treatments. TE: sterilized soil with earthworm inoculation; TO: sterilized soil without earthworm inoculation; UE: unsterilized soil with earthworm inoculation; UO: unsterilized soil without earthworm inoculation.