Biol Fertil Soils
DOI 10.1007/s00374-014-0965-0

SHORT COMMUNICATION

Endogeic earthworms lower net methane production in saturated riparian soils

Maria Kernecker · Joann K. Whalen · Robert L. Bradley

Received: 18 June 2014 / Revised: 22 September 2014 / Accepted: 25 September 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract Methane (CH_4) emissions from soils are erratic, the typical pattern being long periods of imperceptible emissions punctuated by short periods of high CH_4 release. These short peaks are a result of higher gross CH_4 production rates (methanogenesis) relative to gross rates of CH_4 consumption (methanotrophy), and can be induced by rising water table level and periodic flooding in riparian soils. Soil fauna, specifically earthworms, affected soil CH_4 cycling in some studies. We studied the effect of the endogeic earthworm *Aporrectodea turgida* (Eisen) on CH_4 production and consumption activities in saturated and field-moist soils collected from a riparian area in southern Québec, Canada. Saturated (100 % water-filled pore space, WFPS) and field-moist soils (31.5 % WFPS) were incubated at 20 °C, with difluoromethane added to quantify gross CH_4 production (i.e., methanogenesis activity) and consumption rates (i.e., methanogenic activity). The pattern of gross CH_4 consumption followed that of gross CH_4 production, and net CH_4 production was an order of magnitude lower than the gross rate. Gross rates of CH_4 production were 41 to 65 ng CH_4 g^{-1} h^{-1}, gross CH_4 consumption rates were estimated to be 37 to 63 ng CH_4 g^{-1} h^{-1}, and the net CH_4 production ranged from 2 to 4 ng CH_4 g^{-1} h^{-1}. Saturated soils with earthworms had consistently lower net CH_4 production, relative to field-moist and saturated soils without earthworms. This means that earthworms could potentially reduce the impact of periodic flooding on CH_4 emissions from riparian soils.

Keywords Earthworms · Methane · Riparian · Difluoromethane · Methanogenesis · Methanotrophy

Introduction

Soil methane (CH_4) emissions are often erratic, with long periods of being undetected punctuated by short periods of high CH_4 release (Le Mer and Roger 2001). These short periods, or "hot intervals," occur when gross CH_4 production (methanogenesis) exceeds gross CH_4 consumption rates (methanotrophy). High soil moisture due to periodic flooding in riparian soils is conducive to hot intervals of CH_4 emissions (Altor and Mitsch 2008; McClain et al. 2003; Vidon et al. 2010) because lower oxygen (O_2) concentrations (i.e., lower redox conditions) favor CH_4 production and inhibit CH_4 oxidation, in spite of recent evidence that anaerobic CH_4 oxidation may be more prevalent in surface soils than previously thought (Gupta et al. 2013).

Besides soil moisture, net soil CH_4 production may be influenced by biological factors such as plant community composition (Dias et al. 2010), NH_4^+ oxidizing bacteria (Jiang and Bakken 1999), and soil fauna (Ho et al. 2013). An example of the latter are earthworms that may affect soil CH_4 emissions both positively (Kammann et al. 2009) and negatively (Kim et al. 2011), while the underlying mechanisms remain unclear. On the one hand, the earthworm gut and fresh earthworm casts may stimulate methanogenic activity (Depkat-Jakob et al. 2012). Alternatively, earthworm burrows may aerate the soil and favor methanotrophic activity. Hence, the reported effects of various earthworm species on net CH_4 production are site-specific, depending on soil moisture

M. Kernecker · J. K. Whalen (✉)
Department of Natural Resource Sciences, Macdonald Campus, McGill University, 21111 Lakeshore Road, Ste-Anne-de-Bellevue, Montreal, Québec H9X 3V9, Canada
e-mail: joann.whalen@mcgill.ca

M. Kernecker
e-mail: mkernecker@gmail.com

R. L. Bradley
Department of Biology, University of Sherbrooke, 2500 boul. de l'Université Sherbrooke, Sherbrooke, Québec J1K 2R1, Canada
e-mail: Robert.Bradley@USherbrooke.ca

Published online: 10 October 2014

conditions and endemic methanogenic or methanotrophic bacteria (e.g., Kammann et al. 2009; Koubova et al. 2012; Bradley et al. 2012). In order to gain deeper insight into this conundrum, we tested the hypothesis that earthworms exacerbate CH_4 production in riparian soils from southern Québec (Canada) that are flooded (i.e., 100 % WFPS) compared to field-moist soils (31.5 % WFPS). Soil samples were incubated in the presence and absence of difluoromethane (DFM), a gas that selectively inhibits methanotrophic bacteria (Miller et al. 1998). This allowed us to deduce the relative effects of earthworms on gross rates of CH_4 production and consumption simultaneously.

Materials and methods

Aporrectodea turgida is an endogeic species dominating the earthworm communities in riparian zones of northeastern North America (Bradley et al. 2011; Costello and Lamberti 2008). Sexually mature specimen of *A. turgida* were collected from an alfalfa field and depurated on moistened tissue paper at 15 °C for 24 h. Fresh earthworm weights were determined before and after the experiment. A bulk soil sample was collected from the top 15 cm along the bank of a riparian forest near Bedford, Québec (45° 07′ 43″ N, 73° 00′ 21″ W). This soil belongs to the Suffield clay loam series (ca. 40 % sand and 16 % clay) and is classified as a Humo-Ferric Podzol (Soil Classification Working Group 1998) with pH 5.9 and organic C of 24.3 g kg^{-1} in the 0–15-cm layer.

This experiment simulated a flooding event in riparian soil by increasing water-filled pore space (WFPS) to 100 % lasting 24 h. The experiment used a factorial design with repeated measures, testing the effects and interactions of two levels of soil moisture (31.5 vs. 100 % WFPS) and two levels of earthworm densities (presence vs. absence). The lower moisture content corresponded to soil moisture at the time of sampling, whereas the higher moisture content simulated a flooding event lasting 24 h. Experimental units consisted of 1-L Mason jars to which we added 200 g of non-sieved field-moist soil, to maintain as much of the original soil structure as possible. The saturated soil treatment received 50 mL of distilled water, bringing soil moisture to 100 % WFPS. In microcosms with earthworms, we placed two adult *A. turgida* on the soil surface approximately 1 h before jars were sealed. Due to lighting in the laboratory, earthworms rapidly burrowed into the soil before the jars were sealed with lids equipped with septa. Considering the surface area of the Mason jars (76-mm dia.), the earthworm population was equivalent to 440 individuals per square meter, which was similar to field populations in the riparian forest where the soil was collected (Kernecker 2013).

Each treatment was replicated 20 times, and 10 empty jars (controls) were used to assess ambient CH_4 concentrations. Ten replicate jars of each treatment were used to measure net CH_4 production, whereas the other 10 jars of each treatment were injected with difluoromethane (DFM). More precisely, jars containing field-moist soil, saturated soil and the blank controls were injected with 26, 27, and 30 μL DFM, respectively. These quantities provided an effective concentration of 0.04 kPa (±0.01 kPa), which inhibits CH_4 oxidation while ostensibly not affecting CH_4 production (Miller et al. 1998). Methane production in the presence of DFM thus reflected gross CH_4 production rates. Gross CH_4 consumption rates were then calculated as the difference between gross and net CH_4 production rates. All microcosms were then kept at 20 °C for 24 h, during which aliquots of headspace gas (2 mL) were sampled with a needle and syringe at 0, 2, 4, 6, 14, 17, 21, and 24 h via septa in microcosms lids. This amount of gas was chosen to interfere as little as possible with the CH_4 and oxygen concentrations. Methane concentration in these gas samples were determined using a 450-GC System gas chromatograph (Bruker Corp., Bremen, Germany) equipped with an FID and an oven temperature set at 300 °C.

Gross rates of CH_4 production and consumption, and net rates of CH_4 production, were tested for normality using the Shapiro-Wilk test and log-transformed as necessary. Data were analyzed by repeated measures ANOVA, testing for between- and within-subjects effects of earthworms, soil moisture, and time. Due to significant interactions between treatments and time, the treatment effects on gross and net CH_4 transformation rates were analyzed within each sampling time by two-way ANOVA. To test how treatments affected CH_4 rates between individual sampling times, single degree of freedom orthogonal contrasts were used. Two-way ANOVA was used to test the cumulative effects of earthworm × soil moisture interactions on gross and net CH_4 transformation rates over the course of 24 h. Means of the four treatment combinations were compared with Tukey HSD tests, as appropriate. Analyses were performed using the JMP® (2007) interface, version 10, of SAS statistical software (Cary, NC).

Results

Soil water content and DFM did not substantially affect earthworm survival, as >90 % of earthworms in each treatment were alive and active after 24 h. Gross CH_4 consumption rates were higher in saturated than in field-moist soils ($p=0.039$) (Table 1). Earthworms increased average gross CH_4 production and consumption rates during the 24-h incubation by 31 % ($p=0.002$) and 38 % ($p=0.004$), respectively (Fig. 1b, c; Table 1). Earthworms increased gross CH_4 production and consumption rates during the first 6 h of the incubation. Field-moist soil without earthworms had the lowest gross CH_4 rates at measurement times between 14 and 24 h (Fig. 1b, c).

Table 1 Repeated measures analysis of variance testing the overall effect of earthworms (EW) and soil moisture (M) (i.e., between subjects affects), as well as their interactions with time (T) (i.e., within subjects effects), on gross and net CH_4 transformation rates (ng CH_4 g^{-1} h^{-1})

	Gross CH_4 production		Gross CH_4 consumption		Net CH_4 production	
	f value	*P* value	*f* value	*P* value	*f* value	*P* value
Between subjects						
Model	5.39	0.004**	4.98	0.005**	8.27	0.0004**
EW	11.42	0.002**	9.60	0.004**	1.42	0.0192*
M	3.61	0.065	4.58	0.039*	20.93	0.0025*
EW × M	1.15	0.290	0.76	0.387	2.47	0.0127*
Within subjects						
Time (T)	203.60	<0.001***	165.68	<0.001***	7.16	<0.001***
EW × T	10.56	<0.001***	5.25	0.002**	0.72	0.1068
M × T	1.22	0.305	2.07	0.104	4.76	<0.0001***
EW × M × T	5.87	<0.001***	5.33	<0.001***	2.31	0.0409*

Significance levels are denoted *$p<0.05$; **$p<0.01$; ***$p<0.001$

Gross CH_4 transformation rates were approximately one order of magnitude higher than net CH_4 production rates across all treatments (Fig. 1a–c). Net CH_4 production was lower in saturated soil with earthworms (p=0.0127, Table 1; Fig. 1a) than in all other treatments, at seven of the eight sampling times (Fig. 1a).

Discussion

Here, gross CH_4 transformation rates far exceeded net CH_4 transformation rates. This relationship may explain why CH_4 emissions are often quite erratic in nature, since a modest increase or decrease in gross CH_4 production relative to gross CH_4 consumption may cause a relatively large change in net CH_4 production. As such, we found that greater gross oxidation relative to gross CH_4 production in saturated soils led to lower net production in the presence of earthworms. This result underscores the importance of measuring gross rates, since these provide insight to the net results.

Although DFM has been shown to reduce the activities of nontarget microbial groups (Miller et al. 1998), we did not find any literature supporting the idea that DFM was toxic to soil organisms, including earthworms. We were unable to find literature regarding the toxicity or disturbance of DFM to soil organisms, and the identical survival rate of earthworms between DFM and non-DFM-treated microcosms suggest that it did not have any notable effect.

The lower net CH_4 production rates in saturated soils with earthworms confirm that methanotrophy stimulated by earthworm activity offset the CH_4 produced by methanogens in saturated soils. This phenomenon may be linked to O_2 availability in microsites, some of which are anoxic (for CH_4 production) and some of which are oxic (for CH_4 oxidation). We posit that by creating macropores via burrowing and oxygenating water by moving their posterior ends (Fox and Taylor 1955), earthworms facilitated the diffusion of O_2 to the surface of their biostructures (e.g., burrow linings and casts) that serve as favorable anoxic microsites for methanogens. The CH_4 diffusing from the anoxic microsites would be consumed by active methanotrophs living in a relatively more O_2-rich environment at the surface of biostructures and in biopores, and consequently, the net CH_4 production would be reduced by earthworms in saturated soils, as observed (Fig. 1c). Microscale O_2 gradients control the balance of CH_4 production and consumption in flooded rice paddy soil (Reim et al. 2012), and it would appear to be the same phenomenon in flooded riparian soil. Possibly, CH_4 and O_2 diffusion was restricted in saturated soils without earthworms (Yu et al. 2013). Simultaneous CH_4 production and consumption in microsites is often invoked as an explanation for soil CH_4 emissions, but should be quantified with O_2 microprofile measurements such as those performed by Reim et al. (2012).

To understand the observed effects of earthworms on net CH_4 production, we consider that earthworm activity can stimulate the activity of both (a) methanogens, which would generate more CH_4 (Fig. 1b), and (b) methanotrophs, which would consume more CH_4 (Fig. 1c). Earthworms may produce substrates that are metabolized by methanogens, and thus stimulate in situ methanogen activity (Depkat-Jakob et al. 2012). Low molecular weight C sources such as formate, acetate, and methanol are directly metabolized to CH_4 by methanogenic bacteria and archaea (Whitman et al. 2006). In the lumbricid earthworms *Aporrectodea caliginosa*, *Allolobophora chlorotica*, *Lumbricus rubellus*, and *Lumbricus terrestris*, the gut contains byproducts of anaerobic metabolism, including formate and acetate (Horn et al. 2003). Earthworms possibly provided these substrates to

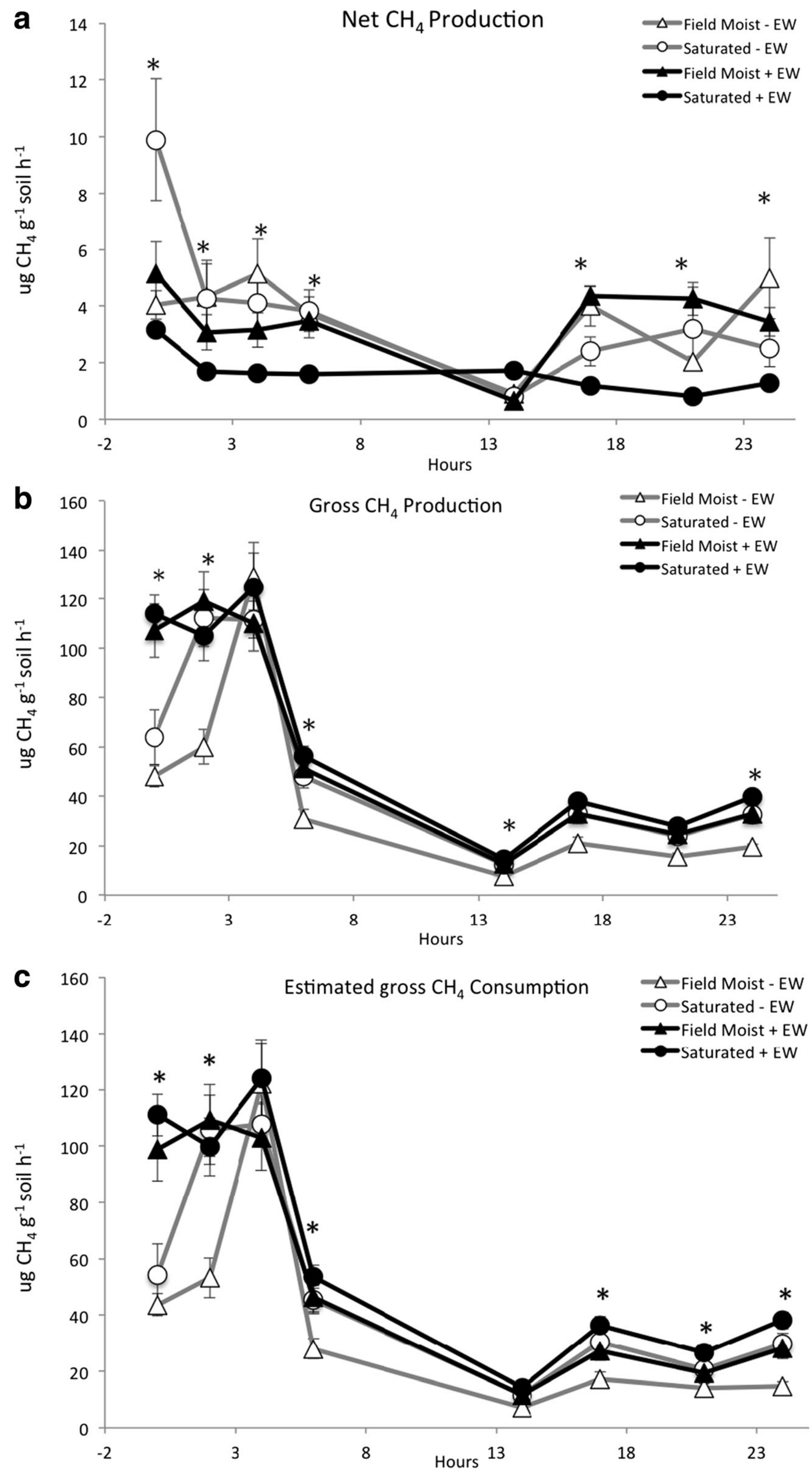

Fig. 1 Mean CH_4 rates over 24 h (n=10). **a** Mean rates of net CH_4 production, *asterisk* indicate significant EW × M interaction effects based on two-way ANOVA ($p<0.05$). **b** Mean rates of gross CH_4 production over 24 h, *asterisk* indicate significant EW × M interaction effects based on two-way ANOVA ($p<0.05$). **c** Mean rates of estimated gross CH_4 consumption over 24 h, *asterisk* indicate significant EW × M interaction effects based on two-way ANOVA ($p<0.05$)

methanogens, leading to higher CH_4 concentrations that in turn could have stimulated methanotrophy (Chan and Parkin 2001), resulting in lower net CH_4 emissions from saturated soils with earthworms.

Conclusions

The mechanisms we propose to explain the direct and indirect effects of earthworms on methanogenesis and methanotrophy provide a framework for further investigation. While we do not claim to go beyond the scope of our study, we do suggest that earthworms are responsible for changing the oxic-anoxic gradient in saturated soils, which is important for CH_4 dynamics in riparian buffer soils.

Acknowledgments The authors appreciate funding from the Centre SÈVE Regroupements stratégiques grant number 144531 and team grant number 131894, both from the Fonds du Québec de recherché Nature et technologies (FQRNT).

References

Altor AE, Mitsch WJ (2008) Pulsing hydrology, methane emissions and carbon dioxide fluxes in created marshes: a 2-year ecosystem study. Wetlands 28:423–438

Bradley RL, Whalen J, Chagnon PL, Lanoix M, Alves MC (2011) Nitrous oxide production and potential denitrification in soils from riparian buffer strips: influence of earthworms and plant litter. Appl Soil Ecol 47:6–13

Bradley RL, Chronakova A, Elhottova D, Simek M (2012) Interactions between land-use history and earthworms control gross rates of soil methane production in an overwintering pasture. Soil Biol Biochem 53:64–71

Chan ASK, Parkin TB (2001) Methane oxidation and production activity in soils from natural and agricultural ecosystems. J Environ Qual 30: 1896–1903

Costello DM, Lamberti GA (2008) Non-native earthworms in riparian soils increase nitrogen flux into adjacent aquatic ecosystems. Oecologia 158:499–510

Depkat-Jakob PS, Hunger S, Schulz K, Brown GG, Tsai SM, Drake HL (2012) Emission of methane by *Eudrilus eugeniae* and other earthworms from Brazil. Appl Environ Microbiol 78:3014–3019

Dias ATC, Hoorens B, van Logtestijn RSP, Vermaat JE, Aerts R (2010) Plant species composition can be used as a proxy to predict methane emissions in peatland ecosystems after land-use changes. Ecosystems 13:526–538

Fox MH, Taylor AER (1955) The tolerance of oxygen by aquatic invertebrates. Phil Trans R Soc B 143:214–225

Gupta V, Smemo KA, Yavitt JB, Fowle D, Branfireun B, Basiliko N (2013) Stable isotopes reveal widespread anaerobic methane oxidation across latitutde and peatland type. Environ Sci Technol 47: 8273–8279

Ho A, Erens H, Mujinya B, Boeckx P, Baert G, Schneider B, Frenzel P, Boon N, van Ranst E (2013) Termites facilitate methane oxidation and shape the methanotrophic community. Appl Environ Microbiol 79:7234–7240

Horn MA, Schramm A, Drake HL (2003) The earthworm gut: an ideal habitat for ingested N_2O-producing microorganisms. Appl Environ Microbiol 69:1662–1669

Jiang Q-Q, Bakken LR (1999) Nitrous oxide production and methane oxidation by different ammonia-oxidizing bacteria. Appl Environ Microbiol 65:2679–2684

JMP® (2007) Version *10*. SAS Institute Inc., Cary, NC

Kammann C, Hepp S, Lenhart K, Müller C (2009) Stimulation of methane consumption by endogenous CH_4 production in aerobic grassland soil. Soil Biol Biochem 41:622–629

Kernecker M (2013) The functional role of earthworms in carbon and nitrogen dynamics in riparian areas under different land use in southern Quebec. Ph.D.Dissertation, McGill University, Montreal, Canada, 135 p. http://digitool.Library.McGill.CA:80/R/-?func=dbin-jump-full&object_id=117140&silo_library=GEN01

Kim TG, Moon KE, Lee EH, Choi SA, Cho KS (2011) Assessing effects of earthworm cast on methanotrophic community in a soil biocover by concurrent use of microarray and quantitative real-time PCR. Appl Soil Ecol 50:52–55

Koubova A, Goberna M, Simek M, Chronakova A, Pizl V, Insam H, Elhottova D (2012) Effects of the earthworm *Eisenia andrei* on methanogens in a cattle-impacted soil: a microcosm study. Eur J Soil Biol 48:32–40

Le Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: a review. Eur J Soil Biol 37:25–50

McClain ME, Boyer EW, Dent CL, Gergel SE, Grimm NB, Groffman PM, Hart SC, Harvey JW, Johnston CA, Mayorga E, McDowell WH, Pinay G (2003) Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6: 301–312

Miller LG, Sasson C, Oremland RS (1998) Difluoromethane, a new and improved inhibitor of methanotrophy. Appl Environ Microbiol 64: 4357–4362

Reim A, Lucke C, Krause S, Pratscher J, Frenzel P (2012) One millimetre makes the difference: high-resolution analysis of methane-oxidizing bacteria and their specific activity at the oxic-anoxic interface in a flooded paddy soil. ISME J 6:2128–2139

Soil Classification Working Group (1998) The Canadian system of soil classification. Agric Agri-Food Can Publ 1646 (Revised), NRC Research Press, Ottawa, 187 p

Vidon PGF, Allan CJ, Burns D, Duval TP, Gurwick NP, Inamdar S, Lowrance R, Okay J, Scott D, Sebestyen S (2010) Hot spots and hot moments in riparian zones: potential for improved water quality management. J Am Water Resour Assoc 46:278–298

Whitman WB, Bowen T, Boone D (2006) The methanogenic bacteria. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes. Springer, New York, pp 165–207

Yu L, Tang J, Zhang R, Wu Q, Gong M (2013) Effects of biochar application on soil methane emission at different soil moisture levels. Biol Fertil Soils 49:119–128