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Carbon dioxide and nitrous oxide fluxes from soil as influenced by anecic and endogeic earthworms

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ABSTRACT

Earthworm–microbial interactions may stimulate CO_2 and N_2O emissions from soil. This study examined the influence of anecic and endogeic earthworms, represented by *Lumbricus terrestris* L. and *Aporrectodea caliginosa* Savigny, on CO_2 and N_2O fluxes, and on the processes (denitrification, nitrification) that lead to N_2O flux from an agricultural soil. Laboratory microcosms, with and without earthworms, were incubated at 15 °C and 40% water-filled pore space, and headspace gases were sampled after 1, 4, 7, 14, 21, and 28 days. Denitrification and nitrification processes were then evaluated in a 24 h acetylene inhibition experiment. Earthworms were responsible for 7–58% of the total CO_2 flux from soil, compared to the control (no earthworms), but did not affect the N_2O flux. The CO_2 flux was greater when more earthworms were present, and in microcosms with mixed *L. terrestris* and *A. caliginosa* populations, suggesting that microbial respiration could be stimulated by the interactions of anecic and endogeic earthworms. Denitrification was the dominant process leading to N_2O production from microcosms with *L. terrestris*, while nitrification was more important in microcosms with *A. caliginosa*. Microcosms with mixed populations produced more N_2O from denitrification than nitrification. Species-specific stimulation of nitrifiers and denitrifiers may be related to unique structures (casts, burrows) produced by *L. terrestris* and *A. caliginosa*, but this remains to be confirmed.

1. Introduction

Soil microbial activities like decomposition, nitrification and denitrification lead to the emission of CO_2 and N_2O, important greenhouse gases linked to climate change (IPCC, 2007). The earthworm gut and associated structures (casts, burrows, middens) represent microhabitats that can support distinct microbial communities and greater microbial activity than the bulk soil (Brown et al., 2000; Drake and Horn, 2006; Marhan et al., 2007). As a result, earthworm–microbial interactions may stimulate CO_2 and N_2O emissions from soil, but this has been difficult to demonstrate in field experiments due to temporal fluctuations in soil moisture content and available substrates (Schindler Wessells et al., 1997; Bertora et al., 2007). In a microcosm study with agricultural soil, Caravaca et al. (2005) found that 40% of the total CO_2 emission from soils with *Eisenia fetida* and composted residues was due to earthworm activity. Microcosm studies using forest (Karsten and Drake, 1997; Borken et al., 2000) and garden soils (Matthies et al., 1999) have likewise shown that earthworms may be responsible for 30–56% of the total N_2O emitted from the soils they inhabit.

Interactions between earthworms and denitrifying microbes have received special attention because *in situ* conditions in the earthworm gut (anoxia, availability of carbon substrates and nitrate/nitrite) stimulate the growth and activity of ingested denitrifiers, leading to N_2O and N_2 emissions from earthworms (Drake and Horn, 2006). *In vivo* N_2O fluxes from *Lumbricus terrestris* and *Aporrectodea caliginosa*

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may range from 0 to 11 nmol N_2O g^{-1} earthworm (fresh wt.) h^{-1}, which is about 0–0.48 μg N_2O g^{-1} h^{-1} (Horn et al., 2006). Earthworms mix soil and organic residues as they feed and burrow, which often stimulates aerobic respiration and may create anaerobic microsites that favor denitrifying bacteria (Burtelow et al., 1998). For example, earthworm casts and burrow linings have greater nitrification and denitrification rates than bulk soil (Svensson et al., 1986; Elliott et al., 1990; Parkin and Berry, 1994,1999). The middens created by *L. terrestris* contain less NO_3–N than bulk soil, possibly due to NO_3–N losses via denitrification (Subler and Kirsch, 1998). Mineralization, nitrification, and denitrification processes are probably affected by earthworm functional diversity, although the interactions between earthworm functional groups, microbial communities and N transformations are complex (Postma-Blaauw et al., 2006; Sheehan et al., 2006). How earthworm functional groups may interact with the nitrifying and denitrifying bacteria that produce N_2O has not yet been fully examined, although this could be done with a differential acetylene inhibition assay. An acetylene concentration of 5–10% (v/v) will block nitrification and nitrate reductase, providing information about $N_2O + N_2$ production, while an acetylene concentration of 0.01% (v/v) is sufficient to block nitrification, giving a measure of N_2O production (Davidson et al., 1986; Tiedje et al., 1989). We hypothesized the following: (1) soils with more earthworms will have greater CO_2 and N_2O fluxes, (2) gaseous fluxes will be affected by earthworm functional groups and soil conditions, and (3) N_2O production from earthworm-worked soils will come mainly from denitrification.

The objective of this study was to determine how earthworm functional groups influenced CO_2 and N_2O fluxes, as well as the processes that lead to N_2O production (nitrification, denitrification). This study was done in microcosms with agricultural soil and we used *L. terrestris* and *A. caliginosa* as representatives of anecic and endogeic functional groups, respectively.

2. Materials and methods

2.1. Earthworms and soil

Earthworms were collected from a field on the Macdonald Campus Research Farm, Ste. Anne de Bellevue, Quebec, Canada (45° 28′ N, 73° 45′ W) in May 2006 by handsorting and formalin extraction. The earthworms were separated by species and kept in 37 l plastic containers with field soil and several grams of dried soybean leaves placed on the surface as a food source. Containers with earthworms were stored in an incubator at 15 °C for 1 month before the experiment began in June 2006. Soil was collected from the same field in May 2006, sieved through a 6-mm screen, and stored in 37 l plastic containers in a laboratory at 20 °C. The soil was a sandy loam, mixed, frigid Typic Endoaquent of the Chicot series. It contained 580 g kg^{-1} of sand, 300 g kg^{-1} of silt and 120 g kg^{-1} of clay, with 34.2 g organic C kg^{-1}, 3.6 g total N kg^{-1} and pH 5.7.

2.2. Microcosms and experimental design

Microcosms were 1 l jars with 500 g of air-dried soil, packed to a bulk density of 1 g cm^{-3} and moistened to 40% water-filled pore space (WFPS) with distilled water. A total of 115 microcosms were prepared and stored overnight at 4 °C, then microcosms were incubated at 15 °C in the dark for 7 days. Ten microcosms were removed to assess baseline soil conditions, and the rest received earthworm treatments. There were 7 earthworm treatments with 15 replicates arranged in a completely randomized design: control (C, no earthworms), *A. caliginosa* (A), *L. terrestris* (L), and both species (AL) at natural (1×) and double (2×) population levels. The number and biomass of earthworms added is provided in Table 1. Earthworms (pre-clitellate to fully clitellate adults) were added to the microcosms after voiding their guts for 24 h so as to minimize the introduction of exogenous soil microorganisms. We prepared 70 microcosms for repeated

Table 1 – Earthworms added to microcosms (Initial) and recovered after 28 days (Final), and the mean CO_2 and N_2O fluxes during the 28 days study

Treatment	Earthworm numbers (individuals per microcosm)		Earthworm biomass (g fresh wt. per microcosm)		CO_2 flux (mg CO_2–C g^{-1} h^{-1})	N_2O flux (μg N_2O–N g^{-1} h^{-1})
	Initial	Final	Initial	Final		
C	0	0	0	0	0.14 ± 0.02^{d}	48 ± 23
A1×	3	3.1 ± 0.1	1.6 ± 0.1	1.4 ± 0.1	0.23 ± 0.04^{bc}	30 ± 14
A2×	6	5 ± 0.5	2.8 ± 0.2	2.4 ± 0.2	0.22 ± 0.02^{bcd}	8.2 ± 8.1
L1×	1	1	3.8 ± 0.4	3.4 ± 0.3	0.15 ± 0.02^{cd}	7.4 ± 5.5
L2×	2	2	7.1 ± 0.4	6.4 ± 0.3	0.22 ± 0.04^{bc}	24 ± 16
AL1×	4	3.3 ± 0.3	4.6 ± 0.3	3.8 ± 0.3	0.25 ± 0.04^{ab}	50 ± 27
AL2×	8	7.5 ± 0.2	9.7 ± 0.9	8.3 ± 0.4	0.33 ± 0.03^{a}	42 ± 18
Contrast analysis						
A versus L					NS	NS
A versus AL					$P = 0.0324$	NS
L versus AL					$P = 0.0021$	NS

Values are the mean ± standard error, $n = 15$ (earthworms) or $n = 5$ (gas fluxes). Within a column, values with the same letter are not statistically different ($P < 0.05$, Tukey test). Treatments: C, control; A, *A. caliginosa*; L, *L. terrestris*; AL, *A. caliginosa* and *L. terrestris* combined; 1×, ambient population, 2×, twice the ambient population.