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Earthworms can increase nitrous oxide emissions from managed grassland: A field study

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ABSTRACT

Earthworms are important in determining the greenhouse gas (GHG) balance of soils. In laboratory studies they have been shown to increase emissions of the potent GHG nitrous oxide (N_2O). Here we test whether these earthworm-induced N_2O emissions also occur in the field. We quantified N_2O emissions in managed grassland in two different seasons (spring and autumn), applying two different types of fertilizer (organic and artificial fertilizer) and under two earthworm densities (175 individuals and 350 individuals m^{-2}) of the species *Lumbricus rubellus* (Hoffmeister). We found an increase in earthworm-induced N_2O emissions of 286 and 394% in autumn for low and high earthworm densities ($P = 0.044$ and $P = 0.007$, respectively). There were no effects of earthworms on N_2O emissions in spring. Fertilizer additions significantly increased cumulative N_2O emissions and grass N content in spring and autumn. For grass N content interactions between earthworm addition and fertilizer type existed in both seasons. Our results suggest that the pathways through which earthworms affect N cycling (and thereby N_2O emission) differ with weather conditions. We postulate that in spring the dry weather conditions overruled any earthworm effects, whereas in autumn earthworms mainly improved soil aeration and thereby increased both plant N uptake and diffusion of N_2O to the atmosphere. While we showed the presence of earthworm-induced N_2O emissions in managed grassland under field conditions for the first time, the nature and intensity of the earthworm effect in the field is conditional on soil physicochemical parameters and thereby on meteorological and seasonal dynamics.

1. Introduction

Earthworms are thought to be important actors in determining the greenhouse gas (GHG) balance of soils. A quantitative review of the overall effect of earthworms on the soil GHG balance reported a significant 42% increase of nitrous oxide (N_2O) emissions due to earthworm activity (Lubbers et al., 2013). Earthworms do not emit N_2O themselves, but rather affect the microbial processes that produce and consume N_2O in the soil through their activity.

These microbial processes are mainly nitrification, denitrification and nitrifier denitrification (Kool et al., 2010; Wrage et al., 2001). Optimal conditions for microbial N_2O production in the soil are controlled by several factors, of which the most important ones are available carbon (C), mineral nitrogen (N), anaerobicity, pH and temperature (Granli and Bøckman, 1994). These controlling factors can be highly variable at the micro scale, both in space and over time. Cumulative soil N_2O emissions are therefore a result of the interactions between biotic and abiotic processes, influencing N_2O production and possibly also reduction through the final step of denitrification. Earthworms can directly influence these controlling factors (*e.g.* by their feeding and burrowing behaviour) and can thereby indirectly affect N_2O emission. They can increase mineral N concentration and available C by mixing organic residues into the soil (Giannopoulos et al., 2010), and they can affect anaerobicity by changing the soil structure through their burrowing and casting activity (Lubbers et al., 2011; Paul et al., 2012; Piron et al., 2012).

Agriculture and associated land use change is estimated to contribute 7.9% to total anthropogenic GHG emissions in the form of N_2O emissions (based on CO_2-equivalents) (IPCC, 2007). The influence of earthworm activity on N_2O emissions is expected to be largest in fertilized grasslands. These grasslands cover approximately 21% of the agricultural land surface in the European Union (Oenema et al., 2005) and are key contributors to global N_2O emissions (Lee et al., 1997). Fertilized grassland soils harbour the greatest numbers of earthworms as they provide a continuous food source (Van Vliet et al., 2007). However, field studies that focus on N_2O emissions from grassland systems induced by earthworm activity have not yet been conducted (Lubbers et al., 2013).

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Statements about the role of earthworms in fertilized grasslands are therefore mainly based on extrapolations from laboratory or greenhouse studies.

The only field study reporting earthworm-induced N_2O emissions that we are aware of is Borken et al. (2000). They found an increase of 57% in cumulative N_2O emissions from repacked forest soil columns applied with beech litter and inoculated with earthworms (*Lumbricus terrestris* L.). Furthermore, a mesocosm experiment with grass growing on a fertilized loamy soil reported a 50.8% increase in cumulative N_2O emissions when earthworms were inoculated (Lubbers et al., 2011). Both studies show large earthworm-induced effects on N_2O emissions. However, translation of these results to realistic field conditions in fertilized grasslands remains problematic. Both studies are still highly manipulative and avoid the earthworm-soil feedback mechanisms that are typical for field studies. For example, earthworms may affect soil moisture levels, a key controlling factor for N_2O emissions (Bremner, 1997; Pihlatie et al., 2004), as their burrowing activity influences drainage. Such an effect would not have been picked up by either of these studies.

The effects earthworms have on the factors controlling microbial N_2O production varies with their ecological strategy. Earthworms are typically classified into three functional groups: (i) *epigeic* species feed on undecomposed litter and its associated microflora, ingesting relatively little mineral soil material; (ii) *endogeic* species feed on mineral soil and associated organic matter and live in non-permanent branching burrows; (iii) *anecic* species feed on fresh surface litter that they pull down into deep, vertical and permanent burrows (Bouché, 1977; Edwards, 2004). Although mesocosm studies have demonstrated that all functional groups are able to increase N_2O emissions (Giannopoulos et al., 2010; Nebert et al., 2011; Rizhiya et al., 2007), the mesocosm study with growing grass showed the largest earthworm effect on N_2O emissions with the epigeic species *Lumbricus rubellus* (Hoffmeister) (Lubbers et al., 2011). It remains to be determined to what extent weather conditions in the field (especially temperature and precipitation) might nullify the effects of (epigeic) earthworms.

The aim of this field study is therefore to test whether the previously observed earthworm-induced N_2O emissions under controlled conditions also occur in the field. We quantified the earthworm effect in managed grassland in different seasons, applying different types of fertilizer (organic and artificial fertilizer) and under two earthworm densities of the species *L. rubellus*. We hypothesized that: (i) higher earthworm densities will lead to increased N_2O emissions; (ii) earthworms will have a larger effect on N_2O emissions in autumn than in spring due to their greater activity in autumn; and (iii) earthworm-induced N_2O emissions will be larger with organic fertilizer than with artificial fertilizer, because earthworms will accelerate nutrient mineralization when ingesting organic fertilizer, thereby further increasing N_2O emissions.

2. Materials and methods

2.1. Experimental set up

We quantified the effect of two earthworm densities and two fertilizer types on N_2O emissions from an agricultural grassland in two different seasons. In spring and autumn 2011, we carried out a field study with intact soil columns over 40 and 43 days, respectively. The selected field sites were both located at the experimental farm "Droevendaal", Wageningen, the Netherlands (51°59′ N, 5°39′ E), and had not been fertilized for at least five years prior to the start of our experiment. Table 1 lists the soil characteristics for the spring and autumn sites. The experiment was laid out as a full factorial design, with the addition of *L. rubellus* and fertilizer type as independent factors. Earthworm treatments included control treatments without addition of earthworms or fertilizer (C), as well as *L. rubellus* applied in average densities for Dutch grassland soils (175 individuals m^{-2} or 5 individuals per column – 175EW) (Didden, 2001); or in extreme densities (350 individuals m^{-2} or 10 individuals per column – 350EW). Fertilizer treatments included no fertilizer; organic fertilizer (slurry; S) and inorganic fertilizer (artificial; A). Both fertilizers were applied at a rate of 170 kg N ha^{-1} yr^{-1}, according to standard Dutch practice on sandy soil for conventional agriculture (MNP, 2007). All treatments are listed in Table 2. With three earthworm treatments and three fertilizer treatments, and five replicates installed in five blocks, the total number of columns was 45. Additional soil columns were installed to allow for quantifying earthworm survival one and two weeks after the start of the studies. Destructive sampling took place on May 30 and June 1 for the spring experiment and on November 12 and 14 for the autumn experiment.

Table 1
Soil characteristics at the spring and autumn site.

[a]Soil characteristics (0–25 cm)	Spring site	Autumn site
Total N (g kg^{-1})	1.30	1.28
Organic matter (%)	2.0	3.1
C/N-ratio	9	14
pH (KCl)	5.2	5.5
CEC (mmol kg^{-1})	76	29

[a] The soil at both sites was classified as a typic endoaquoll (Soil Survey Staff, 1999) with 75% sand, 23% silt and 2% clay.

The columns were constructed of polyvinylchloride (PVC) tubes with an internal diameter of 19 cm and a length of 60 cm. The columns were pushed into the soil to a depth of approximately 40 cm using a crane (the average depth of the profile, below which the sandy Aeolian parent material started). Columns were spaced 20 cm apart. The inside of the columns, just underneath the column top, was lined with adhesive hook tape (part of the 'hook and loop' fastener) to prevent the introduced earthworms from escaping (Lubbers and Van Groenigen, 2013). In both spring and autumn the grass was cut short (approximately 2.5 cm) in the same week the columns were pushed into the soil.

2.2. Earthworm addition, fertilizer application and simulated rainfall

The epigeic earthworm species *L. rubellus* is the most common representative of its functional group in Dutch grassland soils (Didden, 2001). Individuals were collected from park areas in Wageningen, a week before the start of the spring and autumn experiments. They were kept in sandy soil with poplar (*Populus* spp *L.*) leaves as feed, at 15 °C until each experiment started. Collected earthworms were adults or large juveniles and had the contents of their intestines voided 48 h before weighing (Dalby et al., 1996), and were subsequently placed on the soil surface of the columns.

After the earthworms entered the soil, the fertilizer treatments were applied. Artificial fertilizer was applied at a rate of 482 mg N as NH_4NO_3, 317 mg P as KH_2PO_4 and 800 mg K as K_2SO_4 per column, translating to 170 kg N ha^{-1}, 111 kg P ha^{-1} and 282 kg K ha^{-1}. The organic fertilizer was cow slurry and was applied at a rate of 482 mg N, 74 mg P and 462 mg K per column, translating to 170 kg N ha^{-1}, 26 kg P ha^{-1} and 163 kg K ha^{-1}. The N application was split over two dressings, each of 85 kg N ha^{-1}, at days 0 and 20 to reach the total amount of 170 kg N ha^{-1}. The application of fertilizers was done by simulating a rainfall event of 10 mm (284 ml per column): the artificial fertilizer was a 284 ml solution with NH_4NO_3, KH_2PO_4 and KH_2PO_4 dissolved in demineralized water; for the cow slurry the moisture content was determined and the amount of water applied