Soil Biology & Biochemistry 70 (2014) 256–262

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

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

Madhav Prakash Thakur [a,b], Jan Willem van Groenigen [a], Imke Kuiper [a], Gerlinde B. De Deyn [a,*]

[a] Department of Soil Quality, Wageningen University, P.O. BOX 47, Droevendaalsesteeg 4, 6700 AA, The Netherlands
[b] Institute of Ecology, Friedrich Schiller University Jena, Dornburger Str. 159, 07743 Jena, Germany

ARTICLE INFO

Article history:
Received 11 June 2013
Received in revised form
17 December 2013
Accepted 20 December 2013
Available online 17 January 2014

Keywords:
Nitrogen mineralization
Food web
Trophic interaction
Greenhouse gas emissions
Soil biota
Global change

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

* Corresponding author. Tel.: +31 317 482123; fax: +31 317 419000.
E-mail addresses: madhav.thakur@uni-jena.de (M.P. Thakur), janwillem.vangroenigen@wur.nl (J.W. van Groenigen), imke.kuiper@hotmail.com (I. Kuiper), gerlinde.dedeyn@wur.nl (G.B. De Deyn).

0038-0717/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2013.12.020

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

Table 1
Details of the fauna additions per treatment.

Treatment abbreviation	Species	Faunal density		
		# microcosm^{-1}	# m^{-2}	μg dwt per g dry soil
CH	Control with hay	–	–	–
E	*Enchytraeus albidus*	50	2596	96.4
M	Fungivorous mites (*Rhizoglyphus echinopus*, *Acarus siro*)	400	20,769	1.5
EM	*Enchytraeus albidus* and fungivorous mites	450 (50 + 400)	23,365	98
EP	*Enchytraeus albidus* and *Hypoaspis miles*	53 (50 + 3)	2752	96.5
MP	Fungivorous mites and *Hypoaspis miles*	403 (400 + 3)	20925	1.6
EMP	*Enchytraeus albidus*, fungivorous mites and *Hypoaspis miles*	453 (50 + 400+3)	23,521	98

tubes connection to the gas monitor and the headspace of the microcosms, by plugging a hollow needle through each of the septa. The N_2O concentration in ambient air was measured each time after 10 microcosms were measured; these ambient levels were used as a correction factor while calculating fluxes from the microcosms. When measuring N_2O fluxes a soda lime filter was used to minimize interference of CO_2 to maintain accuracy of the N_2O measurements. Measurement of CO_2 fluxes was done in an identical way, but without the soda lime filter.

2.3. Soil parameters

For all the treatments we measured microbial and chemical parameters of the soil at three stages: baseline measurements at the start of experiment (Day 1, Table 2), mid-term harvest measurements at the time of the peak in N_2O flux (Day 35), and final harvest measurements at the end of the experiment when N_2O fluxes had subsided (Day 56). Soil pH, mineral N (NH_4^+ and $NO_3^- + NO_2^-$) and microbial biomass N were measured at all three sampling times. Mineral N was measured in sieved (10 mm) and dried soil subsamples (dried at 40 °C) after extraction with 0.01 M $CaCl_2$ (Houba, 2000). Microbial biomass N was measured from fresh soil subsamples according to Brookes et al. (1985) by the chloroform fumigation method, followed by 0.01 M K_2SO_4 extraction and using a correction factor of 0.54.

2.4. Soil fauna extractions

Soil fauna was extracted from the soils on Day 35, when the treatments were near their peak in N_2O flux, as well as on Day 56, during the final harvest. To extract the enchytraeids we used the Baermann funnel method, whereas for mites we used the Tullgren funnel extraction technique (Petersen and Luxton, 1982). For treatments with only one species, we took a subsample of half the volume of soil per microcosm, whereas for two species we took subsamples from a quarter of the total soil volume. This was necessary because of the different extraction techniques for enchytraeids (wetting) and mites (drying). Abundances and/or biomass were expressed on a per soil weight basis to standardise the parameters. We identified juveniles and adults for enchytraeids based on the presence/absence of visible clitellum. We counted fungivorous and predatory mites stored in ethanol (70%) after the extraction. The individual fresh body weight of enchytraeids was calculated using allometric relations provided by Abrahamsen (1972) for different body lengths. We expressed enchytraeid biomass in dry weight by correcting for moisture using moisture content values given by Maraldo and Holmstrup (2009). For fungivorous and predatory mites, we used individual dry body weight given for functional groups by Vreeken-Buijs (1998). We expressed fauna body weight as dry weight (dw) in mg per g of dry soil.

Table 2
Baseline soil parameter values after incubation period of three days.

Soil parameters	
Microbial biomass N	31.5 mg N kg^{-1}
NH_4^+	5.1 mg N kg^{-1}
NO_3^-	3.6 mg N kg^{-1}
pH-$CaCl_2$	6.4
Bulk density	1.4 g cm^{-3}

2.5. Statistics

We used one-way ANOVA to test the treatment effects of soil fauna additions on cumulative N_2O emissions. Cumulative N_2O emissions comprised the emissions during the entire 56 days of the experiment. To account for differences in soil fauna densities among the treatments at the start of the experiment, we included their initial densities as a covariable in the ANOVA (ANCOVA). Further, we carried out post-hoc multiple comparison (Tukey HSD, $\alpha = 0.05$) to test the differences in N_2O and CO_2 emissions, soil abiotic factors and fauna biomass between the treatments. We carried out Redundancy Discriminatory Analysis (RDA) to find the relation between soil parameters and soil fauna, and actual and cumulative N_2O and CO_2 emissions on Day 35 and Day 56. To test the significance of the canonical axes of the multivariate RDA analysis we used a Monte Carlo permutation test with 999 permutations. N_2O and CO_2 emissions were used as response variables, and microbial and fauna biomass, soil pH, NH_4 and NO_3 levels were used as explanatory variables in the RDA. N_2O and CO_2 emission rates were log-transformed for both RDA on Day 35 as 1*log ($Y_{35} + 1$) and Day 56 as 10*log ($Y_{56} + 1$) where Y_{35} and Y_{56} are gas flux values on Day 35 and Day 56, respectively. We used the statistical software SPSS version 16 to carry out AN(C)OVAs, and for the RDA analysis, we used Canoco for Windows 4.5.

3. Results

3.1. N_2O emissions

Overall, we found a significant effect of the treatments on the cumulative N_2O emissions for the experimental period (One-way ANOVA $F_{6,28} = 26.74, P < 0.001$). However, post-hoc analysis revealed that only the treatment with enchytraeids, fungivorous and predatory mites (EMP) was significantly different. The N_2O emissions from this treatment (482.1 mg N m^{-2}) were on average nearly six times higher than those from the control treatment (CH) (83 mg N m^{-2}), while the other treatments which included one or two types of soil fauna did not differ significantly from the control treatment (Fig. 1). We already observed increased N_2O emissions in the EMP treatment (51. 8 mg N m^{-2}) at the time of the first destructive harvest (Day 35) compared to the control treatment (6.3 mg N m^{-2}).

3.2. Soil fauna and abiotic factors

On Day 35, the RDA showed that 71.7% of the variations in N_2O and CO_2 emissions could be explained by the soil abiotic factors and soil fauna treatments (Fig. 2a). Axis 1 of the RDA plot explained nearly 38% of the variation, and Axis 2 explained a further 17%. Axis

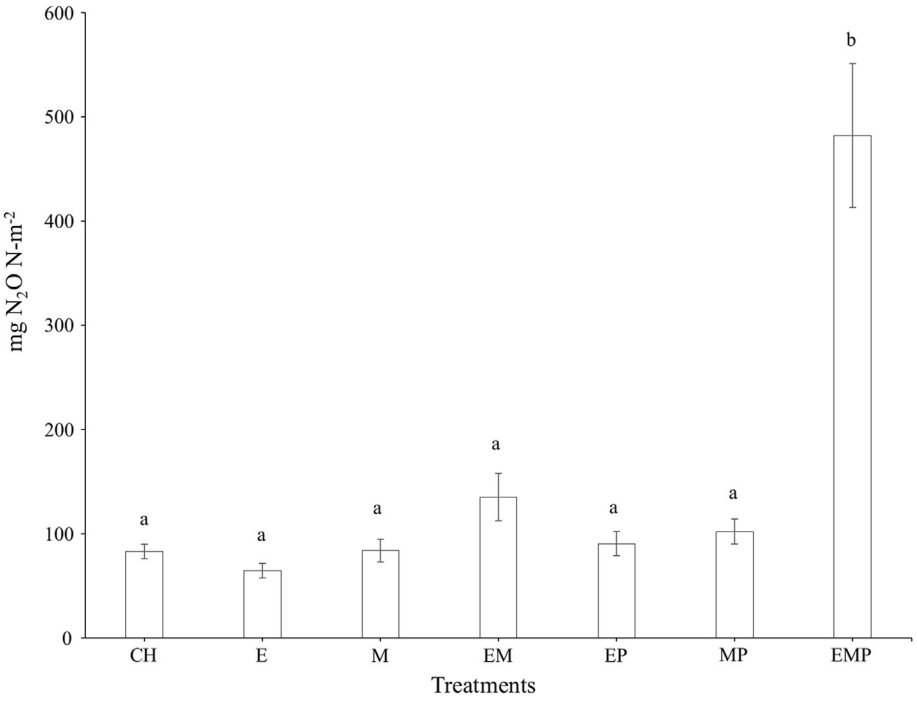

Fig. 1. Cumulative N_2O emission for all treatments at the final harvest (Day 56). The initial faunal density was used as a covariate for the ANOVA test. Bars are means (±1 S.E.), different letters indicate significant differences based on Tukey HSD multiple comparison at $\alpha = 0.05$. Treatment symbols are given in Table 1, in brief CH = control hay, E = enchytraeids, M = fungivorous mites, EM = enchytraeids + fungivorous mites, EP = enchytraeids + predatory mites, EMP = enchytraeids + fungivorous mites + predatory mites.

1 was positively correlated with mineral N (especially NO_3^-) ($r = 0.71$) and enchytraeid biomass ($r = 0.52$) but negatively correlated with microbial biomass N ($r = -0.35$). Axis 2 did not show any strong correlation with explanatory variables. At the final harvest on Day 56, the RDA plot showed changes in the relation between soil abiotic and soil fauna parameters and the actual and cumulative N_2O emissions compared to Day 35 (Fig. 2b). Microbial biomass N, which was negatively related to Axis 1 on Day 35, showed a positive correlation with the first axis on Day 56 ($r = 0.18$), whereas NO_3^- showed a negative relation with Axis 1 ($r = -0.45$) on Day 56. Axis 1 alone explained 77% of the variation in N_2O and CO_2 emissions whereas Axis 2 explained only 3% of the variation. In total, the RDA analysis explained 82.4% of the variation in N_2O and CO_2 emissions on Day 56.

We did not find significant differences in NH_4^+ or in microbial biomass N among soil fauna treatments on both harvest days (Day 35 and Day 56), although a trend of low microbial biomass N in the treatment with enchytraeids, fungivorous mites and predatory mites (EMP) was notable (11.78 mg N per kg soil). The concentration of NO_3^- varied among treatments on both harvest days being highest in treatment EMP on Day 35 (Table 3a). The total biomass of the enchytraeids increased over time and was higher when enchytraeids were living with fungivorous mites, whereas the biomass of the fungivorous mites decreased; for the predatory mites no significant changes over time in biomass were observed (Table 3b).

4. Discussion

Our results show that N_2O emissions are increased by the presence of a combination of enchytraeids, fungivorous and predatory mites (EMP), in line with our second hypothesis. We did not, however, find that the addition of predatory mites increases N_2O emissions when introduced in combination with only enchytraeids or only fungivorous mites (EP and MP). This provides partial support for Hypothesis 2. Further, no significant differences in N_2O emissions between the treatments with single or combined presence of enchytraeids and fungivorous mites were found, hence we reject Hypothesis 1. Our results are in line with the idea that N-mineralization rates enhance when different types of decomposers are combined with their predators (Beare et al., 1995; Brussaard, 1997; Hättenschwiler et al., 2005), which potentially can lead to increased N_2O emissions. Mineral N availability, however, cannot fully explain our results on the enhancement of N_2O emissions, additional explanatory factors such as soil physical-chemical factors must also come into play (Kuiper et al. 2013). We therefore discuss below how interactions between soil fauna can potentially change soil physical–chemical characters which are relevant to increase N_2O emissions. Thereafter we highlight that a combination of biotic interactions in soil together with quantifications of soil physical–chemical characteristics can help to reveal a comprehensive overview of N_2O emissions from soil.

The presence of enchytraeids and fungivorous mites along with predatory mites increased the availability of inorganic N through three level trophic interactions (Table 3a). The feeding of the predatory mites on microbial-feeding soil fauna may prevent the overgrazing of microbes which can lead to higher mineral N availability in the system (Schmitz et al., 2010). Predation pressure by a single species on two prey species can invariably affect prey population (Hixon and Menge, 1991); empirical evidence shows that one prey is often more harmed while the other prey species generally remain unharmed (Toscano et al., 2010). Our observation of the decline in fungivorous mites and increase in enchytraeids in the presence of predatory mites are in line with such argument (Table 3b). This could be due to increased burrowing activities of the enchytraeids in the presence of predators, which results into habitat destruction of fungivorous mites, an example of competitive interactions (Maraun and Scheu, 2000). In turn, fungivorous mites would become exposed

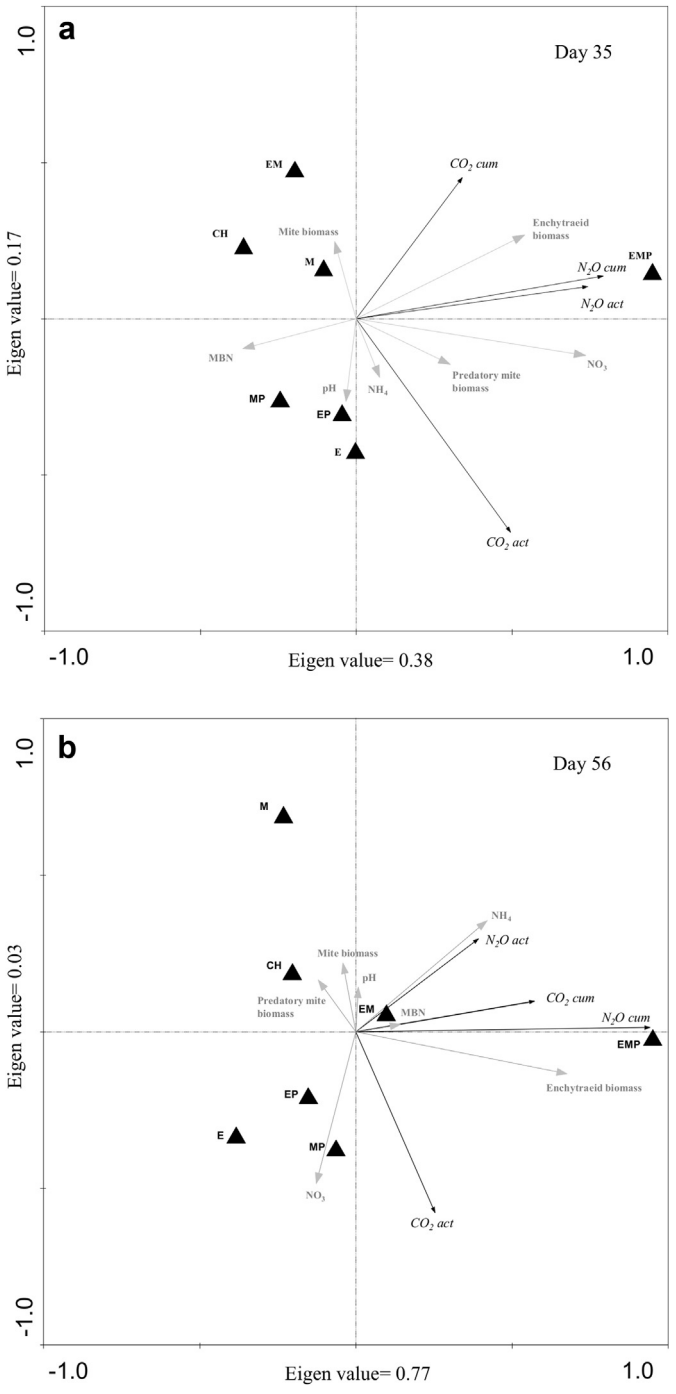

Fig. 2. Biplot based on RDA for N_2O and CO_2 emissions explained by soil fauna and soil physical-chemical parameters on a) Day 35 and b) Day 56. Grey arrows: soil abiotic factors and soil fauna biomass, black arrows: gas emissions with *cum* = cumulative and *act* = actual (on the day of measurement). Closed triangles indicate the treatments; treatment symbols are given in Table 1, in brief CH = control hay, E = enchytraeids, M = fungivorous mites, EM = enchytraeids + fungivorous mites, EP = enchytraeids + predatory mites, EMP = enchytraeids + fungivorous mites + predatory mites.

to higher predation pressure, causing their higher mortality thus an increase in N input from dead tissues which can be colonized and mineralized by soil microbes (Seastedt, 1984).

In cases with higher availability of high quality substrate (i.e. substrate with high N concentration), microbes are accounted to increase the production of NO_3^- (Stark and Hart, 1997; Burger and Jackson, 2003). This increase in NO_3^- availability in combination with soil pockets with low oxygen levels can create ideal conditions for N_2O emissions (Williams et al., 1992). The lower microbial biomass N in treatments with enchytraeids, fungivorous and predatory mites at the time of high N_2O emission was accompanied with a higher availability of NO_3^-, which can be explained by the fact that it was less immobilized in microbial biomass (Table 3a). Also, given that our microcosm favoured the process of denitrification due to soil moisture levels of 70% WFPS during the whole experimental period, the NO_3^- consumption by denitrifiers was likely to be high at higher concentrations of NO_3^- (Bateman and Baggs, 2005).

The presence of enchytraeids enhances the dispersal of microbes through the soil profile (Williams and Griffiths, 1989; Rantalainen et al., 2004). This can additionally favour microbial colonization on dead fungivorous mites and litter, leading to increased N-mineralization. The enchytraeids themselves can also feed on dead animal tissues which may have provided an additional supply of NH_4^+ and consequently NO_3^- in the microcosms (Didden, 1993; Laurén et al., 2012). The higher production of NO_3^- and its higher consumption such as by denitrifying microbes in treatments with the enchytraeids, fungivorous and predatory mites could be a possible reason for higher N_2O emissions. Other treatments such as with enchytraeids and fungivorous mites also increased the NO_3^- concentration in soil over the experimental period (Table 3a). However, N_2O emissions from these treatments were relatively low, possibly due to less favourable soil physical conditions for incomplete reduction of NO_3^- into N_2O (Kuiper et al., 2013).

As dynamics of N_2O emissions always depend on soil abiotic and physical characters (Williams et al., 1992), soil fauna that can influence soil physical structures can considerably influence N_2O emissions (Kuiper et al., 2013). The incomplete reduction of NO_3^- to N_2O is essentially attributed to particular levels of soil aeration, not too oxic and not too anoxic (Williams et al., 1992; Davidson et al., 1993). Enchytraeids for instance can increase porosity in sandy soils which increases aeration and thereby they can decrease N_2O emissions by reducing denitrification rates (Van Vliet et al., 2004). In treatments without enchytraeids, such as with only fungivorous mites, negligible changes in soil structure and soil porosity are expected (Lee and Foster, 1991; Kuiper et al., 2013). On the other hand increased levels of anoxicity may also result from increased biotic activity and total respiration (CO_2 emission), which decreases oxygen availability in soil. Our CO_2 emission data shows that total respiration was comparatively higher in the enchytraeids, fungivorous and predatory mites (EMP) treatment than in the other treatments, albeit not significantly higher (Supporting Information 1).

Our results indicate that different forms of interactions among soil fauna of different feeding guilds, such as one negatively affecting another, can influence N_2O emissions from soil depending on the extent to which such interactions influence soil physical and chemical conditions. Future studies should consider different combinations of soil fauna groups and be able to quantify their impacts on soil physical–chemical factors resulting from their interactions for establishing mechanistic relations between soil food web dynamics and N_2O emissions.

5. Conclusion

We found that the combination of enchytraeids, fungivorous and predatory mites can dramatically enhance N_2O emissions, indicating the importance of soil fauna interactions in N_2O emissions from soil. We argue that accounting for biotic (both trophic and non-trophic) interactions in soil that may alter soil physical–chemical characteristics can increase our understanding of N_2O

Table 3a
Change in soil parameters over experimental period (treatments CH = control hay, E = enchytraeids, M = fungivorous mites, EM = enchytraeids + fungivorous mites, EP = enchytraeids + predatory mites, EMP = enchytraeids + fungivorous mites + predatory mites).

Treatment	MBN [mg N kg^{-1} soil]		NH_4 [mg N kg^{-1} soil]		NO_3 [mg N kg^{-1} soil]	
	Day 35	Day 56	Day 35	Day 56	Day 35	Day 56
CH	17.3 ± 1.36	8.0 ± 3.3	19.8 ± 1.33	8.0 ± 0.53	5.5 ± 0.93 a	20.5 ± 1.51 a
E	21.3 ± 4.64	3.5 ± 1.5	23.6 ± 1.44	7.2 ± 0.59	6.7 ± 0.81 a	32.2 ± 2.28 b
M	24.5 ± 2.60	8.3 ± 0.9	23.5 ± 1.48	7.7 ± 0.48	6.0 ± 0.11 a	21.8 ± 1.74 a
EM	15.8 ± 3.02	1.5 ± 3.3	25.1 ± 1.47	7.6 ± 0.46	8.9 ± 0.74 ab	39.4 ± 1.79 b
EP	19.2 ± 3.92	4.1 ± 0.67	24.7 ± 0.16	7.7 ± 0.47	8.2 ± 0.40 ab	35.7 ± 2.63 b
MP	18.8 ± 3.46	6.3 ± 0.88	23.0 ± 0.10	7.5 ± 0.39	5.2 ± 0.56 a	38.1 ± 1.68 b
EMP	11.78 ± 0.78	7.3 ± 3.8	22.5 ± 1.22	8.82 ± 0.78	10.9 ± 1.47 b	22.2 ± 1.82 a
ANOVA	ns	ns	ns	ns	<0.01	<0.001

Table 3b
Faunal biomass (μg dw per g dry soil) changes over experimental period (treatment symbols see Table 1 and legend of Table 3a).

Enchytraeid biomass			Mite biomass			Predatory mites biomass		
Treat-ment	Day 35	Day 56	Treat-ment	Day 35	Day 56	Treat-ment	Day 35	Day 56
E	520 ± 110 a	820 ± 140 a	M	0.7 ± 0.1 a	0.1 ± 0.03 a	EP	0.04 ± 0.02	0.1 ± 0.03
EM	1400 ± 90 b	1450 ± 170 ab	EM	0.3 ± 0.2 a	0.03 ± 0.01 a	MP	0.1	0.01
EP	960 ± 110 ab	1030 ± 130 ab	MP	0.1 ± 0.03 a	0.1 ± 0.03 a	EMP	0.1 ± 0.02	0.1 ± 0.03
EMP	1410 ± 230 b	1800 ± 300 b	EMP	0.1 ± 0.05 a	0.01 a			
ANO-VA	<0.01	<0.05		<0.05	<0.05		ns	ns

emissions and will enhance our predictive capacity for N_2O emissions from soil.

Acknowledgements

We are grateful to the referees and editor for the very useful comments they provided which greatly improved the manuscript. This study was supported by a personal VIDI grant from NWO-ALW to J.W.v.G., a Marie Curie Intra European Fellowship within the 7th European Community Framework Programme to G.B.D.D. and a Netherlands Fellowship Program grant to M.P.T.. We thank Tamás Salánki for providing the enchytraeids, Iza Lesna and Maurice Sabelis for providing the fungivorous mite species, Jaap Nelemans, Willeke van Tintelen, Harm Gooren and Gerben Bakker for their assistance with laboratory work and Lijbert Brussaard for his comments to improve the earlier version of this manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.12.020.

References

Abrahamsen, G., 1972. Studies on body-volume, body surface area, density and live weight of Enchytraeidae (Oligochaeta). Pedobiologia 13, 6–15.
Bardgett, R., Wardle, D., 2010. Aboveground-Belowground Linkages: Biotic Interactions, Ecosystem Processes, and Global Change. Oxford University Press, New York, USA.
Bateman, E.J., Baggs, E.M., 2005. Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space. Biology and Fertility of Soils 41, 379–388.
Beare, M., Coleman, D., Crossley, D., 1995. A hierarchical approach to evaluating the significance of soil biodiversity to biogeochemical cycling. Plant and Soil 170, 5–22.
Bertora, C., Van Vliet, P., Hummelink, E.W.J., Van Groenigen, J.W., 2007. Do earthworms increase N_2O emissions in ploughed grassland? Soil Biology and Biochemistry 39, 632–640.
Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen – A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17, 837–842.
Brussaard, L., 1997. Biodiversity and ecosystem functioning in soil. Ambio 26, 563–570.
Brussaard, L., 1998. Soil fauna, guilds, functional groups and ecosystem processes. Applied Soil Ecology 9, 123–135.
Brussaard, L., Aanen, D.K., Briones, M.J., Decaens, T., De Deyn, G.B., Fayle, T.M., James, S.W., 2012. Biogeography and phylogenetic community structure of soil invertebrate ecosystem engineers: global to local patterns, implications for ecosystem functioning and services and global environmental change impacts. In: Wall, D.H. (Ed.), Soil Ecology. Oxford University Press, New York, USA, pp. 201–232.
Burger, M., Jackson, L.E., 2003. Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. Soil Biology and Biochemistry 35, 29–36.
Coleman, D., Crossley, D., Hendrix, P., 2004. Fundamentals of Soil Ecology. Elsevier Academic Press, MA, USA.
Davidson, E., Keller, M., Erickson, H.E., Verchot, L.V., Veldkamp, E., 2000. Testing a conceptual model of soil emissions of nitrous and nitric oxides. BioScience 50, 667.
Davidson, E., Matson, P., Vitousek, P., 1993. Processes regulating soil emissions of NO and N_2O in a seasonally dry tropical forest. Ecology 74, 130–139.
De Ruiter, P.C., Veen, J., Moore, J.C., Brussaard, L., Hunt, H.W., 1993. Calculation of nitrogen mineralization in soil food webs. Plant and Soil 157, 263–273.
De Ruiter, P.C., Neutel, M., Moore, J.C., 1995. Energetics, patterns of interaction strengths, and stability in real ecosystems. Science (New York, N.Y.) 269, 1257–1260.
Didden, W., 1990. Involvement of enchytraeidae (Oligochaeta) in soil structure evolution in agricultural fields. Biology and Fertility of Soils 9, 152–158.
Didden, W.A., 1993. Ecology of terrestrial enchytraeidae. Pedobiologia 37, 2–29.
Edsberg, E., 2000. The quantitative influence of enchytraeids (Oligochaeta) and microarthropods on decomposition of coniferous raw humus in microcosms. Pedobiologia 44, 132–147.
Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H., Hättenschwiler, S., 2010. Diversity meets decomposition. Trends in Ecology & Evolution 25, 372–380.
Hättenschwiler, S., Tiunov, A.V., Scheu, S., 2005. Biodiversity and litter decomposition in terrestrial ecosystems. Annual Review of Ecology, Evolution, and Systematics 36, 191–218.
Heemsbergen, D., Berg, M.P., Loreau, M., Van Hal, J.R., Faber, J.H., Verhoef, H., 2004. Biodiversity effects on soil processes explained by interspecific functional dissimilarity. Science (New York, N.Y.) 306, 1019–1020.
Hixon, M.A., Menge, B.A., 1991. Species diversity: prey refuges modify the effects of predation and competition. Theoretical Population Biology 39, 178–200.
Houba, V., 2000. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. Communications in Soil Science and Plant Analysis, 1299–1396.
Kaneda, S., Kaneko, N., 2011. Influence of Collembola on nitrogen mineralization varies with soil moisture content. Soil Science and Plant Nutrition 57, 40–49.
Kool, D.M., Wrage, N., Zechmeister-Boltenstern, S., Pfeffer, M., Brus, D., Oenema, O., Van Groenigen, J.-W., 2010. Nitrifier denitrification can be a source of N_2O from soil: a revised approach to the dual-isotope labelling method. European Journal of Soil Science 61, 759–772.
Kuiper, I., De Deyn, G.B., Thakur, M.P., Van Groenigen, J.W., 2013. Soil invertebrate fauna affect N_2O emissions from soil. Global Change Biology 19, 2814–2825.
Laurén, A., Lappalainen, M., Saari, P., Kukkonen, J.V.K., Koivusalo, H., Piirainen, S., Setälä, H., Sarjala, T., Bylund, D., Heinonen, J., Nieminen, M., Palviainen, M.,

Launiainen, S., Finér, L., 2012. Nitrogen and carbon dynamics and the role of enchytraeid worms in decomposition of L, F and H layers of Boreal Mor. Water, Air, & Soil Pollution 223, 3701–3719.
Lee, K., Foster, R., 1991. Soil fauna and soil structure. Australian Journal of Soil Research 29, 745–775.
Liiri, M., Ilmarinen, K., Setälä, H., 2007. Variable impacts of enchytraeid worms and ectomycorrhizal fungi on plant growth in raw humus soil treated with wood ash. Applied Soil Ecology 35, 174–183.
Loreau, M., 1998. Ecosystem development explained by competition within and between material cycles. Proceedings of the Royal Society B: Biological Sciences 265, 33–38.
Lubbers, I.M., Van Groenigen, K.J., Fonte, S.J., Six, J., Brussaard, L., Van Groenigen, J.W., 2013. Greenhouse-gas emissions from soils increased by earthworms. Nature Climate Change 3, 187–194.
Maraldo, K., Holmstrup, M., 2009. Recovery of enchytraeid populations after severe drought events. Applied Soil Ecology 42, 227–235.
Maraun, M., Scheu, S., 2000. The structure of oribatid mite communities (Acari, Oribatida): patterns, mechanisms and implications for future research. Ecography 23, 374–383.
Mikola, J., Setälä, H., 1998. No evidence of trophic cascades in an experimental microbial-based soil food web. Ecology 79, 153–164.
Moore, J., Walter, D., Hunt, H., 1988. Arthropod regulation of micro-and mesobiota in below-ground detrital food webs. Annual review of Entomology 33, 419–439.
Paul, B.K., Lubbers, I.M., Groenigen, J.W., 2012. Residue incorporation depth is a controlling factor of earthworm-induced nitrous oxide emissions. Global Change Biology 18, 1141–1151.
Petersen, H., Luxton, M., 1982. A comparative analysis of soil fauna populations and their role in decomposition processes. Oikos 39, 288–388.
Rantalainen, M.L., Fritze, H., Haimi, J., Kiikkila, O., Pennanen, T., Setala, H., 2004. Do enchytraeid worms and habitat corridors facilitate the colonisation of habitat patches by soil microbes? Biology and Fertility of Soils 39, 200–208.
Schmitz, O.J., Hawlena, D., Trussell, G.C., 2010. Predator control of ecosystem nutrient dynamics. Ecology Letters 13, 1199–1209.
Schmitz, O.J., Krivan, V., Ovadia, O., 2004. Trophic cascades: the primacy of trait-mediated indirect interactions. Ecology Letters 7, 153–163.
Seastedt, T., 1984. The role of microarthropods in decomposition and mineralization processes. Annual Review of Entomology 29, 25–46.
Solomon, S., Qin, D., Manning, M., Alley, R.B., Berntsen, T., Bindoff, N.L., Tignor, M., 2007. Technical summary. In: Solomon, D., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K., Miller, H. (Eds.), Climate Change 2007: the Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
Stark, J., Hart, S., 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature 385, 61–64.
Thomson, J., Giannopoulos, G., Pretty, J., Baggs, E.M., Richardson, D.J., 2012. Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. Philosophical Transactions of the Royal Society B: Biological Sciences 367, 1157–1168.
Toscano, B.J., Fodrie, F.J., Madsen, S.L., Powers, S.P., 2010. Multiple prey effects: agonistic behaviors between prey species enhances consumption by their shared predator. Journal of Experimental Marine Biology and Ecology 385, 59–65.
Van Vliet, P., Beare, M., Coleman, D., Hendrix, P., 2004. Effects of enchytraeids (Annelida: Oligochaeta) on soil carbon and nitrogen dynamics in laboratory incubations. Applied Soil Ecology 25, 147–160.
Verhoef, H., Brussaard, L., 1990. Decomposition and nitrogen mineralization in natural and agroecosystems: the contribution of soil animals. Biogeochemistry 11, 175–211.
Vreeken-Buijs, M., 1998. Ecology of Microarthropods in Arable Soil. Wageningen University (PhD dissertation).
Wardle, D.A., 2002. Communities and Ecosystems: Linking the Aboveground and Belowground Components. Princeton University Press, New Jersy, USA.
Wardle, D.A., 2006. The influence of biotic interactions on soil biodiversity. Ecology letters 9, 870–886.
Williams, B.L., Griffiths, B.S., 1989. Enhanced nutrient mineralization and leaching from decomposing sitka spruce litter by enchytraeid worms. Soil Biology and Biochemistry 21, 183–188.
Williams, E.J., Hutchinson, G.L., Fehsenfeld, F.C., 1992. NOx and N_2O emissions from soil. Global Biogeochemical Cycles 6, 351–388.