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SHORT COMMUNICATION

Earthworm influence on carbon dioxide and nitrous oxide fluxes from an unfertilized corn agroecosystem

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Abstract Earthworms modify the soil environment through their feeding, casting, and burrowing activities, which may lead to more decomposition and respiration in aerobic microsites and more denitrification in anaerobic microsites. The objective of this study was to determine whether earthworms increase CO_2 and N_2O fluxes from an unfertilized corn agroecosystem. Earthworm populations within field enclosures (2.9 m^2) were reduced by repeatedly applying carbaryl insecticide, then single and mixed populations of *Lumbricus terrestris* L. and *Aporrectodea caliginosa* (Savigny) were added. Gas samples were collected once a week for 14 weeks, from June to September 2005. Carbaryl applications reduced, but did not eliminate earthworms from enclosures. The CO_2 and N_2O fluxes were affected by the sampling date, with peak gas fluxes after rainfall events. Mean CO_2 and N_2O fluxes during the study period tended to be greater from enclosures with added earthworms than the control (no earthworms added), but were not significantly affected by earthworm treatments due to the low survival rate of introduced earthworms. Better control of earthworm populations in the field is required to fully assess the impact of earthworms on CO_2 and N_2O fluxes from temperate agroecosystems.

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Introduction

Earthworms mix large quantities of residues into soil and create aggregates and pores through their casting and burrowing activities. These activities may create favorable microhabitats and stimulate microbial respiration, nitrification, and denitrification processes (Scheu 1993; Parkin and Berry 1999). In a microcosm study with agricultural soil, Marhan and Scheu (2005) observed that CO_2 fluxes were increased by the earthworm *Octalasion tyrtaeum* by factors of 1.2 in unfertilized soil, 1.5 with NPK fertilizer and 1.6 with farmyard manure. In corn agroecosystems amended with organic and inorganic fertilizers, Schindler Wessells et al. (1997) found that plots with added earthworms had higher soil respiration than those with ambient or reduced earthworm populations. However, the effect of earthworms on soil respiration was not consistent during the study period, suggesting that respiration was more strongly influenced by fluctuations in environmental conditions and temporal changes in carbon supply than earthworm activities. In a beech forest, Borken et al. (2000) observed that adding *L. terrestris* to unlimed soil columns increased N_2O fluxes significantly by 57%, but N_2O fluxes were about 8% lower when *L. terrestris* were added to limed soils.

These studies suggest that earthworms may stimulate CO_2 and N_2O fluxes at the field scale, but soil amendments (fertilizer, lime) and seasonal fluctuations in weather affect the earthworm–microbial interactions that lead to gaseous fluxes. However, soils that support larger earthworm populations may also have greater CO_2 and N_2O fluxes.

In a soil column experiment, Kretzschmar and Ladd (1993) observed increasing CO_2 fluxes with increasing *A. trapezoides* numbers. Larger populations of *A. trapezoides* created more burrows that may have permitted more gas diffusion from their soil columns. Frederickson and Howell (2003) reported a positive correlation between N_2O fluxes and an increasing number of *Dendrobaena veneta* in vermicomposting beds. It is not known whether the CO_2 and N_2O fluxes are affected by the size of earthworm populations in agroecosystems.

The objective of this study was to determine whether earthworms increased the CO_2 and N_2O fluxes from an unfertilized corn agroecosystem. The study was conducted in plot-scale field enclosures where naturally occurring earthworm populations were reduced with carbaryl (insecticide) before earthworm treatments were added.

Materials and methods

Study site and experimental design

The study site was located on the Macdonald Campus Research Farm, Ste. Anne de Bellevue, Quebec, Canada (45°28′N, 73°45′W). The soil was a well-drained sandy loam of the Chicot series (fine, mixed, frigid Typic Endoaquent) containing 580 g/kg of sand and 120 g/kg of clay with a pH of 5.9, 24.5 g organic C kg^{-1} and 2.6 g total N kg^{-1}. Field enclosures (2.9 m^2) were made of steel sheets 2.4 m long, 1.2 m wide, and 0.6 m high and installed in April 2004. After burying the sheets to 0.4-m depth, the corners and top edges were bent at right angles to ensure a tight fit between pieces and contain earthworms within the enclosures. Five applications of carbaryl (Sevin XLR Plus, Bayer Group), delivering 0.02 kg a.i. m^{-2}, were made to reduce naturally occurring earthworm populations, soybeans (*Glycine max*. L.) were planted by hand in the center (lengthwise) of the enclosure, earthworm treatments were added in June 2004, and their activity was monitored during the growing season (Perreault et al. 2007).

Beginning in mid-April 2005, earthworm populations in the enclosures were reduced with four applications of carbaryl, which delivered a total load of about 0.04 kg a.i. m^{-2}. A row of silage corn (*Zea mays* L. cv. 'Mycogene 2K350') was planted by hand in the center of each enclosure to simulate a corn agroecosystem with a plant population of about 52,000 plants ha^{-1} on May 31, 2005. One week later, earthworm treatments added; the treatments were re-randomized in 2005 to avoid the confounding effect of previous earthworm manipulation. Briefly, the experiment was a randomized complete block design with seven treatments in four replicate blocks. There was a control that received no earthworms and three treatments with earthworms, namely, *A. caliginosa*, *L. terrestris*, and *A. caliginosa* plus *L. terrestris* added at the ambient population level (1×). There were also three treatments with these same earthworms at double the ambient population level (2×). Further details of the experimental design and the number of earthworms added in each treatment were reported by Eriksen-Hamel and Whalen (2007).

Gas sampling

Gas samples were collected in 2005 only, using a vented closed chamber and collar, based on the methods of Hutchinson and Mosier (1981) and Thompson (1996). Collars (5 cm long) were inserted 3 cm into the soil near the center of each plot, 10 cm from the corn row, on June 17, 2005 and remained in place until 2 days before corn harvest on September 26, 2005. Collars were made of polyvinyl chloride (PVC) pipe (11.4 cm o.d., 10.1 cm i.d.) and had two screws set on opposite sides of the collar. The chambers were held tightly in place on top of the collars by wrapping a rubber band around one screw, pulling it over the top of the chamber, and wrapping it around the second screw. Self-adhesive weather-stripping was set on the bottom of the chamber to form a tight seal with the collar. Chambers were made of PVC pipe (10 cm long, 11.4 cm o.d., 10.1 cm i.d.), with a permanent PVC cap on one end and wrapped with aluminum foil to reflect solar radiation. In the chamber cap was a septum for gas sampling and a 20G1 needle as a vent tube, both secured with silicone to prevent gas leaks.

Gas samples were collected between 11:00 and 13:00 h every 7 days during a 14-week period (missing data for week 5, see Figs 1 and 2). Gas samples (20 ml) were taken with a gas-tight syringe as soon as the chamber was placed over the collar (C_0) and after 1 h (C_1), and injected into pre-evacuated 12-ml exetainers (Labco, High Wycombe, UK). The CO_2 and N_2O concentrations were analyzed on a Varian Model 3800 gas chromatograph (Walnut Creek, CA, USA).

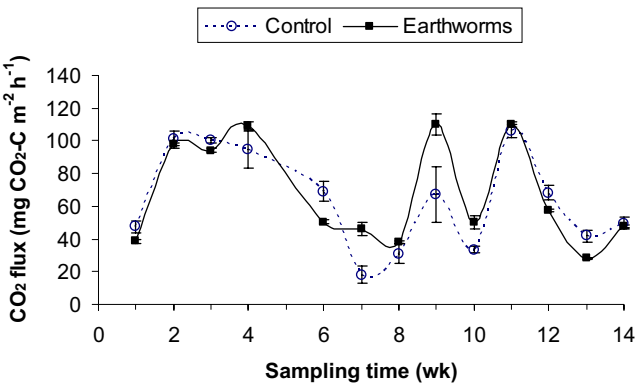

Fig. 1 Dynamics of CO_2 flux from field enclosures without earthworms (control) and with added earthworms (earthworm). Gas fluxes were measured from June 23 to September 23, 2005. *Bars* on data points are the standard error of the mean

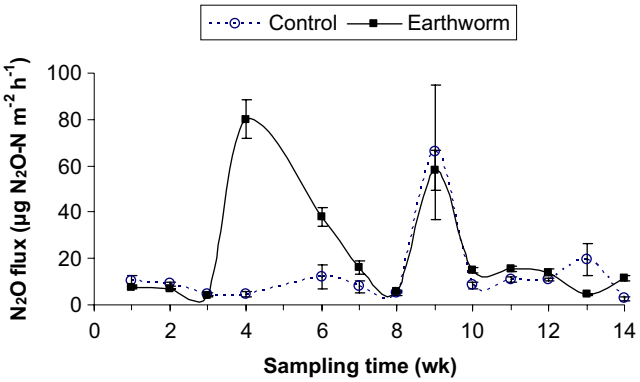

Fig. 2 Dynamics of N_2O flux from field enclosures without earthworms (control) and with added earthworms (earthworm). Gas fluxes were measured from June 23 to September 23, 2005. *Bars* on data points are the standard error of the mean

Fluxes of CO_2 and N_2O were calculated by first converting the gas concentrations in parts per million to milligram per cubic meter using an equation from Holland et al. (1999):

$$C_m = \frac{(C_v MP)}{RT} \quad (1)$$

where C_m is the mass/volume concentration in mg m^{-3}, e.g., mg CO_2 m^{-3}; C_v is the volume/volume concentration in ppm; M is the molecular weight of the trace species, e.g., CO_2 has 12 µg C µmol^{-1} CO_2; P is the atmospheric pressure, 1 atm; T is temperature in K; and R is the universal gas constant, 0.082 L atm mol^{-1} K^{-1}. Then, the flux was calculated using the linear equation of Hutchinson and Mosier (1981):

$$f = \frac{V(C_1 - C_0)}{At} \quad (2)$$

where f is the gas flux in mg m^{-2} h^{-1}; V is the volume of the chamber (0.00104 m^3); $C_1 - C_0$ is the change in concentration in mg m^{-3}; A is the area of soil covered (0.00801 m^2); and t is the time between the first and second gas sample collection (1 h).

Earthworm sampling

The earthworm population in each enclosure was enumerated in October after the corn was harvested. Soil was collected from a pit (0.5×0.3×0.20 m) in the center of each enclosure and hand-sorted, while earthworms from deeper soil layers were collected by formalin extraction (Raw 1959). Earthworms were preserved in 5% formaldehyde solution until they could be identified using the key of Reynolds (1977).

Statistical analysis

Carbon dioxide and N_2O fluxes were tested for normality using the PROC UNIVARIATE function of SAS (Version 9.1, SAS Institute, Cary, NC). Carbon dioxide fluxes had a normal distribution, but N_2O fluxes did not; they were therefore log-transformed before analysis. The effect of selected earthworm treatments on weekly CO_2 and N_2O fluxes were analyzed by repeated measures analysis of variance (ANOVA) using the PROC MIXED function of SAS. The CO_2 and N_2O fluxes were affected significantly ($P<0.05$) by sampling time, but not by earthworm treatments. Results are presented as the mean (with standard error, SE) of weekly gas flux from control enclosures ($n=4$, no earthworms added) and treatments that had significantly ($P<0.05$) more earthworms than the control enclosures ($n=16$, see Table 1).

Table 1 Earthworms populations added in June 2005 and collected in October 2005 from enclosures and the mean number of juveniles and adults of *A. caliginosa* and *L. terrestris* in October 2005

Treatment	Population (individual m^{-2} ± SE)		Mean number of earthworms in October			
			A. caliginosa		*L. terrestris*	
	June	October	Juvenile	Adult	Juvenile	Adult
C	0	93±18 c*	52	20	20	0
A1×	50	132±23 Ab	60	25	47	0
A2×	100	135±33 Ab	92	18	23	0
L1×	15	147±67 a	75	40	27	0
L2×	30	117±45 bc	57	25	32	0
AL1×	65	153±24 a	87	25	40	0
AL2×	130	95±10 c	55	27	12	0
Background[a]		233±15	133	60	23	7

Treatment designations: C, control; A, *A. caliginosa*; L, *L. terrestris*; AL, *A. caliginosa* and *L. terrestris* combined; 1×, ambient population level; 2×, elevated population level

*Values within a column followed by the same letter were not significantly different ($P<0.05$, LSD test).

[a] Background earthworm populations were obtained from two pits adjacent to the field enclosures.

Results and discussion

Earthworms were not eliminated from enclosures with carbaryl applications, although the naturally occurring earthworm population in the control enclosures were lower than the background population in October 2005 (Table 1). Carbaryl is toxic to earthworms and can reduce earthworm numbers and biomass by 90% (Potter et al. 1990), but it has a half-life of 2–9 days in well-aerated sandy soils and is also photodegraded when it remains on the soil surface (Thapar et al. 1995; Nkedi-Kizza and Brown 1998). Earthworms found in the control enclosures in October 2005 may have survived repeated applications of carbaryl or were hatched from cocoons shortly after the last carbaryl application. Adding earthworms to repopulate enclosures did not always result in larger earthworm populations than in the control enclosure (Table 1). It is difficult to manipulate earthworm populations in the field and introduced earthworms often have low survival rates (Bohlen et al. 1995; Boyer et al. 1999; Eriksen-Hamel and Whalen 2007). Therefore, only enclosures with significantly ($P<0.05$) more earthworms than the control in October 2005 were included in the statistical analysis (selected earthworm treatments were A1×, A2×, L1× and AL1×).

Repeated measures ANOVA demonstrated that CO_2 and N_2O fluxes were affected significantly ($P<0.05$) by sampling time, but not earthworm treatments. The peak CO_2 and N_2O fluxes occurred in week 4 (two rainfall events of 60.6 and 23.6 mm occurred the week before this sampling date), week 9 (after 3 days of rain totalling 18.8 mm), and week 11 (after a single event with 73.8 mm of rain). Rainfall is perhaps the most important factor affecting microbial activities that lead to CO_2 and N_2O emission from temperate agroecosystems during the growing season (Rochette et al. 1991). In a 62-day laboratory study, Bertora et al. (2007) reported that cumulative N_2O and CO_2 fluxes were influenced more by the soil moisture content than the presence of earthworms in soil mesocosms. Earthworm and microbial activities were constrained at lower soil moisture levels.

The average CO_2 flux during the 14-week sampling period was 7% greater in enclosures with added earthworms (67.8±3.6 mg CO_2–C m^{-2} h^{-1}) than the control enclosures (63.6±5.1 mg CO_2–C m^{-2} h^{-1}), while the average N_2O flux during this period was 63% greater with added earthworms (21.6±4.3 μg N_2O–N m^{-2} h^{-1}) than the control (13.2±4.7 μg N_2O–N m^{-2} h^{-1}). While consistent with other field studies that suggest a stimulation of CO_2 and N_2O fluxes in the presence of earthworms (Schindler Wessells et al. 1997; Borken et al. 2000), these values were not statistically different due to the seasonal variability in gas fluxes and similarity in earthworm population size within treated and control enclosures.

We could not conclude that earthworms increased the CO_2 and N_2O fluxes from an unfertilized corn agroecosystem during the growing season (June to September). In the future, it could be more informative to study CO_2 and N_2O fluxes from earthworm-worked soils during the entire frost-free period, as earthworms in temperate regions are more active in the spring (from snowmelt to mid-June) and fall (early October to snowfall) rather than during the drier, hotter summer months. It is challenging to manipulate earthworm populations in the field, but additional work on experimental methods to control the size and composition of earthworm communities could provide information on how earthworm populations and functional groups (i.e., anecics, endogeics, and epigeics) contribute to CO_2 and N_2O fluxes. Such efforts will ultimately lead to a better understanding of how earthworm–microbial interactions affect greenhouse gas emissions from agricultural soils.

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