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Interactions between microbial-feeding and predatory soil fauna trigger N_2O emissions

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ABSTRACT

Recent research has shown that microbial-feeding invertebrate soil fauna species can significantly contribute to N_2O emissions. However, in soil food webs microbial-feeding soil fauna interact with each other and with their predators, which affects microbial activity. To date we lack empirical tests of whether or not these interactions play a significant role in N_2O emissions from soil. Therefore we studied how interactions between soil microbes, two groups of microbial-feeding soil fauna (enchytraeids and fungivorous mites) and their predators (predatory mites) affect soil N_2O emissions. We hypothesized that: 1) the presence of two microbial-feeding fauna groups (enchytraeids and fungivorous mites) together increase N_2O emissions more than when only a single group is present; and 2) the addition of predatory mites further enhances N_2O emissions. We assembled soil food webs consisting of soil microbes, enchytraeids, fungivorous and predatory mites in microcosms with sandy loamy soil and sterilised hay as a substrate for the soil microbes. N_2O emissions were measured during 56 days. We found no support for our first yet support for our second hypothesis. Addition of predatory mites to microcosms with enchytraeids and fungivorous mites increased N_2O emissions significantly from 135.3 to 482.1 mg N m^{-2}, which was also significantly higher than the control without fauna (83 mg N m^{-2}) ($P < 0.001$). In presence of enchytraeids, fungivorous and predatory mites, we found much higher nitrate availability at the time of the N_2O peak on Day 35 (10.9 versus 5.5 mg N per kg soil without soil fauna), indicating that the major increase in N_2O emissions in this treatment may be due to increased nitrification. Increased nitrification may be attributed to higher availability of N from the dead tissues of fungivorous mites and increased activity of the enchytraeids that might also have affected soil structure and contributed to increased N_2O emissions. This study demonstrates the importance of interactions between microbial-feeding invertebrate soil fauna and their predators in understanding N_2O emissions.

1. Introduction

Nitrous oxide (N_2O) is a major greenhouse gas, with a global warming potential approximately 300 times higher on a per molecule basis than carbon dioxide (CO_2) (Solomon et al., 2007). The concentration of N_2O in the atmosphere has been increasing by 0.2–0.3% per year in recent times, and this has been attributed mainly to increased use of nitrogen (N) fertilizers in agriculture (Thomson et al., 2012). Soil is the major source of N_2O, a gas which is principally produced by microbial processes in soil such as nitrification, denitrification (Williams et al., 1992) and nitrifier-denitrification (Kool et al., 2010). All these processes are driven by the activity of soil microorganisms and are controlled by soil abiotic conditions such as pH, anaerobicity and temperature, as well as by the availability of inorganic forms of N and labile organic matter (Davidson et al., 2000).

The role of soil fauna in N-mineralization has been well acknowledged (Verhoef and Brussaard, 1990; De Ruiter et al., 1993). However, the potential roles that soil fauna may play in increasing or decreasing N_2O emissions from soil has rarely been explored (but see Kuiper et al., 2013). The main substrates for soil N_2O production are ammonium (NH_4^+) and nitrate (NO_3^-). Soil fauna can affect concentrations of these compounds in various ways: first by feeding on microbes that mineralize, nitrify and/or denitrify; second, by transporting and dispersing the microbes within the soil, thereby stimulating microbial growth and activities; and third by

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increasing the surface area of substrates by shredding of litter which facilitates microbial colonization on the substrates (Petersen and Luxton, 1982; Seastedt, 1984; Verhoef and Brussaard, 1990; Gessner et al., 2010). These interactions between microbes and soil fauna are important with respect to N-mineralization, as suggested by Verhoef and Brussaard (1990) that nearly 30% of N-mineralization in soil is due to the presence and activity of soil fauna, despite the fact that they only encompass a weight of 2.5% of the total soil microbial biomass (Moore et al., 1988). With such a strong influence on N dynamics, soil fauna is likely to have a significant impact on N_2O emissions from soil.

Soil invertebrate fauna comprises a large variety of species living both below and on the soil surface. So far studies on the role of soil fauna in N_2O emissions have focused on earthworms (Bertora et al., 2007; Paul et al., 2012) and enchytraeids (Van Vliet et al., 2004). These studies showed that these soil fauna could increase N_2O emissions, most likely due to their effects on soil structure and their capacity of stimulating microbial activity (Lubbers et al., 2013). A recent microcosm study by Kuiper et al. (2013) revealed that different functional groups of soil fauna can influence N_2O emissions to different extents (decreased, increased, accelerated or delayed) depending on their impact on soil physical conditions and on immobilization of N in microbial biomass. These results trigger the important, yet unanswered question of how interactions between different functional groups of soil fauna affect N_2O emissions.

Two key functional groups other than earthworms in the soil food web that have been well studied with respect to N-mineralization are enchytraeids and microarthropods (De Ruiter et al., 1993; Brussaard, 1998; Wardle, 2002). Enchytraeids are fast-grazing consumers feeding on both detritus and fungi, and they can potentially alter soil physical structure more than any other soil fauna of their size (Didden, 1990; Brussaard et al., 2012). Enchytraeids produce excreta that are richer in NH_4^+ compared to other soil fauna (Didden, 1990), and also soil NO_3^- levels appear to be higher in the presence of enchytraeids than with microarthropods (Edsberg, 2000). This higher NO_3^- production has been linked to increased nitrification potential (Liiri et al., 2007). Enchytraeids have also been recognized as vectors of microbes (Rantalainen et al., 2004), which may influence both nitrification and denitrification processes (Van Vliet et al., 2004). Microarthropods form another large soil fauna group, mostly comprising species of mites and collembola (Brussaard, 1997). A large group of mite species feed on fungi and therefore plays an important role in N-mineralization (Seastedt, 1984; Coleman et al., 2004).

As shown in an experiment with macro-detrivores, combinations of functionally dissimilar soil fauna can increase the N-mineralization rate due to facilitative interactions (Heemsbergen et al., 2004). Such facilitative interactions include one group benefitting from the activity of another group such as through changes in soil structure or litter shredding by isopods promoting microbial growth (Wardle, 2006). Nevertheless, competitive interactions may also positively influence mineralization rates (Loreau, 1998). Predatory mites, which represent another large group of soil mites, feed on fungivorous mites and enchytraeids as well as collembola and nematodes (De Ruiter et al., 1995). Predatory mites can influence microbial activities through trophic cascades (induced positive effects on microbes by feeding on microbial feeders), although empirical evidence of trophic cascades in soil food webs is scarce (Mikola and Setälä, 1998; Bardgett and Wardle, 2010). Presence of predatory mites can potentially influence the behaviour of fungivourous mites and enchytraeids in terms of their feeding rate and spatial distribution, in line with predator–prey relations in other systems (Schmitz et al., 2004). This may potentially cause additional changes in N-mineralization and soil structure, and thereby to N_2O emissions.

The aim of this study was to explore how interactions between soil microbes, microbial-feeding soil fauna and their predators affect soil N_2O emissions. We selected common species of enchytraeids and fungivorous mites as microbial consumers and predatory mites as consumers of enchytraeids and fungivorous mites to test the following hypotheses: 1) the combination of two groups of microbial-feeding fauna (enchytraeids and fungivorous mites) increases N_2O emissions compared to when only one of both groups is present; and 2) addition of predatory mites further enhances N_2O emissions.

2. Materials and methods

2.1. Experimental set-up

We tested our hypotheses in a 56 day microcosm experiment. The microcosms were constructed from polypropylene (diameter = 6.7 cm, height = 15 cm, volume = 500 cm^3) and were filled with soil (loamy sand texture) from the Droevendaal Agricultural Farm near Wageningen University in the Netherlands (51°59′ N, 5°39′ E). After sieving (10 mm mesh size) the soil was dried for 24 h at 70 °C to make the soil free from micro fauna such as nematodes, enchytraeids and micro-arthropods, while minimally affecting microbes present in the soil (Kaneda and Kaneko, 2011). The organic material used in this experiment was hay with a C: N ratio of 13.8 measured in a C/N analyser (LECO CNH-analyser, LECO Europe B.V., Geleen, Netherlands). Prior to its use the hay was cut into small pieces and sterilized by autoclaving for 15 min at 121 °C to remove microbes. Each microcosm was packed with 260 g of dry soil, 39.5 g of distilled water (to reach 70% water filled pore space WFPS) and 1.34 g of dry hay (equivalent to 125 kg N ha^{-1}), which we mixed with the top layer of soil before packing to the set density. Subsequently, the microcosms were pre-incubated for three days in a dark climate room with a constant temperature of 15 °C and 60% humidity to facilitate microbial colonization of the soil and substrate before the fauna inoculation. Distilled water was added every three days in all the microcosms to maintain soil moisture. The microcosms were covered with black woven cotton cloths to facilitate gas exchange whilst minimizing moisture loss.

Enchytraeids (*Enchytraeus albidus*, Henle, 1837) and fungivorous mites (*Acarus siro*, Linnaeus, 1758 and *Rhizoglyphus echinopus*, Fumouze and Robin, 1868) were used from the soil fauna cultures as described in Kuiper et al. (2013). Predatory mites (*Hypoaspis miles*, Berlese, 1892) were bought commercially as Entomite-M (Koppert, Berkel en Rodenrijs, the Netherlands). The faunal treatments for the experiment as well as the number of individuals used per microcosms, their density and total biomass were based on realistic densities as can be found in the field (Table 1). For treatments with enchytraeids, the ratio of adult to juvenile was kept equal. The experiment was set-up using a completely randomized design with five blocks, with each of the five replicates in a separate block. We included three extra replicates for all treatments for destructive sampling on Day 35 of the experiment. The three extra replicates were randomly assigned within three of the five blocks.

2.2. N_2O and CO_2 measurements

We started to measure N_2O and CO_2 fluxes 12 h after soil fauna was added. Both types of gas fluxes were measured two times a week during eight weeks. A photo-acoustic gas monitor (Type 1302, Brüel and Kjaer, Denmark) was used to measure gas fluxes of both CO_2 and N_2O (Kuiper et al., 2013). Before measuring the fluxes, microcosms were closed for at least 45 min with lids equipped with two rubber septa, to allow accumulation of N_2O and CO_2. For measuring gas flux, a sampling circuit was created using Teflon